Volume 121 · Supplement 2 · August 2017

BCPT
Basic & Clinical Pharmacology & Toxicology

37th SEF National Meeting with guest society: the British Pharmacology Society
18–21 June 2017
Barcelona, Spain

WILEY
Blackwell

BASIC & CLINICAL
PHARMACOLOGY & TOXICOLOGY
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Aims and Scope

Basic & Clinical Pharmacology & Toxicology is an independent journal, publishing original scientific research in all fields of toxicology, basic and clinical pharmacology. This includes experimental animal pharmacology and toxicology and molecular (-genetic), biochemical and cellular pharmacology and toxicology. It also includes all aspects of clinical pharmacology: pharmacokinetics, pharmacodynamics, therapeutic drug monitoring, drug/drug interactions, pharmacogenetics/-genomics, pharmacoepidemiology, pharmacovigilance, pharmacoeconomics, randomized controlled clinical trials and rational pharmacotherapy. For all compounds used in the studies, the chemical constitution and composition should be known, also for natural compounds.

 Basic & Clinical Pharmacology & Toxicology ISSN 1742-7843 (Online) is published by John Wiley & Sons Ltd, 9600 Garsington Road, Oxford OX4 2DQ, UK. Tel: +44 1865 776868; Fax: 44 1865 714591.

PLENARY SPEAKERS

PL-1
ROLE OF THE ENDOCANNABINOID SYSTEM IN FOOD-INDUCED ADDICTIVE-LIKE BEHAVIOR

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An increasing perspective conceptualizes obesity and overeating as disorders related to addictive-like processes that could share common neurobiological mechanisms. We aimed at validating an animal model of eating addictive-like behavior in mice, based on the DSM-5 substance use disorder criteria, using operant conditioning maintained by highly palatable chocolate-flavored pellets. For this purpose, we evaluated persistence of food-seeking during a period of non-availability of food, motivation for food, and perseverance of responding when the reward was associated with a punishment. This model has allowed identifying extreme subpopulations of mice related to addictive-like behavior. We investigated in these subpopulations the Epigenetic and proteomic studies have allowed to identify a significant decrease in DNA methylation of CNR1 gene promoter in the prefrontal cortex of addict-like mice, which was associated with an upregulation of CB1 protein expression in the same brain area. The pharmacological blockade of CB1 receptor during the late training period reduced the percentage of mice that accomplished addiction criteria, which is in agreement with the reduced performance of CB1 knockout mice in this operant training. Proteomic studies have identified proteins differentially expressed in mice vulnerable or not to addictive-like behavior in the hippocampus, striatum, and prefrontal cortex. The use of DREADD techniques in this model has now allowed identifying the crucial role of the prefrontal cortex in the development of eating addictive-like behavior. This model provides an excellent tool to investigate the neurobiological mechanisms underlying eating addictive-like behavior.

PL-2
GPCR EXPRESSION IN HEALTH AND DISEASE IDENTIFIES NEW THERAPEUTIC TARGETS

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G protein-coupled receptors (GPCRs) are the most widely targeted class of FDA- and EMA-approved therapeutics but only a small portion (~15%) of known GPCRs are currently targeted with such drugs. We have tested the hypothesis that individual cell types endogenously express previously unrecognized GPCRs that may be drug targets. Our recent efforts have focused on human cells and on defining differential expression of GPCRs on cells from patients with certain diseases, including pulmonary arterial smooth muscle cells/pulmonary arterial hypertension, lung and cardiac fibroblasts/lung and cardiac fibrosis and pancreatic cancer compared to control cells. To test our hypothesis, we have used unbiased approaches (Taqman GPCR arrays and RNA-seq), mining of publicly available datasets (such as The Cancer Genome Atlas, TCGA), and validation studies to assess GPCR signaling and functional activity. We find that most cell types express >100 different GPCRs. Little or no prior functional data exist in such cells for many of the highest expressed GPCRs, numerous of which are "orphans" (without known physiologic agonists). Differentially expressed GPCRs in the disease settings can alter cell function. A major focus has been on pancreatic ductal adenocarcinoma (PDAC) tumors and cells and pancreatic cancer associated fibroblasts (PCAFs). We have identified two orphan GPCRs (Orphan 1 and Orphan A) with high expression in PCAFs and PDAC cells, respectively, and at least 2-fold higher expression in >90% of PDAC tumors (in TCGA) compared to normal pancreas. Orphan 1 and Orphan A regulate functional activity that influences the malignant phenotype. Overall, our results imply that previously unrecognized GPCRs contribute to several diseases and thus may be novel, druggable targets. Moreover, our approach should be readily applicable to other cell types and disease settings. (Supported by NIH grants R21CA202608, R21AG053568, R56AI110505, T32GM007752, T32HL007444, T32HL134632, DoD grant W81XWH-14-1-0372 and a contract with Bristol Myers Squibb).

PL-3
CANCER EPIGENETICS: GENES AND DRUGS

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For the last 25 years an increasing amount of evidence has shown the relevance of epigenetics in cell biology and tissue physiology, being DNA methylation aberrations in cancer the flag-ship for the recognition of its disturbance in human diseases. From the candidate gene approaches, new powerful technologies such as comprehensive DNA methylation microarrays and whole genome bisulfite sequencing has recently emerged that have reinforced the notion of epigenetic disruption in the crossroad of many sickness. From the poster-boy cases of MGMT and GSTP1 hypermethylation in the prediction of alkylating drug response and prostate cancer detection, respectively, to the personalized treatment of leukemia with small molecules targeted to fusion proteins involving histone modifiers such as DOT1L and MLL, the field has walked a long path. The current talk will focus in the epigenetic profiling, basically at the level of DNA methylation and histone modifications, that is starting to provide clinical value in the diagnosis, prognosis and prediction of response to drug therapies, with an emphasis in neoplasia, but without forgetting the novel advances in other human disorders. For cancer, we have already a wide view of the undergoing DNA methylation events that expand beyond classical promoter CpG islands of tumor suppressor genes and we have a growing list of mutated chromatin remodeler genes that contributes to the tumorigenesis process. It is time to apply this knowledge in practical clinical situations like the diagnosis of cancers of unknown primary, the screening of malignancies in high-risk populations or a biomarker selection of the patients that should receive treatment with epigenetic drugs.

PL-4
A NOVEL PHARMACOLOGICAL PARADIGM FOR CHRONIC PAIN

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Pain is the most common complaint for which patients seek treatment from a physician and it has been estimated that nearly half a billion cases are diagnosed each year. Both acute and chronic pain have a major impact on many quality-of-life measures[1]. Chronic pain is

suffered by up to 20% of the adult European population and its incidence is rising as the population ages. For over 50% of these individuals treatment remains unsatisfactory. Despite the prevalence of undertreated chronic pain, the pharmacological armamentarium to prevent or reduce it is surprisingly limited, mainly due to our poor understanding of the genetic, molecular and cellular mechanisms underlying various pain syndromes, along with the inter-individual variation. A large focus has been directed to modulate peripheral receptor operated signalling in nociceptors, developing high affinity modulators. However, these approaches have not rendered yet the expected clinical benefits. We have alternatively focused our pharmacological approach on modulating the disease-induced expression of neuronal receptors. A genomic study revealed that thermoreceptors such as TRPV1 are recruited to the neuronal surface by inflammatory and algesic agents, leading to nociceptor sensitization. Abrogation of TRPV1 recruitment notably reduces neuronal sensitization and produces strong anti-nociception in models of inflammatory and neuropathic pain. The most advanced compound targeting this mechanism is currently in clinical development for chronic pain. Funded by MINECO and GVA (PROMETEO Program).

PL-5
PHARMACOLOGY LEAPING OVER THE GAP: BRINGING TRANSFORMATIONAL MECHANISMS TO DRUG DISCOVERY

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Open Innovation has transformed early drug discovery in a highly multidisciplinary and delocalized scenario, including all the partners sharing knowledge in real time through the entire health ecosystem. The changing face of drug discovery could facilitate to apply, in a holistic way, the pharmacological research path to transformational biomedical research programs. Pharmaceutical companies have created new departments of open innovation in which worldwide best experts are connecting the research on the mechanisms of disease with their pipelines. It is a new world to create 'real' innovation in drug discovery.

This represents a great opportunity for experimental pharmacology to joint to the new scenario, connecting biomedical public research in the mechanisms of disease with clinical, biotechnological and pharmaceutical companies' experts and patients. This change of scientific/technical perspective also implies the creation of innovative figures and models of scientific, legal and financial management.

It is a difficult challenge for this new era and many ingredients like know-how, vision, serendipity, scientific management, in addition to the excellent science are required. For instance, in Spain from more than 1000 programs analyzed in Venture Capital, only 20 were advanced. In any case, deals are coming in the last years (for instance from Esteve, Almirall, Orizon, PaloBiofarma. . .).

Different public-private initiatives aimed at developing transformational pharmacological programs that yield drug will be discussed. Essentially, there are two perspectives: i) to look for the best science across the world in the area of interest of the pharmaceutical companies and incorporate it through the creation of joint laboratories or its absorption in the case of small startups; ii) to search for existing innovative models, already oriented towards therapeutics and to potentiate their innovative trough collaboration/funding, with independent agreements without absorption.

The experience of our lab in both models will be illustrated trough: i) Esteve-USC Joint Research Unit, an effort co-funded by the Galician Government that has launched a special program. Esteve has consolidated its collaboration with the USC by implementing a joint research unit that works as an incubator of in vitro applied pharmacology within compact team sharing public/private research, with a successful outcome both in new methodologies and new chemical entities characterized through that methodologies; ii) Innopharma Platform/Kærtor Foundation, it arises from our experience from 2 years of intensive work with a committee of international experts in open innovation, drug discovery and financial models. Its mission is to manage the reciprocal transfer of private/public knowledge in a 'totipotent model' devoted to leaping the gaps in connection/translation in the whole process of drug discovery. Outcomes include new pharmacology applied programs, connected to 25 agreements with principal investigators/technology transfer offices, 5 agreements with pharmaceutical companies and 1 program with clinical proof of principle finished.

In conclusion, open innovation in drug discovery connecting public and private research represents a challenge but also a great opportunity for experimental pharmacology to move the most innovative disease mechanisms to drugs for unmet medical needs.

PL-6
PRESENT AND FUTURE TREATMENT OF ALZHEIMER′S DISEASE

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Alzheimer′s disease is the most common form of dementia disorder and poses a great challenge for the society. Owing to a steep future increase in the number of elderly worldwide there is a strong urge to find effective targets for treatment of diseases as AD. Drugs with symptomatic effects such as the cholinesterase inhibitors donepezil, rivastigmine and galantamine and the NMDA antagonist memantine are today clinical standard therapy in AD. How should we find disease modifying drugs or even preventive/protective therapy? By modern molecular imaging techniques it is now possible to visualize and measure in vivo proteinopathies already described by Dr Alois Alzheimer as amyloid plaques and neurofibrillary tangles in AD brains. The development of new AD therapies has for more than a decade been dominated by anti-beta amyloid therapies. These include monoclonal antibodies in passive immunizations as well as vaccine in active immunizations. Thus several anti-beta amyloid humanized monoclonal antibodies including bapineuzumab, solanezumab, crenezumab and others have been tested in large clinical trials at different stages of AD but have not met the pre-determined endpoints to alter the course of AD. The beta-amyloid antibody Aducanumab has shown in a phase 2 study of early AD a dose related decrease in amyloid plaque load in brain measured by PET as well as some effects in cognitive tests. Other anti-beta amyloid approaches as the gamma secretase inhibitors failed and the beta amyloid cleavage enzyme 1 (BACE1) inhibitors have still to demonstrate clinical efficacy. Other new interesting approaches are anti tau vaccines. Other strategies are focusing on nerve growth factors (NGF), novel nicotinic receptor agonists and drugs interfering with inflammatory processes in brain.

Key words: Alzheimer's disease, symptomatic drugs, disease modifying therapies, immunizations therapy, beta-amyloid, tau, acetylcholine

SYMPOSIA 1: PHARMACOLOGY AND TOXICOLOGY OF DRUG TRANSPORTERS: RECENT ADVANCES

S1-1
PHARMACOGENOMICS AND EPIGENETIC ALTERATION OF MEMBRANE TRANSPORTERS

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Interindividual variability of expression and function of membrane transporters with consequences on drug response is not only affected by genetic factors but could be also explained by epigenomics (DNA methylation, histone modifications, regulation by miRNA). DNA hypermethylation results in gene silencing by direct inhibition of transcription-factor binding or by recruitment of methylated DNA-binding proteins. There is proof of principle for the clinical value of methylation markers for classification, prognosis and prediction of therapeutic response, and tissue-specific methylation alters the expression of selected ADME genes (Fisel et al. Clin Pharmacol Ther 2016). Systematic knowledge, however, related to human uptake transporters is still limited. Therefore, we investigated the impact of DNA methylation for selected SLC transporters on whether tissue specific expression in normal vs. corresponding tumor tissues is regulated by core gene regions with clinical consequences. DNA methylation regulates gene expression of an important SLC transporter which is the monocarboxylate transporter 4 (MCT4), a metabolic target in tumor biology because it mediates lactate transport across membranes resulting in antiapoptotic effects. MCT4 protein is significantly overexpressed in >85% of renal cancers (ccRCC), and DNA methylation at specific CpGs in the SLC16A3 promoter correlates significantly inverse with MCT4 expression in ccRCC resulting in adverse cancer-specific outcome. Apart from the prognostic potential for patient outcome, the transcriptional regulation of MCT4 by DNA methylation offers novel and attractive opportunities for therapeutic intervention by specific silencing of MCT4 and subsequent targeting of lactate efflux in ccRCC. A second example is the expression of the drug uptake transporters OCT1 in human liver and OCT2 in human kidney which is determined by DNA-methylation. It has been shown that DNA methylation of the hepatic SLC22A1 is associated with downregulation of SLC22A1 in hepatocellular carcinoma (HCC) which might be a novel biomarker for HCC diagnosis and prognosis. Since platinum drugs are substrates of SLC22A1, the modulation of gene expression by pretreatment with demethylating agents may offer novel therapeutic options for anticancer therapy. The large-scale, systematic epigenomic equivalents of GWAS, termed epigenome-wide associations studies (EWAS), are promising tools to determine specific drug-related phenotypes attributable to interindividual epigenomic variation. Thus, there appears to be a promising potential of epigenomics of transporter proteins in determining interindividual variation in drug response and in serving as targets for epidrug intervention.

The work is supported in part by the Robert Bosch Stiftung, Stuttgart, Germany.

S1-2
PBPK MODELLING OF DRUG TRANSPORTERS TO FACILITATE INDIVIDUALIZED DOSE PREDICTION

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Physiologically-based pharmacokinetic (PBPK) modelling is increasingly used as a decision-making tool in different stages of drug development. This approach is particularly important for prediction of complex drug-drug interactions involving interplay between multiple transporters and/or enzymes in different tissues. Current challenges in the translation of transporter kinetic data to in vivo within PBPK paradigm will be illustrated by simvastatin model that incorporates complex lactone-acid inter-conversion. Changes in both simvastatin forms under different clinical scenarios (drug co-administration, genetic polymorphism in OATP1B1 transporter) were simulated at the systemic level, but also in the tissues of interest selected either due to efficacy/interaction concerns (e.g., liver) or safety (e.g., muscle). Presentation will also illustrate areas where PBPK modelling is expected to inform drug labelling in the future (e.g., special populations), highlighting current knowledge gaps from the transporter perspective.

S1-3
ABC TRANSPORTERS IN DRUG-INDUCED LIVER INJURY

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Drug-induced liver injury (DILI) continues to be prominent problem in drug development and patient care. Although our knowledge of DILI mechanisms is incomplete, multiple cellular events and their molecular mediators contributing to DILI have been described. Among these, hepatobiliary transporters, which are key genetic determinants of drug pharmacokinetics and pharmacodynamics, have emerged as important contributors of DILI outcome. DILI is also known to alter the expression and function of drug transporters. The physiological consequences of such an adaptive response are not well understood. We have demonstrated that DILI resulting from acetaminophen (APAP) treatment leads to tolerance to subsequent hepatotoxicant exposure. Our work indicates that this adaptive process is a general response to tissue injury and not a drug-specific event. We have further investigated whether changes in the expression and function of drug transporters contribute to the development of tolerance to APAP hepatotoxicity, with emphasis on the multidrug resistance protein 4 (Mrp4, ABCC4). Research work to be discussed include studies investigating the role of ABCC4 in compensatory cell proliferation following DILI and other models of liver tissue loss and regeneration. This presentation will also highlight the utility of knockout animal models in defining the role of drug transporters in DILI and related liver diseases.

Key words: acetaminophen; drug transporters; liver; drug resistance

S1-4
SCIENTIFIC PERSPECTIVE ON THE EVALUATION OF TRANSPORTER-BASED DRUG–DRUG INTERACTIONS

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Alterations in transporter activities can contribute to variability in pharmacokinetics, pharmacodynamics and efficacy and safety of drugs. Drug transporter-based drug-drug interactions have been increasingly evaluated during drug development (1–5). Regulatory agencies have issued guidance documents (6–8) to suggest when, what and how DDI be evaluated during drug development and labeling implications of these studies. This presentation will discuss scientific issues in the use of in vitro, in vivo and in silico methods of assessing these interactions and will include the following.

1. Various in vitro criteria that have been used to determine whether in vivo studies for P-gp, OATP1B1, OAT1/3, and OCT2-based interactions are needed
2. Availability of substrates and modulators to use in transporter-based DDI in vitro and in vivo (9)
3. The need to consider tissue concentrations in interpreting systemic exposure data from DDI studies.
4. How physiologically-based pharmacokinetic modeling (PBPK) and simulations have been used in the evaluation of DDI- applications and limitations (10–12)
5. Examples of complex DDI (13–14)
6. The need for continued collaborative work to address transporter-based DDI

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SYMPOSIA 2: PAIN

S2-1
PHARMACOLOGICAL ASSOCIATIONS IN PAIN. A REAL-LIFE SCENARIO

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Very few studies and less clinical trials with analgesic drugs reproduce what is the real clinical practice where patients are taking multiple drugs (analgesic and not), either under prescription but also on OTC. Synergistic analgesia but also serious adverse reactions have been described with the associations of different pure analgesics drugs but also with co-analgesics. Except pure opioids agonists, all other analgesics have a ceiling effect, but high and increasing doses are also associated with a higher probability adverse reactions, mainly neurological and gastrointestinal ones. The successful concept of multimodal analgesia, already a standard of care in postoperative pain, takes advantage of the opioid sparing effect of other analgesics like NSAIDs, Paracetamol, Metamizole, local anaesthetics, ketamine and even Gabapentinoids. This concept has been extended to the clinical management of other kinds of acute pain as well as chronic pain, either neuropathic or nociceptive ones. Although the rationale of this approach seems logical even in chronic pain, safety concerns plays a major role in a context of less supervision as home care. The main common adverse reactions due to combinations that deserve supervision are: serotonin syndrome with the association of opioids such as Tramadol and antidepressants (TCA or SNRI) also indicated in neuropathic pain; the combined use of Antidepressants and NSAIDs are generally believed to each increase the risk of abnormal bleeding, even intracranial haemorrhage; the liver toxicity of Paracetamol and Duloxetine; and the potential of serious arrhythmias due to a long QTc that may be elongated with the combination of several antiepileptic drugs or combinations of Methadone.

S2-2
IMPLICATION OF NORADRENALINE IN PAIN AND ITS EMOTIONAL CONSEQUENCES

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The activation of the descending noradrenergic pathway has been implicated in the analgesic effects of antidepressants and anticonvulsants. However, many patients fail to achieve adequate analgesia with these medications or they do not tolerate them. Thus, it is possible that pain produces specific neuroplastic changes that modify the influence of noradrenaline. Furthermore, little is known about the role of the ascending noradrenergic projections on the activity of corticolimbic structures where the emotional aspects of pain are processed.

During this Workshop, novel data will be presented that has been obtained through the combined use of chemogenetics and electrophysiological tools, providing new insights into the noradrenergic system and the amygdala circuits mediating the effects of chronic pain, and pain-induced anxiety. I will show that direct activation of the noradrenergic locus coeruleus (LC) can produce both pro- and anti-nociceptive effects. In addition, the noradrenergic circuit that mediates stress-induced anxiety and aversion will be described, and I will show that the noradrenergic system is a critical hub for the development of the anxio-depressive consequences of long-term pain. These data stimulate interest in novel therapeutic options that modulate the noradrenergic and amygdala circuits.

Key words: noradrenaline; locus coeruleus; amygdala; pain

S2-3
SIGMA-1 RECEPTOR BLOCKADE UNMASK PERIPHERAL OPIOID ANALGESIA

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Opioid receptor agonists constitute one of the pain treatment milestones, but frequently produce centrally-mediated side effects, such as respiratory depression or drug addiction. Ways to minimize these side effects can be to use blood brain barrier-impermeable opioid agonists or to inhibit peripheral brakes for opioid analgesia. Sigma-1 receptor (σ1R) is a chaperone able to interact with several channels and receptors, including mu-opioid receptors. σ1R blockade, either pharmacologic or genetic (σ1R knockout, S1KO), markedly enhance antinociception produced by systemic (sc) administration of opioid agonists. Since σ1R is highly expressed on primary afferent neurons, we hypothesized that at least a part of such enhancement might be produced peripherally. We found that intraplantar (ipl) administration of several opioid receptor agonists (morphine, oxycodone, buprenorphine) was devoid of effect against a mechanical nociceptive stimulus (paw pressure) in control wild-type (WT) mice, but produced a dose-dependent antinociception in S1KO mice. Moreover, σ1R antagonists (S1RA, BD-1063), injected either locally (ipl) or systemically (sc), dose-dependently enhanced the antinociceptive effect of ipl injected opioids. The enhancement was locally-produced, because it did not appear when the opioid agonist was injected in a paw and the σ1R antagonist in the contralateral paw, and was due to σ1R blockade, because it was reversed by a σ1R agonist (PRE-084). These mechanisms are relevant in pathological conditions such as inflammatory pain. During inflammation induced by ipl carrageenan immune cells infiltrate the lesioned tissue, where they release endogenous opioids (such as β-endorphin). In this model, σ1R antagonists inhibited mechanical hyperalgesia and their antihyperalgesic effect was reversed by naloxone-methiodide (a peripherally restricted opioid receptor antagonist) and by local administration of monoclonal antibody 3-E7, which recognizes an N-terminus sequence (Tyr-Gly-Gly-Phe) of most endogenous opioid peptides. Therefore, σ1R is a brake for endogenous and exogenous opioid-induced antinociception and represent an adequate drug-target to enhance peripheral opioid antinociception.

Key words: Sigma-1 receptor antagonists, opioid agonists, endogenous opioids, inflammatory pain

S2-4
ANIMAL MODELS OF PAIN: BEYOND THE EVALUATION OF REFLEXES

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The discovery of novel targets and the development of better analgesics critically depend on the validity of the preclinical animal

models. Preclinical testing of pharmacological agents with a potential for pain modulation has almost exclusively consisted of reflex tests. However, clinical testing of candidate analgesics asses not only changes in pain intensity but also in physical and emotional functioning. In fact, the analgesic-induced recovery of normal physical and emotional functioning is considered at least as important as the analgesic-induced interruption of reflex behaviors evoked by sensory stimuli. It is therefore important to develop animal models to evaluate the non-reflexive component in pain. We have used different behavioural outcomes that depend on higher brain processing to measure how different painful stimuli affect them and how they can be differentially modulated by drugs. We conclude that the implementation of non-reflexive outcomes during analgesic selection and profiling testing will improve preclinical translation.

S3: ISCHEMIA AND REPERFUSION

S3-1
CEREBROVASCULAR PATHOLOGY: NOVEL PHARMACOLOGICAL STRATEGIES

Cuartero M.I., De la Parra J., Pérez-Ruiz A., Bravo-Ferrer I., Medina V., Hernández-Jiménez M., García-Segura J.M., Lizasoain I., Moro M.A.

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Cerebrovascular injury caused by stroke is the major cause of neurological disability and the second cause of dementia. Specifically, the prevalence of developing new dementia among first-ever stroke patients is approximately 10% and the chance of developing delayed post-stroke dementia is as high as 50% among stroke survivors. Stroke is thus a major socio-economic problem for societies and health-care systems world-wide. Despite this high disease burden, there is no specific treatment for post-stroke dementia. One of the reasons for this is that no suitable animal models are available that reliably allow for the investigation of mechanisms of post-stroke cognitive deficits and for the preclinical evaluation of candidate drugs. In our presentation, we will describe an experimental paradigm in which some mice undergoing stroke show cognitive impairments in hippocampal-dependent tests, and that may thus serve for the identification of individual mechanisms underlying cognitive manifestations of vascular cognitive impairment. In this context, hippocampal neurogenesis, through which new neurons integrate into hippocampal circuits driving memory processes, has been stated to contribute to cognitive function. We will report new evidence on the involvement of hippocampal neurogenesis in cognitive impairment in the chronic phase of ischemic stroke. In addition, we will discuss the role of Aryl hydrocarbon Receptor (AhR), a bHLH/PAS ligand-activated transcription factor, as a putative novel pharmacological target in this setting.

S3-2
NOVEL THERAPIES FOR ACUTE ISCHEMIC STROKE: FROM PHARMACOLOGICAL NEUROPROTECTION TO VASCULOPROTECTION. THE URIC ACID EXPERIENCE

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Acute ischemic stroke is a major cause of morbidity and mortality. The most effective treatment is early reperfusion, although less than half of patients who are finally treated obtain permanent benefits. The combination of rapid reperfusion and neuroprotective therapies would be of added value for maximizing the beneficial effects of rapid reperfusion and to reduce the harmful consequences of reperfusion injury. Given the relevance of oxidative stress in the physiopathology of brain ischemia, the use of antioxidant molecules, such as uric acid, could translate into effective neuroprotective effects. Uric acid (UA) is the most potent natural antioxidant, and its exogenous administration in experimental models of brain ischemia is neuroprotective and has synergistic effects when administered alongside alteplase, a thrombolytic drug. Additionally, UA also attenuates vessel-wall changes induced by ischemia by reducing local oxidative stress and inflammation thus suggesting the relevance of additional vascular protection to direct effects on neurons to enhance post-stroke recovery. In humans, UA therapy is safe and has measurable neuroprotective effects, especially in patients with pre-treatment hyperglycemia or in women. Given the encouraging preclinical and clinical data regarding the potential neuro/vasculoprotective effects of UA administration in combination with reperfusion in acute brain ischemia, the development of adequately powered pivotal confirmatory clinical trials are warranted.

S3-3
OXYCYTE: AN INTRAVENOUS OXYGEN CARRIER CAPABLE OF DIAGNOSING AND TREATING ACUTE ISCHAEMIC STROKE

Macrae I.M.[1], *Deuchar G.A.*[2], *Shirley R.*[1], *Roy L.*[1], *Brennan D.*[2,3], *Holmes W.M.*[1], *Gonzalez L.*[1], *McCabe C.*[1], *Muir K.W.*[3], *Dani K.*[3], *Santosh C.*[2,3]

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Glasgow Oxygen Level Dependent (GOLD) technology is a novel stroke management product in development by Aurum Biosciences, which offers unique theranostic benefits through simultaneous diagnostic and therapeutic applications. MRI-based techniques are combined with an intravenous oxygen-carrying emulsion (Oxycyte®) and an oxygen challenge (OC- increased inspired oxygen) to identify and support the survival of ischaemic penumbra, injured but potentially salvageable hypoperfused tissue.

Proof-of-concept for the diagnostic was established using 100% O_2 alone in rodent stroke models[1,2] with T2*OC MRI translated to 3T clinical scanners and tested in stroke patients[3]. Limitations identified when using 100% O_2 were overcome by incorporating Oxycyte nanoparticles (30–40 times smaller than red blood cells) injected i.v.

Oxycyte (4.5 ml/kg, i.v) combined with hyperoxia (40–50% O_2) improves identification of T2*OC defined penumbra on MRI scans[4] and improves sensitivity to detect changes in penumbra lactate levels.

Oxycyte + hyperoxia also improves oxygen transport through the ischaemic microcirculation via any remaining plasma flow, improving oxygen levels in penumbra and thereby limiting damage. Serial MRI scanning following stroke reveals brain damage is significantly reduced in rats treated with 3 ml/kg Oxycyte + hyperoxia compared to normoxic controls or hyperoxia alone. Oxycyte + hyperoxia reduces infarct size and improves functional outcome in rat stroke models (transient & permanent middle cerebral artery occlusion) and shows no contra-indications when combined with t-PA, MRI or CT contrast agents in a rat embolic stroke model.

Conclusions: GOLD shows potential to improve acute ischaemic stroke patient management: diagnostically providing a single stratified measure of tissue viability via MRI-based metabolic imaging while therapeutically supporting penumbra survival by improving oxygen delivery.

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Oxycyte® provided by Tenax Therapeutics Inc. (Morrisville, NC, USA).

Key words:
stroke; MRI; penumbra; rat.

S3-4
PROTECTION AGAINST MYOCARDIAL REPERFUSION INJURY BY TARGETING MITOCHONDRIAL SUCCINATE DEHYDROGENASE

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Background: Previous studies have demonstrated the importance of reactive oxygen species (ROS) generation during flow restoration after ischemia in reperfusion injury. In a recent study we analyzed the role played by reverse electron transfer from mitochondrial complex II to complex I in ROS generation during reperfusion. For this purpose, we reversibly inhibited succinate dehydrogenase with malonate, and analyzed its effects on infarct size.
Methods and results: Before testing the effects of succinate dehydrogenase inhibition in reperfusion injury, we assessed malonate actions in normoxic hearts. Isolated mice hearts were treated, under normoxic conditions, with increasing concentrations of disodium malonate (0.03–30 mM, $n = 4$). Malonate induced a concentration-dependent decrease in left ventricular developed pressure (EC50 = 8.05 $\pm$ 2.11 mM). In isolated mice hearts submitted to 35 min of ischemia followed by reperfusion (60 min), malonate, given continuously from 15 min before ischemia to the end of reperfusion, at either 3 or 10 mM ($n = 7$–8/group) reduced infarct size, an effect associated with reduced LDH release and improved functional recovery during reperfusion. To assess its effects on reperfusion injury, malonate 3 mM was given only during the first 15 min of reperfusion. Under these conditions, malonate was still able to reduce infarct size (24.57 $\pm$ 2.32 vs. 39.84 $\pm$ 2.78 in controls, $p = 0.001$, $n = 7$–8/group) and LDH release (125.41 $\pm$ 16.82 vs. 189.20 $\pm$ 13.74 U/g dry tissue/15 min, $p = 0.015$) and to improve functional recovery at the end of reperfusion. Furthermore, malonate increased myocardial succinate content (^{1}H-NMR), reduced tissue ROS production (MitoSOX staining) during reperfusion, and reduced mitochondrial permeabilization (calcein retention). These data have been recently confirmed in an in situ pig model of transient coronary occlusion (infarct size of 59.62 $\pm$ 4.00% of area at risk in control animals vs. 36.46 $\pm$ 5.35% in animals treated with 10 mM malonate).
Conclusion: Succinate dehydrogenase inhibition with malonate at the onset of reperfusion reduces reperfusion injury in mice and pig hearts.

SYMPOSIA 4:G PROTEIN-COUPLED RECEPTORS PHARMACOLOGY

S4-1
"THE DRUG INVENTION TEMPLATE: FROM "CRAFTED ROUND THE NATURAL HORMONE" TO "SCULPTED TO FIT THE RECEPTOR"

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Over the last 60 years receptors for extracellular messengers have moved from being entirely theoretical concepts (black boxes) to molecules whose structure is being considered in 3D and time. In this introduction to the symposium on 7TM/GPC Receptors I will discuss how drug invention following the key and lock analogy (agonist and receptor) has changed. Initially we knew only about the key, with no knowledge of the lock, and modified the key in an attempt to disable the lock. Now that we have some knowledge of the structure of the lock, does that increase the efficiency of designing keys?

S4-2
NEW APPROACHES TO MODULATE G PROTEIN-COUPLED RECEPTORS

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Despite the proven success of GPCRs as drug targets, useful ligands do not exist for the majority of them. The main reason being that the orthosteric binding sites across members of a GPCR subfamily for a particular endogenous ligand are often highly conserved, making it difficult to achieve high selectivity for specific GPCR subtypes. Thus, the emerging knowledge of the structure and physiological functions of GPCRs has begun to alter the approaches for drug discovery. These novel approaches to modulate GPCRs involve, in addition to traditional drug discovery programs dominated by efforts to develop compounds that act as agonists or inverse agonists by binding to the orthosteric site for endogenous hormones, the discovery of bitopic ligands that bind the orthosteric site as well as a less conserved site at the extracellular entrance, multivalent ligands that target physiologically relevant GPCR hetero-oligomers, or allosteric ligands that bind at allosteric sites and act in conjunction with the endogenous ligand. These allosteric ligands, which have been proven highly successful for ligand-gated ion channels, do not bind to the orthosteric ligand binding site but instead act at an alternatively located binding site (allosteric site) that is distinct from the orthosteric site. Allosteric modulators are categorized in negative (NAM) or positive (PAM) depending on their ability to decrease or increase the action (affinity and/or efficacy) of the orthosteric ligand.
In this presentation, we will explore new approaches to modulate GPCRs and the challenges associated with the design of new type of ligands.

S4-3
OPTOPHARMACOLOGY: LIGHTING UP G PROTEIN-COUPLED RECEPTORS

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G Protein-coupled receptors (GPCR) constitute the largest group of cell-surface receptors involved in signal transduction and the most important pharmaceutical drug targets. Numerous drugs targeting GPCR have been shown to exert beneficial therapeutic effects, but in many cases, they also provoke undesired adverse effects. Thus, a major aim in pharmacological research consists of developing novel drugs that may have effectiveness but with limited side effects. Optopharmacology, which is based on the use of light-activable compounds that can elicit their effect only upon light activation, is a promising approach. We have developed light-dependent GPCR (i.e. adenosine, glutamate receptors) ligands that allow photocontrolling, both *in vitro* and *in vivo*, receptors' activity. Interestingly, we challenged these light-activable compounds in heterologous systems, neuronal cultures and in mouse models, and demonstrated the ability of precisely, in time and space, deliver the active compound at the desired targets. Overall, optopharmacology may permit achieving better benefit/risk therapeutic balances when using GPCR-based drugs.
This work was supported by grants MINECO/ISCIII (SAF2014-55700-P, PCIN-2013-019-C03-03 and PIE14/00034), the Catalan government (2014 SGR 1054), ICREA (ICREA Academia-2010) and Fundació la Marató de TV3 (Grant 20152031) and FWO (SBO-140028). We thank Esther Castaño and Benjamín Torrejón from the Scientific and Technical Services (SCT) group at the Bellvitge Campus of the University of Barcelona for their technical assistance.

S4-4
PROBING THE MOLECULAR MECHANISM BEHIND THE COGNITIVE IMPAIRMENT INDUCED BY THC

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There is high medical interest in harnessing the beneficial effects of delta-9-tetrahydrocannabinol (THC). We found that the serotonin 2AR receptor (5-HT2AR), a major target for anti-psychotics, is necessary for the amnesic- and anxiolytic-like effects of THC, but not for its hypolocomotor, hypothermic, anxiogenic and analgesic effects. While investigating the underlying molecular mechanism we discovered that CB1 cannabinoid receptor (CB1R) and 5-HT2AR form heteromers. Remarkably, we found that the formation of this heterocomplex leads to a switch in G-protein coupling for 5HT2AR from Gq to Gi proteins

and to a reduction in cell signaling. We demonstrated with behavioral experiments that via this heteromer complex, antagonists of 5-HT2AR can mitigate the amnesic and anxiolytic effects of THC. We then mapped which amino acids are responsible for these interactions and in so doing provide a framework for how receptor crosstalk can occur. These genetic, molecular and pharmacological data imply that the detrimental side effects of cannabis use can be avoided without affecting its beneficial properties through a specific modulation of the CB1R-5-HT2AR heterocomplex. This potential for modulation, either in the form of disruption of the heteromer or its pharmacological blockade represent a new mechanism for both THC and potentially anti-psychotics and clearly indicates that CB1R-5-HT2AR heteromers are new targets for drug design.

S5: CARDIOVASCULAR AND SMOOTH MUSCLE PHARMACOLOGY

S5-1
NEW DRUGS IN THE TREATMENT OF ACUTE HEART FAILURE: HOPE OR HYPE?

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Acute heart failure (AHF), defined by a rapid or gradual onset of signs and symptoms of HF requiring urgent therapy, is a heterogeneous, multifactorial and progressive syndrome presenting a wide spectrum of phenotypes often associated with multiple comorbidities. AHF represents a major health problem due to its high prevalence, morbidity, mortality and significant healthcare costs, and a therapeutic challenge for the clinician. In the last 25 years, multiple potential therapeutic targets involved in the genesis/progression of AHF have been identified and many promising new drugs were investigated. However, the treatment of AHF has not changed much over this time as new drugs improved signs and symptoms, but failed to improve HF outcomes when compared to placebo or conventional therapies. These considerations provide the stimulus for the development of new drugs that target the underlying pathophysiological processes leading to progressive myocardial dysfunction and unfavourable remodelling and improve long-term outcomes of patients with AHF. The development of new safer and more effective drugs for AHF should be based on well-supported hypotheses, robust preclinical data and well-designed randomized controlled trials. Interestingly, a repeated finding in AHF treatment has been the discrepancy between the positive results found in preclinical studies and the lack of efficacy and safety in phase II and III clinical trials. In this presentation the mechanisms of action, efficacy and safety of new drugs under development for the treatment of AHF (inotropes, vasodilators, hormones, atrial natriuretic peptides and NO-independent stimulators and activators of soluble guanylate cyclase, ß-arrestin-biased AT1R ligands) and the possible explanations for the discrepancy between preclinal and phase II-III trials will be analyzed. A better understanding of AHF triggers and pathophysiology will allow to identify new targets and develop novel therapeutic approaches that might prevent the progression of myocardial dysfunction and improve outcomes.

S5-2
NOVEL MEDIATORS OF INFLAMMATION IN VASCULAR DAMAGE IN HYPERTENSION

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Hypertension is a global burden that significantly contributes to target organ damage and is a major risk factor for cardiovascular diseases. Despite this knowledge recent evidence suggest that over the past 25 years, the number of individuals with prehypertension or established hypertension has increased substantially and this is associated with the estimated number of deaths. Different factors and mechanisms are involved in hypertension development and maintenance but at vascular level specific alterations are found in both patients and hypertension models. In general, endothelial dysfunction, vascular remodeling and increased vascular stiffness are key hallmarks of hypertensive vascular disease and they predict target organ damage and the development of future cardiovascular disease. Different factors and mechanisms have been involved in the vascular alterations observed in hypertension and it is now well accepted that low-grade inflammation triggered by several components of the innate and adaptive immune systems plays a key role in promoting a misbalance between vasoconstrictor/growth promoter and vasodilator/growth inhibitor factors in hypertension. Proinflammatory cytokines, oxidative stress derived from novel sources such as lysyl oxidase, prostanoids from the inducible isoform of the microsomal prostaglandin E synthase-1 and other mediators seem to have a role in vascular alterations in hypertension through their effects in NO availability, vascular smooth muscle cells proliferation or extracellular matrix deposition.

Key words: Hypertension; inflammation; endothelial dysfunction; vascular remodeling.

S5-3
DRUGS FOR PULMONARY HYPERTENSION: OLD STORIES OF PARTIAL SUCCESS, PARTIAL FAILURE AND NEW PERSPECTIVES

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Pulmonary arterial hypertension is a rare life-threatening progressive disease characterized by vasoconstriction, vascular remodeling and thrombosis. Over the last 20 years we have witnessed the introduction of an unprecedented large number of drugs belonging to different classes which are directed mainly to reduce the vasoconstrictor component. They restore endothelial function by mimicking or inhibiting endothelial derived factors: NO (inhaled NO, phosphodiesterase 5 inhibitors and soluble guanylyl cyclase stimulators) prostacyclin (epoprostenol and its analogues), and endothelin-1 (endothelin-1 receptor antagonists). Unfortunately, there are no approved specific therapies for the most common "non-arterial" forms of PH: PH due left heart disease (group 2) and associated to lung diseases and hypoxia. Current therapies improve symptoms and prolong survival but the disease remains fatal. Vasodilators are limited by several factors. First, they lack pulmonary selectivity, thus their systemic effects leading to hypotension often preclude the use of effective doses. Second, despite most approved drugs show antiproliferative effects to some extent, aggressive proliferative phenotypes are often drug-resistant. However, attempts to directly address proliferation with kinase inhibitors have failed in clinical trials. Third, vasodilators are often unable to induce effective vasodilation depending on the underlying vasoconstrictor mechanism. Finally, they may uncouple ventilation-perfusion ratio. Hypoxic pulmonary vasoconstriction (HPV) represents a crucial protective mechanism that redistributes blood flow away from diseased (hypoxic) lung tissue to the best oxygenated alveoli at the expense of elevated pulmonary pressure. Vasodilators, by inhibiting HPV, may also increase blood flow to poorly-ventilated areas of the lung decreasing arterial oxygenation.

S5-4
PLEIOTROPIC ACTIONS OF LOSARTAN METABOLITES IN HYPERTENSION

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Losartan is an antihypertensive drug that exerts its effects by the blocking of the angiotensin II type 1 (AT_1) receptor, which also exhibit a variety of pleiotropic effects. Losartan is a prodrug metabolized by the cytochrome-P-450 pathway in the liver producing 2 metabolites, EXP3174 and EXP3179. EXP3174 is the final metabolite and the pharmacological blocker of the AT_1 receptor by which losartan exerts its antihypertensive actions. EXP3179 is an aldehyde intermediate metabolite that has no AT_1 receptor blocking properties and mediates a variety of AT_1 receptor-independent, non-hemodynamics actions. Thus EXP3179 metabolite (i) mediates the anti-inflammatory properties of losartan by abolishing cyclooxygenase-2 upregulation, (ii) stimulates endothelial NO synthase and suppresses tumor necrosis factor-α-induced apoptosis, (iii) acts as a peroxisome proliferator-activated receptor-γ-agonist, (iv) inhibits collagen-dependent platelet activation, and (v) blocks the NADPH oxidase in phagocytic and vascular cells, an effect that appears to depend on a protein kinase C-dependent mechanism. Traditionally, losartan has been demonstrated to reduce myocardial fibrosis and left ventricular stiffness in hypertensive patients, effects associated with the reduction of the content of myocardial collagen fibers. Several recent studies have underlined the key role that the degree of collagen-cross linking (CCL) within the collagen fibers, a process catalyzed by the enzyme lysyl oxidase (LOX), plays on cardiac stiffness and resistance to fibers degradation. In a model of experimental hypertension, rats treated with N^G-nitro-L-arginine methyl ester, EXP3179 metabolite prevents LOX, CCL increase, as well as fibrosis. This effect is obtained without normalization of blood pressure. In contrast, 3174 metabolite prevents blood pressure increase and attenuates fibrosis but do not modify LOX and CCL. Thus, and despite a lower antihypertensive effect, EXP3179 shows higher anti-fibrotic efficacy than EXP3174.

Key words: Losartan; Hypertension; Nitric oxide; Myocardial fibrosis.

S6: NATURAL PRODUCTS IN PHARMACOLOGY

S6-1
QUALITY OF HERBAL MEDICINAL PRODUCTS: CONTRIBUTION OF THE EUROPEAN PHARMACOPOEIA

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Herbal medicinal products (HMP) and Traditional HMP contain herbal drugs (HD) or herbal preparations (HP) (extracts, essential oils, etc.) as active pharmaceutical ingredients. The quality of HMP is a challenge, mainly due to the difficulties linked to the variability and complexity of HD and HP, the limited knowledge of their active constituents, the possible adulterations and contaminations, as well as the influence of the production processes. Overcoming this challenge requires to have the quality as an objective from the beginning of the production chain, to establish strict controls on all stages, to improve the knowledge on the product, to optimize and validate the production processes, to work under accepted international guidelines and to establish quality specifications [1,2].

In setting quality specifications, the European Pharmacopoeia (Ph.Eur.), an organization in the European Directorate for the Quality of Medicines and Health Care (EDQM) of the Council of Europe, is a key tool for maintaining the high levels of quality of HMP in Europe. This is because the Ph. Eur. is an updated and transparent regulatory document, harmonized and agreed by 38 countries, which provides a unique collection of methods of analysis and *ca.* 330 monographs of HD and HP [2,3]: plant parts (61%), extracts (14%), essential oils (10%), exudates, starches, gums and mucilages (8%) and fatty oils and waxes (7%). Three permanent groups (13A, 13B and 13H) and several working parties (TCM, Extracts, Pesticides, etc.), comprising more than 70 experts of 21 countries, from industry or private sector (46%), universities or public research centres (31%) and medicines agencies (23%), together with the staff of the EDQM, make possible these achievements.

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S6-2
FLAVONOIDS IN THE TREATMENT OF MENTAL DISORDERS

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Several mental disorders like dementia and age-associated cognitive decline could be preventable depending on different aspects of lifestyle, in particular exercise and diet, that may affect neural function and consequent cognitive performance throughout the life course. A balanced diet and regular exercise can protect the brain and ward off mental disorders. Among minor dietary components, flavonoids, found in a variety of fruits and vegetables, are a group of promising bioactive compounds capable of influencing different aspects of brain function, including cerebrovascular blood flow and synaptic plasticity. Flavonoids health benefits can be considered in the context of diet or isolated from food matrix and used at doses higher than those provided by diet in the search of targeted pharmacological effects. This presentation will focus on the use of flavonoids as nutraceuticals.

Flavonoids can exert direct vs. indirect effects via different mechanisms of action. They are known to interact directly with neuronal receptors (i.e. estrogenic or glutamate) and kinase signaling pathways (i.e. DYRK1A) which are key to neuronal activation and communication and synaptic strengthening. Alternatively or concurrently, flavonoids can positively affect peripheral and cerebrovascular blood flow, which may be an indirect effective mechanism impacting brain health and cognition. Examples will be provided of the therapeutic potential of flavonoids for the improvement of cognitive function and adaptive functionality in intellectual disabilities like Down and Fragile X syndromes (epigallocatechin gallate), the treatment of substance abuse disorders like the fetal alcohol spectrum disorders (epigallocatechin gallate) or cocaine addiction (isoflavones) and the deceleration of cognitive decline (flavanones).

S6-3
MARINE DERIVED MEDICINES FOR THE TREATMENT OF CANCER: THE EXPERIENCE OF PHARMAMAR

Fudio S.

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Marine biodiversity is almost unlimited and yet to be broadly discovered. Marine exploration for pharmaceutical purposes is quite recent (<30 years old) and there are already some successful results, such as in the field of oncology. In this regard, when a tumor screening hit is detected on crude extracts from marine organisms, chromatographic purification of active samples is carried out to attempt isolation of a new chemical entity. This natural candidate may not be necessarily a drug-like molecule. While a cost-effective route of supplying the molecule needs to be made available, it should have the potential to reach the site-of-action in man, at the required concentration, for the necessary duration and with an adequate safety window. Therefore, structural modification and/or drug delivery technologies may be put in place, along with the characterization of the adequate dose, regimen and route of administration in the clinical setting. Finally, a positive balance of benefits over risks in a specific unmet medical need should be considered of clinical relevance in order to apply for marketing approval.

During the last 30 years, PharmaMar has been focused in the search of oncology therapies from macro- and micro-organisms of marine origin and, as a result, has developed the first marine-derived compound to reach the oncology market.

S6-4
DEVELOPMENT OF NEUROPROTECTIVE THERAPIES WITH PHYTOCANNABINOIDS FOR NEURODEGENERATIVE DISORDERS

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Phytocannabinoids are compounds derived from cannabis plant, which, together with endogenous signalling lipids and synthetic derivatives, form a singular family of pleiotropic compounds active in the CNS,

but also in the periphery, and having promising therapeutic applications, including cytoprotection in neurodegenerative disorders. Such application derives from their capability to preserve, rescue, repair and/or replace neurons, astrocytes, oligodendrocytes and their precursor cells against a myriad of insults that deteriorate the homeostasis and integrity of these neural cells. These cytoprotective effects are facilitated by the location of specific targets for the action of phytocannabinoids (e.g. CB_1 and CB_2 receptors, endocannabinoid inactivating enzymes, non-endocannabinoid targets) in key cellular substrates (e.g. neurons, astrocytes, resting and reactive microglia, perivascular microglial cells, oligodendrocytes and oligodendrocyte precursor cells, and neural progenitor cells) and structures (e.g. blood-brain barrier [BBB]). Such cellular locations enable phytocannabinoids to exert selective control over the specific functions fulfilled by these cells in degeneration, protection, and/or repair. For example, in the case of CB_1 receptors, their activation inhibits glutamate release limiting excitotoxic damage, enhances metabolic support exerted by astrocytes for neurons, and promotes remyelination by increasing oligodendrocyte precursor cell maturation. In the BBB, CB_1 receptor activation improves vascular supply and limits peripheral cell infiltration. In the case of CB_2 receptors located in astrocytes and reactive microglia, their activation reduces the generation of fractalkine, TNF-α, IL-1β and other proinflammatory mediators by these activated glial cells. FAAH and MAGL enzymes, and GPR55 and PPAR receptors have been also identified in key cellular substrates being also proposed to mediate the cytoprotective activity of phytocannabinoids. In summary, the multiplicity of active targets for phytocannabinoids places this family of natural compounds in a promising position for developing novel cytoprotective therapies using a broad-spectrum phytocannabinoid or combinations of compounds, a circumstance essential in neurodegenerative disorders, in which neuronal damage is the result of a concerted action of different neurotoxic processes (e.g. excitotoxicity, oxidative stress, protein aggregation, mitochondrial failure, glial reactivity, inflammation) demanding a multi-target strategy capable to limit all these processes at the same time.

Supported by MINECO (SAF2015-68580-C2-1-R) and CIBERNED (CB06/05/0089).

S7: BEING IN THE RIGHT PLACE IN THE RIGHT TIME: TARGETING OXIDATIVE STRESS

S7-1
MEASURING LIGAND CONCENTRATION WHERE IT MATTERS: ASSESSING THE "MICRO PK/PD" OF ADENOSINE A2A RECEPTOR LIGANDS

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We have recently demonstrated that, dependent upon their physiochemical properties, β_2-adrenoceptor ligands can interact directly with phospholipids, increasing their local concentration and directly influencing the measured association rate constant at the receptor (Sykes et al, 2014). The aim of this current study was to expand upon this observation by directly measuring the local ligand concentration and binding kinetics of two fluorescent adenosine A2a receptor ligands with identical pharmacophores (xanthine amine congener [XAC]) and fluorophores (BODIPY 630/650-X-SE), but varying linker regions to modulate the physicochemical properties. The first of these two ligands, XAC-X-BY630, is more lipophilic than the second, CA600245.

The local aqueous concentration of each ligand was measured using fluorescence correlation spectroscopy (FCS) in a detection volume of ~0.25 fL at distances 2–200 μm above live CHO cells. Ligands were added at 10-fold below their K_D concentrations (XAC-X-BY630 at 5 nM, CA600245 at 15 nM). The binding kinetics of the two ligands was assessed by measuring the time-resolved fluorescence energy transfer (TR-FRET) between the terbium-labelled A2a receptor and fluorescent ligand over time. The aqueous concentration of both ligands was dramatically higher in the local vicinity of the plasma membrane (2 μm above) than in bulk aqueous phase (200 μm above). For example, at 3 μm above the membrane, the measured concentration of CA600245 was 83.8 ± 23.1 nM (n = 6), whilst the more lipophilic XAC-X-BY630 was even higher at 656.1 ± 212.7 nM (n = 6). This increased local concentration influenced the association kinetics of the XAC derivatives with XAC-X-BY630 having a significantly faster on-rate (1450 ± 450/mM/min; n = 4) than CA600245 (462 ± 109/mM/min; n = 4) ($p < 0.05$, unpaired t-test).

These data demonstrate that lipophilicity can increase the local concentration of drug immediately above the cell membrane, potentially distorting the measured pharmacological values. We propose it is critically important to consider this "micro PK-PD" when determining the pharmacology of novel receptor ligands.

Sykes et al (2014) *Mol Pharmacol* 85(4):608–17

S7-2
MOLECULAR AND FUNCTIONAL IMPACT OF OXIDATIVE STRESS IN COPD

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Oxidative stress is plays a major pathophysiological role in many lung diseases, and is considered to be the principle predisposing factor in the pathogenesis of COPD. Oxidative stress can cause alterations in cellular redox status, activate redox sensitive kinase signaling pathways and transcription factors. Oxidative stress can also impact on chromatin modification enzymes resulting in reduced corticosteroid efficacy, as well as triggering epigenetic changes that lead to increased pro-inflammatory responses. Some of the manifestations of oxidative stress arise as a result of tissue damage by free radicals, through lipid peroxidation for example, forming reactive carbonyls or carbonyl stress. This in turn can lead to protein modification which can affect protein and cellular function, along with wider systemic impacts that may lead to associated co-morbidities with COPD. This presentation will highlight how oxidative stress arises in the lung and leads to increased carbonyl stress, and how this impacts on inflammation and autoimmunity and the development of emphysema and small airway disease in COPD. Finally, a number of studies have shown that there is no single "magic bullet" to combat oxidative stress, but instead a combination therapy, targeting oxidative stress in the various sub-cellular compartments, may prove to be more effective in COPD, with several promising approaches on the horizon.

Key words: Oxidative stress; COPD; Inflammation; autoimmunity.

S7-3
TARGETING MITOCHONDRIA IN OXIDATIVE STRESS BY MODULATION OF POTASSIUM CHANNELS

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Alteration of mitochondrial metabolism and calcium homeostasis is highly associated with the pathology of neurodegenerative diseases. Small conductance calcium-activated potassium (SK) channels provide protection in different paradigms of neuronal cell death. Recently, these channels were identified at the inner mitochondrial membrane, however, their particular role in the observed neuroprotection remains unclear. In this study we aim to investigate the distinct role of SK channels in protection against glutamate toxicity, and to identify a potential role of SK channels in the regulation of mitochondrial metabolism and calcium uptake, two key players in neuronal cell death. Our results demonstrate that overexpression of mitochondria-targeted SK2 channels enhanced CyPPA-mediated mitochondrial resilience by reducing mitochondrial ROS formation. These effects were inhibited by overexpression of a mitochondria-targeted dominant-negative SK2 channel mutant. Real-time analysis of neuronal cells revealed that SK2 channel activation, or enrichment with mitochondrial SK2 channels, attenuated mitochondrial respiration and mitochondrial calcium uptake. These findings strongly suggest that mitochondrial SK2 channels provide neuroprotection by regulation of the mitochondrial metabolism and mitochondrial calcium homeostasis in conditions where sustained mitochondrial damage determines progressive neuronal death.

S7-4
MITOCHONDRIAL DYSFUNCTION IS A KEY DRIVER OF FIBROBLAST SENESCENCE. SO WHAT DO WE TARGET?

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Introduction and Rationale: The limited success in identifying and targeting important drivers of idiopathic pulmonary fibrosis (IPF) is likely because of the disease's complexity and heterogeneity. Previous studies have compared gene or protein expression from biopsies or

random samples of IPF lung to normal or disease controls. However, regional variation and typical progression of IPF provides a unique opportunity to investigate disease pathogenesis.
Hypothesis: Region-specific cues from the microenvironment are central to the pathogenesis of IPF.
Methods: We are integrating multiple samples isolated from the same individual lung; including pan-omics based profiling and imaging with cell-based studies with the severity of pathology in human disease, in order to comprehensively map the dynamic processes that regulate current fibrosis and predict future disease.
Results: Preliminary MALDI/MS imaging identified the spatial distribution of 198 protein signals from different non-fibrotic lung sections and 163 protein signals from the corresponding IPF sections, including immunoglobulins and ECM fragments. Cell culture data suggest that fibroblasts also show region-specific profiles of senescence, ECM production and proliferation.
Conclusions: The use of an individual lung for systems-based approaches offers unique insight into the pathogenesis of IPF, with the added advantage of minimizing disease co-morbidities and genetic heterogeneity.
Key words: idiopathic pulmonary fibrosis (IPF), lung disease.

S8: INFLAMMATION

S8-1
L-SELECTIN SHEDDING IS THE "GO" SIGNAL FOR NEUTROPHIL EFFECTOR FUNCTION

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We have reported that ectodomain shedding of L-selectin in monocytes occurs specifically during transendothelial migration (TEM) and not before (1). L-selectin shedding is critical in regulating front-back polarity of monocytes entering the subendothelial space, which in turn facilitates interstitial chemotaxis. We have recently extended our focus towards neutrophils and find that L-selectin shedding is similarly regulated during TEM (unpublished). In vivo, neutrophils are typically challenged with conflicting gradients of intermediate chemokines (e.g. IL-8) and end stage chemoattractants (e.g. mitochondrial fMLF) and are therefore required to prioritise their responses to such gradients. In this context, we explored the in vivo importance of L-selectin shedding in mediating neutrophil chemotaxis towards different ranking chemoattractants. By challenging the cremasteric muscle: first with chemokine (MIP-2/KC) and then with superfused fMLF (a damage/pathogen associated molecular pattern – DAMP/PAMP), or laser injury (to promote neutrophil swarming), we noticed that shedding of L-selectin was only ever apparent in neutrophils responding to DAMPs but not chemokines. Modelling these inflammatory responses in vitro revealed that human neutrophil chemotaxis through 3D collagen scaffolds towards fMLF gradients, but not gradients of the chemokine IL-8, was significantly attenuated when L-selectin shedding was genetically or pharmacologically blocked. Furthermore, release of superoxide in response to fMLF stimulation or phagocytosis of opsonised zymosan particles were also significantly attenuated when L-selectin shedding was blocked. Taken together, we conclude that L-selectin shedding is the "go" signal for neutrophil chemotaxis towards end-stage chemoattractants (e.g. fMLF) and effector function (e.g. phagocytosis and superoxide production). Future work will explore whether blocking L-selectin shedding is a suitable therapeutic target for minimising unwanted neutrophil effector function in the setting of acute sterile injury, such as myocardial infarction.

(1) L-selectin shedding is activated specifically within transmigrating pseudopods of monocytes to regulate cell polarity in vitro. Rzeniewicz K, Newe A, Rey Gallardo A, Davies J, Holt MR, Patel A, Charras GT, Stramer B, Molenaar C, Tedder TF, Parsons M, Ivetic A. Proc Natl Acad Sci U S A. 2015 Mar 24;112(12):E1461–70

S8-2
ADIPOCYTOKINES: NOVEL THERAPEUTIC TARGETS IN ENDOTHELIAL DYSFUNCTION AND VASCULAR INFLAMMATION?

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The adipose tissue is not simply a storage site of fat mass, but also an organ that secretes a heterogenous group of substances, the so-called adipocytokines, which may exhibit paracrine or endocrine actions. Adipocytokines range from classic cytokines, such as interleukin (IL)-1β or tumour necrosis factor-α, to vasoactive peptides or metabolic regulators, such as leptin. They can be released not only by the visceral or subcutaneous adipose tissue, but also by epicardial or perivascular fat depots. In certain conditions, such as type 2 diabetes and obesity, an imbalanced production and release of adipocytokines has been proposed to negatively impact on vascular homeostasis, thus contributing to the development of vascular disease. In this context, adipocytokines have gained interest as potential pharmacological targets to retard or attenuate vascular complications associated to metabolic disorders. In recent years, we have explored the capacity of selected adipocytokines, such as IL-1β, visfatin/Nampt or soluble dipeptidyl peptidase-4 (sDPP4), to directly promote features of vascular damage, including vascular cell inflammation, endothelial dysfunction, impaired vascular reactivity or premature cell senescence, as well as the respective mechanisms of action involved. Moreover, we have observed that the pro-inflammatory signalling triggered by IL-1β in human vascular cells is exaggerated by the presence of extracellular glucose levels. This effect seems to rely on a higher uptake of glucose favoured by the adipocytokine. Once inside the cell, part of the excess glucose is diverted via the pentose phosphate pathway, which derives in the over-production of oxygen reactive species resulting in an over-activation of pro-inflammatory signalling. The deleterious vascular effects exerted by different adipokines can be pharmacologically blocked *in vitro* or *in vivo* by drugs that are either under development or already available in clinical practice for non-vascular indications. This opens new therapeutic horizons for the treatment of cardiometabolic complications.

Keywords: vascular, inflammation, endothelial dysfunction, adipocytokines

S8-3
CXCL16/CXCR6 AXIS BLOCKADE IMPAIRS METABOLIC SYNDROME-ASSOCIATED ENDOTHELIAL DYSFUNCTION AND ABDOMINAL AORTIC ANEURYSM (AAA) DEVELOPMENT

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Introduction and Objectives: Clinical evidence indicates that metabolic syndrome (MS) is a precursor of endothelial dysfunction and an independent predictor of abdominal aortic aneurism (AAA). Since in both pathologies angiotensin-II (Ang-II) is a relevant player, the functional role of CXCL16/CXCR6 axis in MS, AAA formation and the mechanisms involved in Ang-II-induced CXCL16 arterial expression were investigated.

Methods and results: Flow cytometry revealed that MS patients showed increased platelet activation and percentages of circulating CXCR6-expressing platelets, CXCR6-expressing platelet-bound neutrophils, monocytes and $CD8^+$ lymphocytes compared with aged-matched controls leading to enhanced CXCR6/CXCL16-dependent adhesion to the dysfunctional (Ang-II- and TNFα-stimulated) arterial endothelium determined by the dynamic model of the flow chamber. Endothelial Ang-II stimulation (1 μM) for 24 h caused significant CXCL16 up-regulation but its neutralization only significantly inhibited (49%) mononuclear leukocyte-adhesion to cells from arterial but not from venous origin. This response was found to be dependent on Nox5 expression and subsequent RhoA/p38-MAPK/NFκB activation. Ang-II-induced AAA formation in apolipoprotein E-deficient mice

(apoE$^{-/-}$, 1000 ng/kg/min, 28 days) showed AAA higher incidence, increased macrophage, CD3$^+$ and CXCR6$^+$ cell infiltration and enhanced neovascularization than unchallenged animals. These effects were accompanied by increased CCL2, CXCL16, CXCR6 and VEGF mRNA expression within the lesion and enhanced levels of circulating soluble CXCL16. These parameters were reduced in AT_1 receptor antagonists-treated animals (losartan, 30 mg/kg/day). Similar reductions in these parameters were found in apoE$^{-/-}$ mice lacking functional CXCR6 receptor (CXCR6$^{GFP/GFP}$). Therefore, CXCR6 expression on platelet-bound monocytes and CD8$^+$ lymphocytes may constitute a new membrane-associated biomarker for adverse cardiovascular events and pharmacological modulation of this axis may positively affect cardiovascular outcome in metabolic disorders linked to rennin-angiotensin system activation.

This study was supported by grants SAF2011-23777, SAF2014-57845R and PI15/00082 from the Spanish Ministry of Economy and Competiveness, Carlos III Health Institute, the European Regional Development Fund.

S8-4
INCREASED PLATELET AND T CELL ACTIVATION IN PATIENTS WITH PRIMARY HYPERCHOLESTEROLEMIA IS ASSOCIATED TO ENHANCED LEUKOCYTE-ARTERIAL ADHESION

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Introduction and Objectives: Primary hypercholesterolemia (PH) is associated with a marked increase of low-density lipoprotein cholesterol (LDL-C) which has been linked with augmented risk of premature cardiovascular disease (CVD). A low grade of systemic inflammation is often associated with PH and systemic inflammation is the main driver of premature atherosclerosis development. However, the systemic inflammatory response in PH has been poorly investigated. Therefore, we have determined the activation state of platelets and different T lymphocyte subsets and their atherogenic consequences.

Materials and Methods: Whole blood from 22 PH patients and 20 age-matched controls was analysed by parallel-plate flow chamber assay to evaluate platelet-leukocyte and leukocyte adhesion to TNFα-stimulated arterial endothelium. Flow cytometry was employed to measure platelet activation (P-selectin expression and circulating PAC-1$^+$ platelets). CD69 expression, a marker of leukocyte activation, was also determined in different lymphocyte subsets.

Results: Parallel-plate flow chamber revealed enhanced platelet-leukocyte and leukocyte adhesiveness to TNFα-stimulated arterial endothelial cells in PH patients vs. age-matched controls. PH patients presented greater numbers of activated circulating platelets (PAC-1$^+$) with increased P-selectin expression than age-matched controls. Additionally, PH patients showed augmented numbers of platelet-lymphocyte aggregates than age-matched control subjects in different lymphocytes subsets (CD3, CD8, Th1, Th2 and Th17). Similarly, the activation state of CD4 lymphocytes such as Th1, Th2 and Th17 as well as CD8$^+$ cells was significantly greater in PH patients than in age-matched controls.

Conclusions: We have provided evidence that increased platelet and lymphocyte activation in PH patients leads to enhanced arterial leukocyte adhesiveness. Therefore, since platelet/leukocyte- and leukocyte-arterial adhesion precedes atherogenic process development, it is plausible that the activation state of these immune players may predict the likelihood of suffering further cardiovascular events in PH patients.

S8-5
DPP4 DELETION IN ADIPOSE TISSUE PROMOTES HEPATIC INSULIN SENSITIVITY VIA IGF1

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Besides a therapeutic target, dipeptidyl peptidase 4 (DPP4) is an adipokine secreted by adipose tissue (AT) whose serum levels are enhanced in obese and insulin-resistant patients. DPP4 might represent a missing link between increased AT mass and obesity-associated metabolic diseases. To explore the role of AT-derived DPP4 in diet-induced obesity, we generated an AT-specific DPP4 knockout (AT-DPP4-KO) mouse. Under high fat diet (HFD), circulating DPP4 levels (340 $\pm$ 32 ng/ml vs. 487 $\pm$ 30 ng/ml; $p < 0.01$) were reduced in KO mice, indicating AT as a relevant source of soluble DPP4. However, fasting and glucose stimulated serum concentrations of glucagon-like peptide-1 and gastric inhibitory peptide were similar in wild type and AT-DPP4-KO animals on HFD. KO animals under HFD showed improved oral glucose tolerance and suppression of endogenous glucose production during hyperinsulinaemic-euglycemic clamps. Although AT-DPP4-KO under HFD displayed increased body weight and fat mass, a beneficial remodeling was observed in a AT consisting of smaller adipocytes, enhanced M2 macrophage markers and decreased fibrosis markers. Upon HFD, knockout mice had lower IGF binding protein 3 (IGFBP3) levels in both AT and serum, while serum concentrations of free IGF1 were increased. Accordingly, exposure of human hepatocytes to recombinant IGFBP3 resulted in impaired insulin signaling and insulin-induced suppression of glucose production. Interestingly, type 2 diabetes patients treated with the DPP4 inhibitor sitagliptin, displayed increased serum free IGF1 and free IGF1/total IGF1 ratio. In conclusion, our study highlights a key role of adipose DPP4 in obesity-related metabolic disorders. Adipose DPP4 deletion under HFD triggers increased body weight followed by beneficial AT remodeling. In this scenario, adipose IGFBP3 production is reduced, leading to increased free IGF1 promoting hepatic insulin sensitivity. In summary, specific DPP4 deletion or inhibition arises as a novel therapeutic strategy in obesity to increase IGF1 and thus preserve hepatic insulin sensitivity.

Keywords: DPP4; adipokine; obesity; hepatic insulin sensitivity

S9: PSYCHOPHARMACOLOGY

S9-1
THE PROLYL OLIGOPEPTIDASE INHIBITOR IPR-19 AMELIORATES COGNITIVE DEFICITS IN MOUSE MODELS OF SCHIZOPHRENIA

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Cognitive decline associated with schizophrenia is considered a key feature of the disease since it might precede the onset of the disease and continues after psychosis, being a predictor of the disorder evolution. Current antipsychotic drugs for positive or negative symptoms have little or no effect on cognitive deficits. Furthermore, an exacerbation of cognitive deficits by some antipsychotic drugs is plausible.
Prolyl oligopeptidase (POP) is an 81-kDa monomeric serine protease that is expressed in brain and other tissues. Recent advances suggested that this enzyme participates in protein-protein interactions that control brain functions and cognitive processes. POP inhibition shows neuroprotective, anti-amnesic and cognition-enhancing properties in some rodent models. Here, we evaluated for the first time the potential of this target for the treatment of cognitive impairment associated with schizophrenia (CIAS). IPR-19, a potent and selective POP inhibitor, was tested in three cognition impairment mouse models associated with schizophrenia based on pharmacological subchronic phencyclidine and acute dizocilpine administration, and offspring from mothers with immune reaction induced by polyinosinic: polycytidylic acid administration during pregnancy. Acute IPR-19 administration (5 mg/kg, ip) was able to reverse the cognitive performance deficits of the three mouse models in the novel object recognition, T-maze and eight-arm radial maze tests. Although is not considered a behavioral test, IPR-19 also had positive effects on the prepulse inhibition test. The mode of action of IPR-19 differs greatly from other classical mechanisms unsuccessfully explored for the treatment of CIAS. The findings presented here become particularly valuable as a novel pharmacological mechanism option for the treatment of cognitive dysfunctions. Taking into account that CIAS represents the most treatment-elusive symptoms of schizophrenia, POP inhibition opens avenues for the treatment of this unmet clinical need.

S9-2
ROLE OF SEROTONIN 5-HT3 RECEPTORS IN THE TREATMENT OF DEPRESSIVE DISORDERS

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Depression is a chronic, recurring and potentially life-threatening mental illness with estimated lifetime prevalence up to 16% of the population. Despite current antidepressant treatments are generally safe and effective, less than a half of patients reach full remission. Therefore there is still a great need for faster acting, safer and more effective pharmacological treatments for depression. During the last decades, the monoamine hypothesis of depression has centre the development of new antidepressants. Thus, the increase of serotonin (5-HT) and/or noradrenaline (NA) availability in the synapse by the inhibition of their respective reuptake transporters underlies the therapeutic activity of the most widely prescribed antidepressant drugs. In this context, the 5-HT3 receptors are located postsynaptically on GABAergic interneurons in the brain stem regulating several neurotransmission systems. The activation of 5-HT3 receptors induces an inhibition of the release of 5-HT and NA. Therefore, it was proposed that the antagonism of these receptors would lead to an increase in the release of monoamines that could improve symptoms of depression. This effect would be mediated by increasing pyramidal neuron activity through blockade of 5-HT3 receptor-mediated activation of GABA interneurons. Our own research group demonstrated that the coadministration of a 5-HT3 antagonist with selective serotonin reuptake inhibitors (SSRIs) increased monoamine levels in the rat frontal cortex. Moreover, it was suggested that the blockade of 5-HT3 receptors in coadministration with SSRIs could be a potential strategy for avoiding counter-therapeutic effects of SSRIs in the early stages of treatment.
Mirtazapine and the new antidepressant vortioxetine are examples of antagonists of the 5-HT3 receptors. Vortioxetine presents 5-HT3 receptor antagonism and selective serotonin reuptake inhibition in the same molecule. Several studies with vortioxetine have demonstrated that the 5-HT3 receptor antagonism potentiated the increase in 5-HT extracellular levels produced by the serotonin reuptake inhibition.
Key words: 5-HT3 receptors, depression, antidepressants, vortioxetine

S9-3
PHARMACOLOGY OF NEW PSYCHOACTIVE SUBSTANCES

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The use of new psychoactive substances (NPS) has increased in recent years. Until December 2016, more than 700 NPS had been reported by 102 countries to United Nations since 2008. NPS are defined as "substances of abuse, either in a pure form or a preparation, that are not controlled by the 1961 Single Convention on Narcotic Drugs or the 1971 Convention on Psychotropic Substances, but which may pose a public health threat". The term "new" does not necessarily refer to new inventions, but to substances that have recently become available on the market. Since NPS are not controlled their legal status can differ widely from country to country. NPS are cheap, easy to obtain, and often legally available. NPS appeared in the market by terms such as "legal highs", "herbal highs", "bath salts" and "research chemicals".
The main substance groups of NPS are synthetic cannabinoids, phenethylamines (including 2C-derivatives), synthetic cathinones (including mephedrone), aminoindanes, ketamine and phencyclidine-type substances, piperazines, tryptamines, benzodiazepines, opioids and plant-based substances.
In general, side effects of NPS range from seizures to agitation, aggression, acute psychosis and cardiovascular effects. NPS users have frequently been hospitalized with severe poisonings including cases of death. Information about pharmacology, safety and toxicity in animals and humans of most NPS are not available or very limited, in addition, long-term adverse effects or risks are still largely unknown.
In this conference, recent results of the presence of NPS in the Spanish market and the human pharmacology of mephedrone, 2C-B and synthetic cannabinoid JWH-018 will be presented and discussed.
Acknowledgements: Founded in part by grants from Instituto de Salud Carlos III (ISCIII, FIS-FEDER, PI14/00715), ISCIII-Red de Trastornos Adictivos (RTA RD16/0017/0003 and RD16/0017/0010), Ministerio de Sanidad, Servicios Sociales e Igualdad (Plan Nacional sobre Drogas-PNSD, 2015I054). Esther Papaseit is a Juan Rodes fellow (ISC-III, JR16/00020).
Key words: New psychoactive substances, synthetic cathinones, synthetic cannabinoids, phenethylamines, intoxication

S9-4
ROLE OF INFLAMMATORY PROCESSES IN DEPRESSION

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A large dataset documents the role of immune-to-brain interactions in the pathophysiology of depressive disorders. Inflammatory cytokines released peripherally by activated immune cells during the host response to pathogen invasion play a major role in these interactions. To further elucidate the role of inflammatory processes in the development of depression, our group has used models of chronic inflammation, both at the clinical and preclinical levels. Findings have contributed to characterize the phenomenology of depressive symptoms that occur in contexts of inflammation and to describe the underlying mechanisms and biomarkers associated with these effects. In particular, they revealed the key contribution of alterations in enzymatic pathways involved in the biosynthesis of neurotransmitters, together with changes in neuroendocrine activity and neurocircuitry. Altogether, these data provide important information regarding the role of immune mediators in the pathophysiology of depression and highlight potential new therapeutic targets.

S10: THERAPEUTIC INNOVATION

S10-1
P2Y12 INHIBITION IN ACS: PLATELET AND BEYOND PLATELET EFFECTS

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$P2Y_{12}$ receptor antagonists have potent effects inhibiting the ADP-induced activation of platelets. Dual antiplatelet therapy with the cyclo-oxygenase (COX) inhibitor aspirin and a purinergic $P2Y_{12}$ receptor inhibitor is at present the standard of treatment in acute coronary syndromes (ACS). Different clinical trials have shown the relative efficacy of clopidogrel, prasugrel and ticagrelor in ACS patients. Recently it has become increasingly accepted that $P2Y_{12}$ inhibitors exert off-target effects that protect against myocardial injury. Yet, the extent of cardioprotection differs among the different $P2Y_{12}$ antagonists likely because their off-target properties. As such, administration of $P2Y_{12}$ inhibitors has demonstrated, at both experimental and clinical levels, to reduce the size of infarction. This is of key importance since infarct size is a major determinant of post-STEMI morbidity and mortality. However, $P2Y_{12}$ inhibitors have been shown to differ in their ability to limit the size of infarction regardless of their antiplatelet efficacy. In this regard, we have recently demonstrated in a preclinical animal model of MI-induction and by CMR analysis, that although clopidogrel and ticagrelor administration prior-MI reduces the size of infarction assessed acutely post-MI, this effect was significantly greater in ticagrelor-administered animals. Most interestingly, ticagrelor cardioprotective effects were associated with the attenuation in oedema formation, an effect not observed in clopidogrel-treated pigs. These differences suggest that $P2Y_{12}$-related cardioprotection may exhibit a class effect (clopidogrel/thienopyridine vs ticagrelor/cyclopentyl triazolopyrimidine) helping to partly explain the differences in the clinical benefit found in the PLATO trial. Although the underlying molecular mechanisms by which platelet $P2Y_{12}$ inhibitors mediate cardioprotection remain to be elucidated they have been shown to exhibit an ischemic conditioning-like effect. Particularly, ticagrelor has shown to protect the heart against MI though adenosine-related mechanisms. By blocking the ENT-1 transporter, ticagrelor prevents erythrocytes reuptake of the adenosine released upon the ischemic insult leading to the consequent local adenosine increase. In a follow-up study we have demonstrated that ticagrelor protects also against adverse cardiac remodelling.

Key words: platelets, ACS, cardioprotection, ticagrelor.

S10-2
PHARMACOECONOMICS APPLIED TO BIOLOGICAL DRUGS IN THE MANAGEMENT OF SEVERE ASTHMA

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Asthma currently affects around 300 million people globally, being one of the most prevalent chronic diseases, affecting 5% of the adult population in Spain. Approximately 10% of patients with asthma suffer from severe asthma, which has an increased risk of death. Achieving good disease control involves taking good control of symptoms and reducing the number of exacerbations. Approximately 50% of patients with severe asthma receiving maintenance therapy, through the combination of inhaled corticosteroids (IC) and long-acting β 2 -adrenergic agonists (LABA) do not achieve good disease control, as evaluated through the administration of the Asthma Control Questionnaire. In addition, these poorly controlled patients are the ones that generate the greatest cost to the National Health System and those that show a greater affectation of the quality of life. The management of these patients in specialized units of asthma is cost-effective (improving asthma control and reducing exacerbations and hospitalizations) and this is achieved with therapeutic modifications that sometimes include addiction to the treatment of biological drugs. Biological therapy in severe asthma has a beneficial impact on disease control, provided that patients are adequately selected. The results obtained in the published studies suggest that severe asthmatics treated with biological agents (after appropriately selecting the appropriate therapeutic target for each patient) can improve their symptoms and increase the number of QALYs, consume less health resources and have a lower loss of productivity. All these variables should be considered in real practice when choosing an effective treatment and improving the quality of life of patients with severe asthma.

Key words: uncontrolled severe asthma; biological drugs; quality of life; pharmacoeconomics.

S10-3
TARGETED IMMUNOTHERAPY FOR CANCER TREATMENT, DISCOVERING A NEW ERA IN ONCOLOGY

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In the last few years modern immunotherapy has emerged as a powerful tool to successfully treat a large number of solid and hematologic malignancies. Monoclonal antibodies targeting immunomodulatory proteins of the immune synapses like cytotoxic T-lymphocyte associated protein 4 (CTLA-4) or programmed cell death protein 1 (PD-1) represent a breakthrough therapy that have reported impressive results in a wide set of different tumours such as melanoma, lung, kidney, head and neck cancer, Hodgkin lymphoma and others. Nowadays, this topic is an area of expanding clinical and translational research, with uncountable clinical trials ongoing. However, despite the awesome results achieved, a considerable fraction of patients does not respond to immunotherapy and thus the great challenge that lies ahead is to identify predictive biomarkers that would allow using these new therapies in the patients most likely to benefit, and therefore using them in a more efficient way.

S11: GASTROINTESTINAL AND HEPATIC PHARMACOLOGY

S11-1
THE IMPORTANCE OF THE TRANSPORTOME IN HEPATIC AND GASTROINTESTINAL DISEASES

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Epithelial cells express a broad variety of membrane transporter proteins which coordinately determine nutrient supply but also modulate other tissue functions, including purinergic responses and cell proliferation. These membrane transporter proteins, understood as a whole functionally integrated system is known as the transportome. Transportome alterations may be relevant to the physiopathology of particular diseases but can also determine response to therapy. Transportome alterations in Crohn's disease with a particular emphasis on the purinome will be discussed. Purinergic signaling in inflammation is in part modulated by selected membrane transporters implicated in adenosine removal. How the microbiota can contribute to restore transportome integrity will be also briefly summarized. Similarly, the network of transportome encoding genes in gastrointestinal tumor cell models will be also reviewed, focusing on how selected genes in these networks may be modulating response to selected anticancer drugs. Although alterations in transporter membrane proteins can indeed determine drug bioavailability and action, recent evidence supporting the view that loss of selected transportome members may contribute to oncogenesis will be also briefly reviewed.

S11-2
PHARMACOLOGICAL TARGETS FOR NASH

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NAFLD is one of the most common causes of chronic liver disease worldwide, and particularly, the main chronic liver disease condition in the Western world. NAFLD is a clinical pathological term that includes a spectrum of alterations that range from simple triglyceride accumulation in the hepatocytes (steatosis) to hepatic steatosis with inflammation (nonalcoholic steatohepatitis or NASH), which may or not have associated fibrosis. NAFLD is tightly linked to obesity, insulin resistance, type 2 diabetes, dyslipidemia and hypertension, all of them risk factors to the metabolic syndrome. Metabolic syndrome is a clustering of different risk factors that collectively increases the probability of developing cardiovascular disease and diabetes. NAFLD can be considered the hepatic manifestation of the metabolic syndrome, being a key factor predisposing to it.

The prevalence of NAFLD in developed countries is 20–30% of adults. It is more frequent among people with diabetes (30–50%) and obesity (80–90%), and almost universal when combining both factors. In the case of children, there is a prevalence of 3–10% rising up to 40–70% among obese children. Even though NAFLD is the most common liver disorder in developed countries, current therapies are restricted to lifestyle modifications whereas pharmacological approaches remain scarce and experimental. Indeed, it has been shown that 7–10% of weight loss slows the progression of NAFLD and may reverse some of the steatosis and necroinflammation. There are few clinical trials in NAFLD and no specific therapy can be firmly recommended. For this reason, drug therapy should be reserved for patients with progressive NASH or with high risk of disease progression (diabetes, metabolic syndrome, persistently increased ALT or high necroinflammation) to prevent disease progression.

Our group have shown that knocking out enzymes involved in S-adenosylmethionine (SAMe) metabolism, the main biological methyl donor in humans that is abundant in the liver, will lead to NASH development in mice. New data from humans have also suggested that these enzymes play a role in the pathogenesis of NAFLD and that some of SAMe cycle metabolites may serve as noninvasive biomarkers of NASH and potentially as NASH treatment. Novel druggable therapeutic targets will definitely impact the huge health and economic burden NAFLD-related.

S11-3
IMMUNE THERAPY OF LIVER CANCER

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Several approaches to enhance the immune response against tumor cells have failed to provide treatment benefit in advanced hepatocellular carcinoma (HCC) including dendritic cell vaccination, cytokine-induced killer cells or oncolytic viruses expressing immunostimulating cytokines. Following the proof of concept in melanoma patients, it has been shown that T-cell checkpoint modulation may break the barrier that different tumor types create to evade the attack from the immune system.

In fact, enhancing T cell function by targeting cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4), programmed death 1 (PD-1) and programmed death ligand 1 (PD-L1) using monoclonal antibodies has revolutionized the field of cancer immunotherapy. Tremelimumab (anti-CTLA-4) has provided the first signal of activity and favorable safety profile in a phase II clinical trial targeting HCC patients with chronic HCV infection. In a small group of 21 patients, Tremelimumab produced an overall response rate of 18% while 59% of patients had a stable disease that in almost half of the cases lasted for more than 6 months. A median time to progression of 6.5 months provided further evidence of antitumor activity. These results paved the way for other checkpoint inhibitors.

Nivolumab (anti-PD-1) has very recently provided strong signal of antitumor activity in a phase I/II clinical trial. A dose-escalation cohort was followed by a large expansion cohort, and uninfected patients as well as patients with chronic HCV infection or chronic HBV infection under control by antiviral agents were recruited worldwide. Altogether, a large population of nearly 250 patients with fairly advanced HCC has been treated, most of them already exposed to Sorafenib. Following Nivolumab, 15% of patients reached an objective tumor remission (including several complete responses) and an additional 50% had stable disease that was frequently durable. Regarding survival, a 9-month survival rate of 70% was reported in the large expansion cohort while a median overall survival of 14 months (irrespective of prior Sorafenib treatment) was reported in the dose-escalation cohort with a longer follow-up. Importantly, these results were obtained with only a 1% rate of intense (CTCAE grade ≥ 3) symptomatic adverse events. Other checkpoint inhibitors have started clinical development and, following reports of synergistic effects in melanoma, a lot of interest is focused on combinations. CTLA-4 plus PD-1 or PDL-1 blockade is

being explored in two different trials using the combination of Ipilimumab plus Nivolumab or Tremelimumab plus Durvalumab, and their results are eagerly awaited.

S11-4
PURINE RECEPTORS AND SMOOTH MUSCLE FUNCTION IN THE GASTROINTESTINAL TRACT

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Gastrointestinal motility (GI) is accomplished by the interaction between the enteric nervous system and pacemaker cells. Enteric neurons, glial cells and interstitial cells of Cajal (ICC) interact with smooth muscle to coordinate motor patterns such as gastric emptying, propulsion, segmentation and sphincter accommodation among others. Purine receptors including several subclasses of P2X and P2Y receptors are present in different cell types and modulate gastrointestinal motility. They are both pre- and post-junctional located and can contribute to the modulation of nerve mediated excitatory and inhibitory responses. Nerve-mediated relaxation is accomplished by Nitric Oxide (NO) and ATP or a related purine that are probably released by the same inhibitory motor neuron as inhibitory co-transmitters. The $P2Y_1$ receptor has been recently identified as the receptor responsible for purinergic smooth muscle hyperpolarization and relaxation in the gut. Both orthosteric (MR2179, MRS2279 and MRS2500) and allosteric (BPTU) $P2Y_1$ antagonists concentration dependently decrease nerve-mediated inhibitory responses. Purinergic neurotransmission is absent and transit time is impaired In $P2Y_1$-deficient mice. The dynamics of the co-transmission process is frequency dependent: low frequencies of nerve stimulation cause $P2Y_1$ mediated responses whereas high frequencies of stimulation cause NO responses, suggesting a different role for each co-transmitter. A direct communication from nerve to muscle is possible. However, specific cell types such as ICC or fibroblast-like cells expressing PDGFRα and $P2Y_1$ receptors are intercalated between nerve and muscle. Accordingly inhibitory neurons modulate smooth muscle contractility through other cells that are coupled each other and contribute to pacemaker function. The contribution of purinergic neurotransmission in neuromuscular diseases affecting GI motility is still not understood. In this presentation, we will focus on the physiological mechanisms responsible for nerve-mediated purinergic relaxation providing the functional basis for possible future clinical and pharmacological studies on GI motility targeting purine receptors.

S12: TEACHING PHARMACOLOGY

S12-1
ARE TV MEDICAL DRAMAS USEFUL TO TEACH CLINICAL PHARMACOLOGY? AN EMPIRICAL STUDY USING HOUSE M.D.

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TV series with medical plots have always had a great success in general public and they have been used as a teaching tool in medical students. However, their use has not reached clinical pharmacology courses and empirical data do not exist about their pedagogical effectiveness. Therefore, we performed an initial content analysis study to evaluate the 22 chapters of the first season of *House MD*. Later, we carried out a multicenter study in three universities to assess its pedagogical value to learn clinical pharmacology in medical students. To this aim, we compared how seeing and discussing a chapter improved the factual knowledge. Students were randomly assigned to two groups, each of them seeing a different episode, and answered a multiple choice questionnaire that included questions related to both chapters. Finally, we asked the students to rate the interest of the experience for learning the discipline. The content analysis showed that 43 primary and 136 secondary issues likely to be of interest in teaching clinical pharmacology were chosen. The evaluation of the pedagogical interest identified five episodes of great interest, 13 of medium interest and four low interest. Four episodes of the first group were used in the comparative study. The evaluation with students showed that two chapters allowed the improvement of knowledge in all universities, one was not useful in any of them and contradictory results were seen with the fourth. However, students showed a high satisfaction with their experience and the interest in improving their knowledge in all study groups. In conclusion, the project showed that *House MD* contains elements of pedagogical interest in the field of clinical pharmacology. The view of some episodes allowed to obtaining a better knowledge of the discipline but not in all cases. Consequently, its use should consider prior knowledge when choosing the episode.

S12-2
SPANISH INTER-UNIVERSITY NETWORK FOR INNOVATIVE EDUCATION IN PHARMACOLOGY: A COMMON SPACE TO IMPROVE LEARNING?

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Changes in Pharmacology teaching have been introduced in the last years that include not only changes in the curriculum but also in the way teaching is delivered to facilitate learning. In this context, building materials for innovative education in Pharmacology is a task that needs to be envisaged. Until now, problem based learning, simulated practical works, peer assessment, interactive computer learning, virtual environments and multidisciplinary activities have been implemented in different universities, but collaboration between teachers on a global scale could be an effective strategy to optimize the necessary changes to improve learning.

We initiated a Spanish inter-university network to create a common space for sharing not only the teaching material but mainly the implementation experiences and research results obtained on different aspects of innovation. Fourteen Spanish universities that teach pharmacology to different Degrees of Health Sciences are associated to this project. A Moodle platform has been organized, presented to the network members in a meeting and used to share teaching materials. Until now, shared material includes: pharmacological cases and problems for seminars or practical sessions, videos for flipped class or as an alternative of animal use, discussion activities based on films or medical series visualization or literary texts and press news reading, use of social network, gamification, . . .as well as other resources to practical sessions as computer-based simulation software, practice notebooks. Another shared resources were the different evaluation and self-assessment systems used in each institution. Along with the teaching material, a description with the experience of its use and the evaluation of results, have been added in the Moodle interface.

With the experience accumulated during the last and the present year, we will evaluate the contribution of the network to the improvement of results in pharmacological education.

Funded by Vice-Rectorate for Training Policies and Educational Quality (UVEG) (UV-SFPIE_GER16-418743).

Key words: Interuniversity network; pharmacology teaching; innovation

S12-3
AN EXPERIENCE OF USING E-LEARNING IN THE TEACHING OF PHARMACOLOGY

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The aim of our experience consists of developing E-learning strategies to carry out a functional teaching in Pharmacology. The student should be able to use the acquired knowledge in their profession. Instead of retaining information that would remain inert or forgotten, they must perform activities to allow them using the knowledge of Pharmacology to solve real situations. A new E-learning tool based on thematic units was implemented; students achieve theoretical knowledge and develop skills such as collaborative work and self-learning, which increases if thematic units are delivered before theoretical class.

The application of the thematic units, before the theoretical class increases the degree of self-study with a similar academic performance, when thematic units is working after class attendance.

The assessment of the methodology by the students is very positive, mostly expressing their utility when not only in the resolution of enforcement activities (situations similar to real clinical cases), but also with regard to the possibility of working group.

We have prepared a video that shows the methodology used to conduct E-learning activities and assessment activities, wishing that can be useful to other teachers.

The video is in the following sections: introduction, presentation of the methodology for students and formation of working groups, theoretical class, working group session, interviewed customized for students to obtain its opinion on the methodology and conclusions.

*This work was carried out with the aid granted by the UB our Consolidated Teaching Innovation Group (GINDOC-UB/094)

Key words: e-learning; functional teaching; self – learning; collaborative work

S12-4
AN ACADEMIC SERVICE-LEARNING ACTIVITY IN HIGHER EDUCATION TO PROMOTE THE RESPONSIBLE USE OF ANTIBIOTICS

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Service-learning is an educational approach that integrates students' academic learning with community service. The aim of this service-learning activity is to promote a responsible use of antibiotics and to improve students' academic learning related to their own contents of Pharmacology and Audiovisual Projects as well as the ones related to transversal competences. The service-learning activity began with the identification of a real need: to avoid the spread of antimicrobial resistance due to the irresponsible use of antibiotics. Next, Medical students had to prepare their plan for action: to encourage best practices amongst the general public and health workers about antibiotics consumption. An audiovisual media campaign was chosen in order to improve awareness and understanding of antimicrobial resistance. For that purpose, it was necessary to establish a partnership with the students of the Degree in Audiovisual Studies. With this action, it was expected students to learn how to communicate scientific subjects in a language style appropriate to the subject and audience, to detect some common mistakes in antibiotics prescription or therapeutic compliance, to make audiovisual and multimedia productions and to acquire some abilities to network with experts from another field of knowledge. Students analysed the most suitable information about antibiotic consumption to be included in the audiovisual product and chose the audiovisual product that best fit the sector of the population targeted. Moreover, an agreement with a cooperation entity was signed. After the action was made, a period of reflection started. The experience, knowledge and skills acquired were evaluated for the students. However, reflection could also occur before, during and after implementation of the action. Finally, service-learning project was globally assessed: the extent to which the initial objectives have been accomplished.

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S13: NEUROPHARMACOLOGY

S13-1
VULNERABILITY FACTORS THAT PREDISPOSE TO COCAINE ADDICTION

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A large number of people experiment with drugs of abuse, but only a few become addicts. This individual variation in the propensity to become addicted is driven by both genetic and environmental factors. Many studies have investigated vulnerability factors that predispose to cocaine addiction, but few have focused on adolescence—a critical period for neurobehavioral plasticity and vulnerability to drug use. This symposium will present pre-clinical data from our laboratory which demonstrated timing-specific cocaine exposure effects during early-mid adolescence (i.e., vulnerable window: postnatal day PND 33–39) on inducing immediate neurotoxic effects on particular brain regions and long-term consequences on addiction-like behavior (i.e., stimulus-reward learning) and negative affect (i.e., psychiatric comorbidity). Supported by 'Delegación del Gobierno para el Plan Nacional sobre Drogas' (MSSSI, Grants 2012/011, 2016/002) and 'Ramón y Cajal' Program to M.J.G.-F. (MINECO), and by RTA-RD16/0017/0010.

Key words: adolescence; cocaine; long-term consequences; negative affect

S13-2
POTENTIAL VALUE OF MICRORNA-30C AS BIOMARKER AND THERAPEUTIC TARGET FOR NEUROPATHIC PAIN

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Neuropathic pain is a prevalent and debilitating chronic syndrome highly refractory to current analgesics. MicroRNAs (miRNAs) are single-stranded ~22nt long non-coding RNA transcripts involved in the post-transcriptional regulation of gene-expression. Recently, a number of studies have reported aberrant expressions of several miRNAs in nociception-related structures, which are associated to neuropathic pain development in rodent models. Herein, we have exploited the anti-allodynic phenotype of mice lacking BAMBI (BMP and Activin membrane bound inhibitor) to identify, by next-generation sequencing, new miRNAs potentially valuable as therapeutic targets and/or biomarkers for neuropathic pain states. Among then, miR-30c overexpression in pain-relevant areas (spinal cord, dorsal root ganglion) and body fluids (cerebrospinal fluid (CSF) and plasma) showed a direct correlation with the severity of the mechanical allodynia developed by rats subjected to sciatic nerve injury. Administration of LNA miR-30c inhibitor into the cisterna magna delayed neuropathic pain development and reversed fully established allodynia. In contrast, administration of miR-30c mimic accelerated allodynia development after the nerve injury. After witnessing the relevant contribution of miR-30c to neuropathic pain in rats, we aimed to assess the potential translation of the experimental data into humans. For this purpose, miR-30c was determined in plasma and CSF samples from patients suffering from neuropathic pain associated to severe leg ischemia. Patients with no evidence of neuropathic pain, with either leg ischemia or without ischemia served as controls. As observed in the experimental animal, patients affected by neuropathic pain presented significantly higher levels of miR-30c in both plasma and CSF in comparison with patients free of pain. Logistic regression analysis and receiver operating characteristic (ROC) curves identified circulating miR-30c, either in plasma or in CSF, diabetes mellitus and age as significant positive predictors of neuropathic pain occurrence. The predictive model obtained if diabetes and age were combined with circulating miR30c in plasma presented the highest area under the ROC curve [AUC = 0.93 (95% CI 0.87–0.99)], with a sensitivity of 80% and a specificity of 94%. Overall, our results support that miR-30c is involved in neuropathic pain establishment and maintenance after neural injuries. Our findings in patients support the notion that the level of circulating miR-30c, either in plasma or in CSF, in conjunction with clinical parameters, could help to create an individual risk profile disclosing the potential for neuropathic pain in patients suffering from chronic peripheral ischemia. We propose that (i) targeting miR-30c could constitute a therapeutic approach in chronic pain processes and (ii) miR-30c circulating levels could be useful biomarkers of disease prognosis. (Supported by SAF2013-47434-R).

S13-3
BEHAVIORAL AND MOLECULAR ALTERATIONS INDUCED BY MATERNAL ALCOHOL BINGE CONSUMPTION IN OFFSPRING MICE

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Maternal alcohol consumption leads to a range of long-lasting morphological and behavioral deficiencies known as fetal alcohol spectrum disorders, associated with neurodevelopmental disabilities. Alcohol activates neuroimmune system that is a critical contributor to brain injury outcome. We sought to test whether alcohol binge drinking during the prenatal period, or in combination with lactation, promotes long-term behavioral alterations and whether such effects are associated with neuroinflammation and myelin dysfunctions. Pregnant C57BL/6J female mice underwent a model procedure for binge alcohol drinking either during the gestation period or both the gestation and lactation periods. Subsequently, adult male offspring were assessed for their cognitive function and motor coordination. Recognition memory was not significantly affected by maternal binge alcohol drinking, but executive functioning was impaired in prenatal and lactation alcohol-exposed mice. The behavioral effects were associated with up-regulation of pro-inflammatory molecules (Toll-like receptor 4, Nuclear factor-*kappa* B p65 and Interleukin-1β) together with a reduction in several structural myelin proteins (MAG, MBP, PLP) in both the prefrontal cortex and hippocampus of adult mice exposed to alcohol. Finally, histone acetylation levels (H3K9, H4K5 and H4K12) and an imbalance in histone acetyltransferase and histone deacetylase activities were found to increase in the prefrontal cortex of both groups of mice exposed to alcohol, with more discrete effects in the hippocampus. Altogether, our results reveal that maternal binge-like alcohol consumption induces neuroinflammatory damage causing long-term myelin and epigenetic modifications, effects that might underlie the long-term cognitive and behavioral impairments observed in fetal alcohol spectrum disorders.

Key words: fetal alcohol spectrum disorders, neuroinflammation, myelin, mice

S13-4
THERAPEUTIC POTENTIAL OF CONJUGATED SIRNAS FOR THE TREATMENT OF MAJOR DEPRESSIVE DISORDER

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The high prevalence and socioeconomic impact of major depressive disorder (MDD) is not paralleled by marked improvements in its treatment. Antidepressant drugs, including the widely prescribed monoamine (serotonin-5-HT and norepinephrine-NE) reuptake inhibitors, show slow onset of clinical action and limited efficacy, which reduces the quality of life of MPP patients and increases suicide risk. The reasons for the limited efficacy of monoamine-based drugs are manifold, including neuroadaptive mechanisms and genetic factors.

RNAi strategies may avoid some of these limitations by directly modulating the expression of genes involved in clinical response. However, despite its enormous potential, *in vivo* use of RNAi is limited due to the difficulty to deliver oligonucleotide sequences (e.g., small interfering RNA; siRNA) to the desired neurons/circuits in mammalian brain. Our strategy has been to develop conjugate siRNA molecules (C-siRNA) in which the siRNA sequence is covalently bound to the SSRI sertraline in order to selectively accumulate it by the dense network of 5-HT axon terminals in brain. Hence, the intracerebroventricular (i.c.v.) or intranasal (i.n.) administration of the sertraline-conjugated siRNA (Ser-C-siRNA) directed against the serotonin transporter (SERT) or 5-HT_{1A}-autoreceptors evoked robust antidepressant-like responses in mice,[1,2] mimicking the effects of the local application of unmodified siRNA in the raphe nuclei.[3,4] Interestingly, 1-week i.n. administration of small amounts (2.1 nmol/day) of a Ser-C-SERT-siRNA evoked antidepressant-like responses similar to those induced by 1-month treatment with 10 mg/kg/day fluoxetine. Likewise, acute 5-HT_{1A} autoreceptor knockdown also evoked rapid antidepressant-like effects in mice, due to a lesser self-inhibition of serotonergic neurons. We recently extended this strategy to knockdown TASK3, an acid-sensitive two pore-domain potassium channel (K2P) selectively in serotonergic or noradrenergic neurons using sertraline- and reboxetine-conjugated siRNA, respectively.

Overall, these results support the usefulness of our approach to elicit rapid and robust antidepressant-like effects in rodents using RNAi, showing a high potential translational value.

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S14: PHARMACOLOGY OF ANTI-CANCER DRUGS

S14-1
ABTL0812 AN AUTOPHAGY-ANTICANCER DRUG IN PHASE II CLINICAL TRIALS THAT INHIBITS PI3K/AKT/MTOR PATHWAY BY INDUCING TRIB3 EXPRESSION

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ABTL0812 is a first-in-class anti-cancer agent with a unique mechanism of action via a PPARα/γ-dependent TRIB3-mediated AKT/mTOR pathway inhibition that leads to autophagy-induced cell death. The antitumour activity of ABTL0812 has been demonstrated in *in vitro* models including squamous and adenocarcinoma non-small cell lung cancer, endometrial cancer, pancreatic cancer, breast cancer and prostate cancer, even in treatment-resistant cellular models such as doxorubicin-breast cancer resistant cells. *In vivo* studies showed that ABTL0812 reduced tumour growth to a similar or greater extent than erlotinib in NSCLC cells. Notably, when ABTL0812 was administered in combination with standard-of-care chemotherapies, it exhibited increased antitumour activity in different models such as docetaxel or paclitaxel/carboplatin in squamous NSCLC xenograft models, paclitaxel in an orthotopic endometrial cancer model, paclitaxel/carboplatin in an endometrial PDX model, paclitaxel/gemcitabine in a pancreatic cancer xenograft model or cisplatin in a neuroblastoma xenograft model. In these cases, the efficacy of the combined therapy was higher than that observed for the single agents; however, no potentiation of toxic effects was observed.

All this preclinical work led to the first-in-human dose escalation phase I clinical trial where ABTL0812 was administered as a single agent in adult patients with advanced solid tumours. The study included twenty-nine patients and showed that ABTL0812 has an excellent safety profile. AKT phosphorylation in platelets was used as a surrogate pharmacodynamic biomarker that permitted the determination of the recommended phase 2 dose by pharmacokinetic-pharmacodynamic modelling. Additionally, preliminary signs of efficacy were observed in five patients presenting with stable disease, including one cholangiocarcinoma (80 weeks), one endometrial cancer (60 weeks) and three sigmoid colon adenocarcinomas (28 and 22 and 20 weeks). A phase 2 clinical trial is currently ongoing in patients with endometrial or squamous NSCLC cancer where ABTL0812 is administered as first-line therapy in combination with standard-of-care chemotherapy.

Key words: ABTL0812, autophagy, PI3K/Akt/mTOR pathway, Phase I/II, lung cancer, endometrial cancer

S14-2
NEW THERAPEUTIC PROPOSALS EMERGING FROM PATIENT-DERIVED PRECLINICAL PLATFORMS OF PEDIATRIC SOLID TUMORS

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Most pediatric solid tumors are characterized by homogeneous genetic properties and unique biomarkers. Examples of specific alterations of pediatric tumors are the high expression of the disialoganglioside (GD2) in neuroblastoma and osteosarcoma, the EWS/FLI1 fusion gene of Ewing sarcoma, or the K27M mutation in the H3.3 histone of diffuse intrinsic pontine gliomas. These unique properties enable the design of novel strategies to target pediatric tumor cells. However, preclinical studies in pediatric oncology are challenging because pediatric solid tumor models are scarce. The translational research program at Hospital Sant Joan de Déu Barcelona contributes to generate clinically relevant models of pediatric solid tumors, including patient-derived xenografts, which are useful for the validation of novel therapeutic approaches, the identification of biomarkers and the design of clinical trials.

S14-3
THE CANCER GENOME INTERPRETER: A TOOL TO ANNOTATE THE BIOLOGICAL AND CLINICAL RELEVANCE OF TUMOR ALTERATIONS

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The difficulty to interpret the somatic variants constitutes a major hurdle to the identification of those genomic events more likely to be biologically and/or therapeutically relevant in each individual tumor. The information on genomic alterations that are validated as oncogenic and those that are found to influence the response to anti-cancer therapies is fragmented across the literature and several specialized resources. Moreover, there is no framework to automatically match the variants in a individual's tumor alterations to the available knowledge. Finally, most of the alterations observed in a tumor, even those occurring in cancer genes, remain of uncertain significance. Here we will present the Cancer Genome Interpreter (http://www.cancergenomeinterpreter.org), a resource that combines extensive expert curation and computational analyses to annotate the significance of alterations in tumor genomes according to distinct levels of relevance that respond to different use cases. The tool is aimed to support a broad range of applications in both pre-clinical and translational settings. As a proof-of-concept of its use, we will present the results of applying the tool to (a) systematically analyse 7000 tumors and reveal the landscape of genomic drivers and genomic-directed therapeutic opportunities –including potential repurposing opportunities– in cancer as it stands today; and (b) support the allocation of patients to the most appropriate clinical trial or to comprehensively explore off-label opportunities for genome guided therapies in patients unresponsive to standard-of-care treatment in two real-world clinical scenarios.

S15: JAK.STAT SIGNALING: NEW TARGETS AND THERAPEUTICS FOR MULTIPLE DISEASES

S15-1
INFLAMMATORY RE-TUNING OF IL-6 MEDIATED JAK-STAT SIGNALING

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Interleukin (IL)-6 is recognized as major therapeutic target for the treatment of certain autoimmune diseases and cancer. Studies show that IL-6 has a broad effect on immune cells and structural stromal cells where it controls context-dependent pro- and anti-inflammatory activities. Clinical experience with IL-6 inhibitors has raised various questions about how and when to block this cytokine to improve disease outcome and patient benefit. Here, a better understanding of the receptor signaling events holds the potential to improve patient diagnosis, stratification and the choice of clinical intervention. To address this issue studies have considered the complexity of IL-6 receptor signaling in model systems. With a primary focus on IL-6 activities in inflammatory arthritis, studies will consider how cytokine control of the latent transcription factors Signal Transducer and Activator of Transcription (STAT)-1 and STAT3 affects immune outcomes. Attention will be given to IL-6 signaling in CD4 T-cells, and how T-cell activation re-tunes the signaling characteristics IL-6 to affect changes in T-cell proliferation, survival and effector function.

Funding: Arthritis Research UK Programme Grants (20770, 19796) Hoffmann la Roche Pharmaceuticals.

S15-2
THE STAT3 PARADOX IN BREAST CANCER: AN INDUCER OF CELL DEATH AND AN ONCOGENE

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STAT3 is well established as an oncogene and is frequently constitutively activated in breast cancers. Established breast cancer cell lines with high levels of STAT3 phosphorylation often exhibit addiction to STAT3 and undergo cell death when STAT3 is inhibited or ablated. Conversely, Stat3 is specifically activated in mouse mammary gland at the onset of post-lactational regression (involution) and conditional deletion of Stat3 in mammary epithelial cells abrogates cell death and delays involution. We have demonstrated that Stat3 induces a lysosomal pathway of cell death and switches cell fate from milk secretion to a phagocytic function. We are currently investigating the mechanism by which Stat3 mediates these effects. Given the challenges of developing drugs that specifically target STAT3, we anticipate that insights gained from these studies will be informative for the design of therapeutics that target essential components of the STAT3 pathway.

S15-3
JAK-STAT PATHWAY IN MYELOPROLIFERATIVE NEOPLASMS: FROM JAK2 V617F TO CALRETICULIN MUTANTS

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Myeloproliferative neoplasms (MPN) are diseases of the hematopoietic system whereby hematopoietic stem cells acquire mutations that give a major clonal advantage to myeloid progenitors leading excessive production of functional red blood cells, platelets and granulocytes. MPNs can accelerate and transform into acute myeloid leukemia, which is very difficult to treat. The most prevalent MPNs are Polycythemia Vera (PV), Essential Thrombocythemia (ET) and Myelofibrosis (MF), where patients suffer from excessive red blood cell, platelet and scarring of the marrow, respectively. Together PV, ET and MF are five times more prevalent than chronic myeloid leukemia induced by BCR-ABL. The molecular basis of MPNs is represented by acquired mutations that lead to persistent, non-regulated activated of JAK2 and the pathways downstream, STAT5/STAT3/STAT1, MAP-kinase and PI-3'-kinase. 70% of MPN carry the unique acquired somatic JAK2 V617F mutation, where a pseudokinase domain mutation removes inhibitory effects of pseudokinase domain on kinase domain, and also, activates a circuit via the SH2-JH2 linker to the kinase domain. We identified the first conformational event, formation of the aromatic stacking F595-F617 interaction, which triggers an activating circuit to the kinase domain. This circuit is conserved in JAK1 and TYK2, where homologous of V617F activate in a similar manner. The next somatic mutation playing a driver role is represented by W515L/K/A mutations in the thrombopoietin receptor (TpoR), which are present only in ET and MF (3–7%, respectively). We have identified the W515A mutation as being active, and showed that the W515 is part of an inhibitory amphipathic helix, that is required to maintain TpoR inactive. In the case of TpoR W515 mutations, it is JAK2 wild type that is pathologically activated. Importantly, we have also shown that 17 out of the 20 amino acids can activate at TpoR W515, thus a complete sequencing will be necessary. At the end of 2013, 20–35% of ET and MF cases were denoted double negative as they did not carry JAK2 V617F or TpoR mutations. The groups of R. Kralovics and T. Green discovered mutations in the calreticulin gene uniquely in ET and MF (15% and 25%, respectively). We have examined the mechanism of MPN induction by these CALR mutations, where deletions and insertions in exon 9 shift the frame by +1, resulting invariably in the same novel C-terminal positively charged tail, where the natural KDEL endoplasmic reticulum retention sequence is deleted. We have then shown in collaboration with the Kralovics and Vainchenker laboratories that CALR mutants exhibit the ability to activate the TpoR, by binding to its extracellular domain, promoting traffic in an immaturely N-glycosylated form through the secretory pathway to the cell surface. Thus, CALR mutants activate constitutively TpoR, and this pathologic signaling can be inhibited by JAK2 inhibitors. On the other hand, careful signaling experiments in cell lines and treatment with combinations of inhibitors show that JAK2 and MAP-kinase are key for CALR mutant effects, while for JAK2 V617F, JAK2 and PI-3'-kinase were key for transformation. Since mutant CALR proteins activate TpoR signaling from intracellular compartments and from the cell-surface, the next challenge is to assess the different contributions of these localizations to the overall phenotype. In summary, the transforming effects of CALR mutants represents a novel oncogenic mechanism, whereby a mutated chaperone activates its client and induces pathologic traffic.

S15-4
TARGETING STAT5 IN LEUKEMOGENESIS – WHAT'S NEW?

Sexl V.

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JAK-STAT signalling has been identified as one of the core cancer pathways. Mutations in JAK kinases are considered drivers of malignancies, STAT transcription factors have been recognized as important factors for tumor maintenance and progression. As a consequence intensive efforts are ongoing to develop drugs targeting this pathway. One of the key players in haematopoietic cancers is STAT5, which is considered essential for several forms of leukemia. Novel insights in the role of STAT5 in leukemia but also in NK cell mediated tumor surveillance will be discussed.

POSTERS PRESENTATIONS

P-1
NEUROPROTECTIVE EFFECT OF NOVEL IMIDAZOLINE I_2 RECEPTOR LIGANDS IN THE SENESCENCE ACCELERATED MOUSE PRONE 8 (SAMP8)

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Cognitive impairment associated with age is characterized by changes in neurons such as a reduction in the dendrite arborization and dendritic length, jointly with changes in a number of cell membrane receptors. Some of these receptors have a prominent role in brain functions as they are binding sites for the neurotransmitters modulating neurons' responses. Recently, imidazoline I_2 receptors (I_2-IRs) have been suggested as a potential therapeutic target for several CNS disorders such as Alzheimer's disease (AD).

We have recently reported the pharmacological characterization of a new family of (2-imidazoline-4-yl)phosphonates, prepared by a multicomponent microwaved-assisted reaction that fulfil the principles of green chemistry. Radioligand binding studies showed that they displayed an outstanding affinity for I_2-IRs. Moreover, they showed also neuroprotective effects.

In the present work, we present further studies on the neuroprotective properties of these compounds. To this end, 12 months-old SAMP8 female mice were distributed in three groups: Control (n = 10), compound 1 (n = 8), and compound 2 (n = 8). Mice were treated for 4 weeks with compounds 1 and 2 at the dose of 5 mg/kg/day added to drinking water.

Both compounds induced a significant increase in motor activity, measured by open field, and cognitive improvement, through novel object test. These changes were accompanied by a decrease in Tau hyperphosphorylation and amyloid precursor protein (APP) processing. A significant Bax and Calpain protein level diminution, and Bcl-2 increase, indicated modifications in apoptosis process. Finally, qPCR measured gene expression of *IL-6, IL-1*β and *Cxcl10* inflammation markers as well as the oxidative stress markers *Tnf-*α*, iNOS, Hmox1* and *Aldh2* were reduced in the treated mice compared to the control groups. Altogether these results demonstrated the neuroprotective effect of these novel I_2-IRs ligands in a neurodegenerative disease animal model of AD.

Keywords: Neurodegeneration, (2-imidazoline-4-yl)phosphonates, imidazoline receptors I_2, aging.

Acknowledgments: This study was supported by the Ministerio de Economia y´ Competitividad and Fondo Europeo de Desarrollo Regional (MINECO-FEDER) (Projects BFU2015-66030-R and SAF2016-77703-C2-1-R)

P-2
CHRONIC Δ^9-THC ADMINISTRATION LEADS TO FUNCTIONAL 5HT2A RECEPTORS SUPERSENSITIVITY MEDIATED BY INHIBITORY GI/O PROTEINS IN MOUSE BRAIN CORTEX

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Several findings suggest that 5HT2A receptors (5HT2AR) may be involved in the molecular mechanisms responsible for psychotic symptoms. Hallucinogenic drugs acting as 5HT2AR agonists, such as psilocybin, lysergic acid diethylamide (LSD) and dimethoxyiodoamphetamine (DOI) produce psychosis-like symptoms in healthy subjects. On the other hand, cannabis intoxication induces in healthy subjects cognitive alterations similar to those seen in schizophrenia and worsens the symptoms in schizophrenic patients. These data may suggest a link between cannabis and psychosis. Our aim was to investigate the status and functionality of 5HT2AR in brain cortex of adolescent mice chronically treated with Δ^9-THC.

Mice were treated during adolescent period with Δ^9-THC (10 mg/kg daily, 30 days, i.p.) or vehicle. Prepulse inhibition test (PPI) was performed in both groups at basal conditions and after acute DOI injection (0.5 mg/kg, i.p.). Displacement curves of specific [^{3}H]ketanserin binding (2 nM) by DOI (10^{-12} to 10^{-3} M) were also carried out in brain cortex membranes. Moreover, specific stimulation of different Gα proteins by DOI (10^{-5} M) following a [^{35}S]GTPγS binding assay combined with immunoprecipitation was determined.

Chronic Δ^9-THC significantly potentiated ($p < 0.05$) the DOI-induced reduction in the PPI. Additionally, although no changes were found in the density of 5HT2AR, a significant increase ($p < 0.01$) in the affinity of G-protein pre-coupled population of 5HT2AR was observed in brain cortex of Δ^9-THC-treated mice. Moreover, a significant increase ($p < 0.05$) in the 5HT2AR-mediated stimulation of $G_{\alpha i1}$, $G_{\alpha i3}$, $G_{\alpha o}$ and $G_{\alpha z}$ protein subtypes but not of $G_{\alpha s}$ or $G_{\alpha q/11}$ subunits was found in Δ^9-THC-treated mice compared to controls.

Our results show that, in mice brain cortex, chronic exposure to Δ^9-THC during adolescence enhances the 5HT2AR ability to activate specific pathways of inhibitory G_α protein subtypes. This finding suggests that chronic cannabis exposure induces a pro-hallucinogenic 5HT2AR conformation that may facilitate the development of psychosis.

Key words: 5HT2AR, Δ^9-THC, mouse brain.

P-3
IDENTIFICATION OF PHARMACOLOGICAL CHAPERONES FOR THE CELLULAR PRION PROTEIN BY DYNAMIC MASS REDISTRIBUTION SCREENING

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Genetic prion diseases are dominantly inherited neurodegenerative disorders linked to mutations in the *PRNP* gene encoding the prion protein (PrP). One of these mutations, D178N, is likely to be causal[1] and may trigger the conversion of the cellular prion protein (PrP^{C}) into an aggregated form (PrP^{Sc}) that self-propagates in the brain by imposing its abnormal conformation[2]. This concept provides a rationale for tackling aggregation by stabilizing the PrP^{C} monomeric protein precursors by increasing the Gibbs free energy barrier (ΔG) required for the initial misfolding events. This goal could potentially be achieved with ligands of PrP^{C} that act as pharmacological chaperones. In order to identify such compounds, we developed a novel screening method based on Dynamic Mass Redistribution (DMR), a label-free, fully automated biophysical technique, capable of detecting small molecule-protein interactions. Two High Throughput Screening (HTS) campaigns were carried out, first with Prestwick® chemical library (1280 compounds) and second with a diversity library (3888 compounds). The diversity library was designed selecting compounds with an optimal molecular weight to bind with PrP^{C} and which cross the blood-brain barrier.
Active compounds were selected for cherry picking and triaged by Protein Misfolding Cyclic Amplification assay[3], a technique that mimics prion replication in vitro. DMR-based HTS campaign identified 13 active compounds in Prestwick® chemical library which stabilize the conformation of human PrP^{C}. CBG030937 stands out from others for its high affinity. Moreover, it inhibits in a dose-dependent manner the conversion of PrP^{C} into PrP^{Sc}. HTS of diversity library is ongoing.
Our results demonstrated that DMR is a reliable platform for the identification of novel pharmacological chaperones targeting the cellular prion protein.

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P-4
GENDER DIFFERENCES ON THE EFFECTS OF PRENATAL STRESS AND PERINATAL DIET ON THE BEHAVIORAL PROFILE OF AGED RATS

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Epidemiological studies have provided compelling evidence that chronic exposure to stress is associated with significantly increased risk of developing psychiatric disorders in adulthood, such as depression or schizophrenia. Gender is one major variable that appears to confer differential vulnerability to stress. On the other hand, previous studies have reported nutrition as a factor that can modify the stress response and even more, maternal nutrition, could also influence fetal programming. The present work investigates whether chronic perinatal stress (PS) influences the long-term sex-dependent neuropsychological status of offspring and the effects of an early dietary intervention in the dam. To this end, dams were fed either a high fat sugar diet (HFSD) or methyl donor supplementation diet (MDSD). PS procedure did not affect body weight of the offspring. MDSD induced decreases in body weight both in male and female offspring (1 month) that were still present in aged rats. HFSD induced an increase in body weight both in male and female offspring that did not persist in aged rats. In the Porsolt forced swimming test, only young PS males showed depressive-like behavior. This increase in immobility time was reversed by MDSD. In both old male and female rats (20 months), PS induced cognitive impairment in the Morris Water Maze that was reversed by MDSD. HFSD induced cognitive impairment in both control and PS old rats, but there was no additive effect of PS and HFSD. It is proposed here that the diversity of symptoms following PS could arise from programming effects in early brain development and that these effects could be modified by the dietary intake of the dam.

Key words: chronic perinatal stress; methyl donor supplemented diet; high fat sugar diet; cognition.

P-5
EXOCYTOSIS AND MITOCHONDRIAL ULTRASTRUCTURE AND FUNCTION IN CHROMAFFIN CELLS OF THE $SOD1^{G93A}$ MOUSE MODEL OF ALS AT A PRE-DISEASE STAGE

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Paralysis and respiratory failure in patients of amyotrophic lateral sclerosis (ALS) are due to the selective loss of motor neurons. Excess synaptic glutamate release and Ca^{2+}-dependent excitotoxicity has been hypothesized to be involved in ALS pathogenesis. We recently found that in chromaffin cells of a $SOD1^{G93A}$ mouse model of familiar ALS, the fusion pore kinetics and cell excitability are diminished at postnatal age P100-120, when the disease is already established (Calvo-Gallardo et al., Am J Physiol Cell Physiol 2015;308:C1-C19). Here we have investigated whether exocytosis and mitochondrial ultrastructure are altered at early disease stages (P30-P40) in CCs of mouse $SOD1^{G93A}$. Transmission electron microscopy indicated that with respect to wild-type adrenal medullary CCs, mitochondria from $SOD1^{G93A}$ CCs showed the following alterations: 1, more number and small sized; 2, increased mitochondrial intermembrane space; 3, swollen cristae. These ultrastructural changes were accompanied by lower ATP levels and a higher rate of reactive oxygen species (ROS) generation. In spite of the low ATP, the kinetics of the exocytotic fusion pore SOD1G93A CCs have not been dramatically changed, as we observed once the paralysis is already established in P100-120 mice. This suggests that ultrastructural alterations and mitochondrial dysfunction in $SOD1^{G93A}$ occurring at early predisease stages (P30-P40), have lower physiological relevance than in later stages, but these results could generate some clues about the initiation and progression of the disease.

Key words: Amyotrophic Lateral Sclerosis; $SOD1^{G93A}$; mitochondrial ultrastructure; chromaffin cell.

P-6
DOPAMINERGIC DEGENERATION PARTIALLY MODIFIES THE RESPONSE OF THE *LOCUS COERULEUS* TO NOXIOUS STIMULUS

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Pain is a major complaint for up to 85% of Parkinson's disease (PD) patients; however, its pathophysiology is still poorly understood. There is an increasing evidence suggesting that the noradrenergic system, in particular the *locus coeruleus* (LC) nucleus, is directly related to the development of pain. Given that previous studies have demonstrated that PD is associated with dysfunctional neuronal activity of the LC, we examined the implication of this nucleus in pain modulation in a rat model of PD.

The aim of the present study was to investigate if LC response to noxious stimulus was modified in 6-hydroxydopamine (6-OHDA) lesioned rats. To do that, single-unit extracellular recordings were carried out under chloral hydrate anesthesia in sham and 6-OHDA lesioned rats before and after noxious stimulus (ipsilateral and contralateral paw compression). Neuronal activity at baseline and parameters of noxious stimulus-evoked response were analyzed. In a set of experiments L-DOPA effect was also analyzed.

Dopaminergic denervation did not modify the spontaneous activity or the noxious stimulus-evoked discharge parameters (duration, suppression period and frequency) of LC neurons. However, the incidence of the noxious stimulus-evoked response in LC neurons after contralateral paw compression was significantly lower in 6-OHDA lesioned rats compared to that obtained in sham rats. L-DOPA administration (24 mg/kg, plus benserazide 15 mg/kg; intraperitoneal) decreased significantly the incidence of the noxious stimulus-evoked response in LC neurons after paw compression in 6-OHDA lesioned and sham rats.

This work provides evidence that the LC-noradrenergic system is involved in the alteration of pain modulation after dopaminergic denervation. Both, 6-OHDA lesion and L-DOPA exogenous administration modify the incidence of neuronal response to noxious stimulus.

Supported by SAF2016-77758-R (AEI/FEDER, UE) and IT 747-13.
Elena Paredes-Rodriguez has a predoctoral fellowship for the Training of Personal Research from the Basque Government.
Key words: locus coeruleus, 6-OHDA lesion, Parkinson's disease, pain

P-7
BRAIN SPECIFIC CARNITINE PALMITOYLTRANSFERASE 1C (CPT1C) IS INVOLVED IN THE HYPOTHALAMIC REGULATION OF THERMOGENESIS BY ENDOCANNABINOIDS-DEPENDENT MECHANISMS

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Brain-specific carnitine palmitoyltransferase-1C (CPT1C), despite its minimal CPT1 catalytic activity, is implicated in central regulation of food intake and energy homeostasis. CPT1C knock-out (KO) mice show an increased susceptibility to diet-induced obesity and an impaired regulation of food intake in response to leptin or ghrelin. However, it is completely unknown whether CPT1C is involved in hypothalamic regulation of thermogenesis. Here we explore the role of CPT1C in leptin- and diet-induced thermogenesis in brown adipose tissue (BAT) and the mechanisms involved.

CPT1C KO and WT mice were fed a standard or high fat diet (HFD) for 7 days. In WT mice, HFD significantly increased interscapular BAT (iBAT) temperature and thermogenic markers expression, while these parameters were lower in CPT1C KO mice. Also, KO animals evidenced higher body weight gain and adiposity as well as hyperleptinemia, suggesting an earlier onset of leptin resistance. 4 h-Central leptin injection induced a sustained increase of iBAT temperature and upregulation of thermogenic markers in WT mice, whereas these responses were significantly attenuated in KO mice. Phenotype reversion studies identified the mediobasal hypothalamus (MBH) as the area of CPT1C activity responsible for those effects.

Evaluation of the mechanisms involved revealed a dysregulation of leptin signaling markers (i.e. p-AMPK, p-STAT3 and SOCS3) in MBH from CPT1C KO mice compared to WT mice. KO mice also showed increased hypothalamic levels of the endocannabinoid 2-arachidonoylglycerol (2-AG), which is associated to an obesogenic phenotype. In relation to this, proteomic and FRET analysis confirmed the interaction of CPT1C and ABHD6, which is a 2-AG hydrolase involved in the hypothalamic regulation of thermogenesis. Therefore, our data demonstrate that CPT1C is crucial in the hypothalamic regulation of BAT thermogenesis by leptin signaling and 2-AG-dependent mechanisms in the MBH and suggests CPT1C as a potential central target to fight against obesity and its metabolic complications.

P-8
REPEATED ELECTROCONVULSIVE SHOCK INCREASED NEWLY BORN GENERATED CELLS AND NEUROPLASTICITY MARKERS IN THE RAT HIPPOCAMPUS

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Electroconvulsive shock (ECS) is a widely used and effective treatment in psychiatry, especially in major depressive disorders, but the basis for its therapeutic effect is still unknown. Thus, the aim of this study was to evaluate in male Sprague-Dawley rats the differential effects of a single (1 session) or repeated (5 sessions, 1 per day) exposure to ECS (95 mA, 0.6 s, 100 Hz, via earclip electrodes) on hippocampal plasticity markers. Earclip electrodes were also applied to control rats but without electrical current (sham-control). Rats were sacrificed 24 h after the last ECS session by decapitation. The left hippocampus was cryostat-cut and slide-mounted for ascertaining cell genesis markers (i.e., Ki-67 for recent cell proliferation and NeuroD for immature neurons) by immunohistochemistry and the right hippocampus was freshly dissected for studying neuroplasticity markers (i.e., BDNF, FADD and p-FADD) by western blot analyses. The main results showed that repeated ECS (vs. sham-controls) increased hippocampal: (1) cell proliferation (Ki-67 positive: (+464 $\pm$ 34%, $p < 0.001$); (2) mature BDNF protein content (+618 $\pm$ 80%, $p < 0.001$), which is associated with neuronal survival, growth and differentiation; and (3) p-FADD protein content (59 $\pm$ 25%, $p < 0.05$), which is considered a neuroplasticity/anti-apoptotic marker. Taken together, the enhanced cell proliferation, accompanied by the upregulation of BDNF and p-FADD contents, indicate neuroplastic effects of repeated ECS in the rat hippocampus. NeuroD immunohistochemisty is currently being analyzed and ongoing immunofluorescence experiments are being carried out to assess the possible association (co-localization) of these neuroplasticity markers with particular stages of cell genesis. Supported by 'Delegación del Gobierno para el Plan Nacional sobre Drogas' (MSSSI, Grant 2016/002), 'Ramón y Cajal' Program to M.J.G.-F. (MINECO) and RTA-RD16/0017/0010.
Key words: electroshock; rat; hippocampal neurogenesis; neural plasticity

P-9
NOVEL COUMARIN DERIVATIVES AS SELECTIVE MAO-B INHIBITORS: *IN VITRO* AND *IN VIVO* ASSAYS

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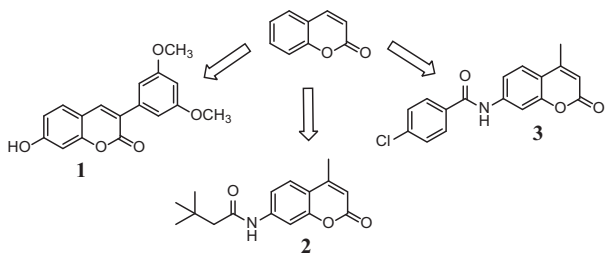
As a consequence of an increasingly aging population, the number of people affected by neurodegenerative disorders is rapidly increasing. Although the etiology of these diseases has not been completely defined, common molecular mechanisms including neuroinflammation, excitotoxicity, oxidative stress, mitochondrial dysfunction and deficits in neurotransmitters have been confirmed and can be targeted therapeutically. Among these disorders, Parkinson's disease (PD) is one of the most prevalent characterized by degeneration of nigrostriatal dopaminergic neurons, aggregation of α-synuclein and motor symptoms[1].
Current dopamine-replacement strategies provide symptomatic relief, however their effectiveness wear off over time and their prolonged use leads to disabling side-effects in PD patients. There is therefore a critical need to develop new drugs and drug targets to protect dopaminergic neurons from degeneration in PD[2].
In this research, natural or synthetic coumarins appropriately substituted have shown interesting MAO inhibitory activity[3]. In recent years, our group have evaluated more than one thousand coumarin derivatives and confirmed that substitution at 3 or 7 position leads to potent



and selective *in vitro* hMAO-B inhibitors[4].
Our next goal is to know the *in vivo* possibilities of these derivatives. Therefore, we have selected three new molecules which exhibited a promising IC_{50} hMAO-B (1: 1.05 μM; 2: 0.246 μM; 3: 0.312 μM) as well as adequate theoretical ADME-related physicochemical/structural parameters (miLogP 1: 3.275; 2: 3.518; 3: 3.935) for *in vivo* administration. Moreover, *in vivo* assays have shown that none of these coumarin derivatives exhibits toxicity in a 100 mg/Kg intraperitoneal administration in mice and all the compounds significantly modify parameters studied: speed, % movement and distance moved.
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Key words: Neurodegenerative disease; Parkinson's disease; coumarin; MAO-B inhibitors

P-10
GENE-BASED ASSOCIATION ANALYSIS OF GENE NETWORKS FOR THE STUDY OF EXTRAPYRAMIDAL SYMPTOMS INDUCED BY ANTIPSYCHOTICS

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Introduction: Acute extrapyramidal symptoms (EPS) induced by antipsychotic (AP) treatment are frequent and serious adverse reactions of AP drugs. Pharmacogenetic studies identified several associations between EPS and genetic markers in candidate genes, however, these associations did not explained the whole variability of the EPS. In the present study, we applied an integrative approach to overcome some of the limitations detected in pharmacogenetic studies of EPS.
Material and Methods: Microarrays data from different models will be integrated to create a protein-protein interaction (PPI) network that will represents the molecular signature of AP induced EPS. The network will be analyzed to identify key components. SNPs in regulatory elements of these genes will be genotyped. Finally, gene-based algorithms will be applied in a discovery sample (243 chronic psychiatric patients) and replicated in an independent sample (109 first episode psychotic patients).
Results: The integration of omics data allowed the construction of a PPI network with 17.868 nodes, of whom 1.368 nodes where common to all models and served as a base for the network analysis. After PPI network was analyzed 28 candidate genes were selected, and 137 SNPs in regulatory elements of these genes were genotyped. Gene based analysis in the discovery sample identified nine genes associated with EPS (*OAS1*, *ADARB2*, *DMD*, *ARPP21*, *KALRN*, *SHROOM3*, *MAST4*, *PINX1*, *ABL1*), and four where consistency replicated in the replication sample (*OAS1*, *DMD*, *PINX1*, *ABL1*).
Discussion: The replication of gene-based analysis in two independent population demonstrated the suitability of the strategy presented here to identify new candidate genes for pharmacogenetic studies of EPS. Genes associated to EPS where involved in cytoskeleton regulation (*DMD*, *ABL1*) and RNA editing (*OAS1*, *ABL1*), and where previously associated to movement disorders, such as parkinsonism and muscular dystrophy, and also to metabolic disorders, such as diabetes mellitus type 1 and rheumatoid arthritis.

P-11
THE EFFECT OF BUSPIRONE ON THE BASAL GANGLIA OUTPUT NUCLEI IS RELATED TO THE DOPAMINERGIC SYSTEM INTEGRITY IN RATS

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The basal ganglia (BG) nuclei form an integrative subcortical network directly related with the motor performance. The pathophysiology of Parkinson's disease (PD) and L-DOPA-induced dyskinesia (LID) is associated with dysfunctional neuronal activity and changes in the oscillatory activity and synchronization of the cortical-BG-thalamus circuits. Serotonin-based therapies have promising results in the treatment of PD and LID. Interestingly, buspirone, a partial agonist of 5-HT_{1A} receptors, has shown antidyskinetic properties in humans and different animal models of PD, but the mechanisms involved in this therapeutic effect are not fully understood.
The aim of the present study was to investigate the effect of buspirone on the neuronal activity of the BG output nuclei; the substantia nigra *pars reticula* (SNr) and entopeduncular nucleus (EP). To do that, single-unit extracellular recordings were carried under urethane anaesthesia in control and 6-hydroxydopamine (6-OHDA) lesioned rats. Neuronal activity parameters, oscillatory activity and synchronization between these nuclei and the motor cortex were analyzed.
In the EP, local (0.25–2 nmol) and systemic (0.625–5 mg/kg i.v.) administration of buspirone consistently reduced neuron activity in control and 6-OHDA lesioned rats with a moderate denervation (between 60% and 80% of optical TH-immunoreactivity density). However, the systemic effect was absent in 6-OHDA lesioned rats with complete nigrostriatal denervation. The inhibitory effect was rapidly reversed by an antagonist of the 5-HT_{1A} receptors, WAY-100635 (1 mg/kg i.v.). In the SNr, local application of buspirone (0.25–2 nmol) also produced an inhibitory effect in lesioned, partially lesioned and control rats, but not when systemically (0.625–5 mg/kg i.v.) applied. Furthermore, the synchronization and low-frequency oscillatory activity in both nuclei or in the motor cortex remained

unaltered after drug application in 6-OHDA lesioned and control situations. Altogether, these results indicate that at least in the EP, buspirone produces an inhibitory effect, which is mediated by a complex mechanism that involves DA system as we had previously shown in the activity of the subthalamic nucleus.

Supported by SAF2016-77758-R (AEI/FEDER, UE) and IT747-13
Sergio Vegas-Suárez has a predoctoral fellowship for the Training of Personal Research (UPV/EHU)
Key words: 5-HT_{1A} receptors, 6-OHDA lesion, Electrophysiology recording, Oscillatory activity

P-12
EFFECT OF ABCB1 C3435T POLYMORPHISM ON PHARMACOKINETICS OF ANTIPSYCHOTICS AND ANTIDEPRESSANTS

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Background: P-glycoprotein, encoded by *ABCB1* gene, is an ATP-dependent drug efflux pump which export substances outside the cell. The T allele of C3435T polymorphism located in this gene is frequently associated with a slower metabolizer phenotype, so homozygous T/T individuals could show higher plasma levels of drugs. The aim of the present study was to evaluate the effect of this polymorphism on pharmacokinetics (PK) of 4 antipsychotics (olanzapine, quetiapine, risperidone and aripiprazole) and 3 antidepressants (trazodone, sertraline and citalopram).
Material and Methods: Four hundred and forty-five healthy volunteers receiving a single oral dose of the mentioned drugs were genotyped by Real Time PCR for C3435T SNP. Plasma concentrations of the drugs were measured by high performance liquid chromatography with tandem mass spectrometry detection (LC/MS/MS). PK parameters were calculated using non-compartmental analysis. Differences in PK between individuals were analyzed by a parametric univariate analysis, ANOVA or T-test (to compare T allele carriers vs non-carriers).
Results: As shown in the following table, C3435T polymorphism had no significant effect on PK of olanzapine, quetiapine, sertraline or citalopram. As expected, T allele carriers showed a higher AUC and $T_{1/2}$ for aripiprazole. Surprisingly, Cl of risperidone and trazodone was higher in T allele carriers.

	ABCB1 genotype					
	C/C			C/T + T/T		
Drug (*n*)	AUC (ng·h/ml)	Cl/F (ml/h·kg)	$T_{1/2}$ (h)	AUC (ng·h/ml)	Cl/F (ml/h·kg)	$T_{1/2}$ (h)
Olanzapine (61)	300.1	0.26	32.8	292.8	0.25	32.6
Quetiapine (79)	172.0	2.37	4.0	200.0	2.14	3.91
Risperidone (70)	64.1	0.64	6.9	34.3*	1.1*	3.6
Aripiprazole (148)	1514.8	66.62	47.1	1675.1*	62.12	54.4*
Trazodone (36)	9023.7	0.18	9.01	9876.1†	0.16†	9.46†
Sertraline (34)	924.8	1.98	26.05	794.8	1.93	24.24
Citalopram (17)	1142.5	0.38	32.8	1350.9	0.37	34.1

*$p < 0.05$ comparing C/T plus T/T vs C/C.

†$p < 0.05$ comparing T/T vs C/T and C/C.

Conclusions: ABCB1 C3435T polymorphism can affect the pharmacokinetics in different ways, increasing the elimination in some drugs and reducing it in others.

P-13
ENVIRONMENTAL ENRICHMENT IN THE ABSENCE OF PHYSICAL EXERCISE PRODUCES BENEFICIAL BEHAVIOURAL EFFECTS AND ANTI-OXIDATIVE EFFECTS IN RATS

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Early sensory experience is crucial on brain development and in the capacity for learning in adult life. It has been shown that both environmental enrichment (EE) and physical exercise can produce a variety of long term effects at neuroanatomical, neurochemical, and behavioural levels in different species. In a recent study with rats (Chamizo et al., 2016) the animals had been reared in either EE plus wheel running or control conditions. The results revealed beneficial effects of both EE and wheel running: EE rats learned more and showed less anxiety than controls. However, an undeniable weakness of this previous study is that there is not a clear distinction between EE and physical exercise (i.e. voluntary wheel-running). The present work, with male rats, addresses this problem. After weaning, the rats were housed in pairs in enriched or in standard cages, for two and a half months in the absence of wheel running. When the rats were approximately 90 days old they were tested in the Morris pool (as in Rodríguez et al., 2010). EE rats reached the platform faster than control animals and performed better on a subsequent test trial without platform with the geometry cue individually presented. Behavioural anxiety was also measured by means of thigmotaxis. Then, the rats were sacrificed and the hippocampus was extracted (Marmol et al., 2015). Overall, the measures taken in the hippocampus revealed that EE rats showed higher values for antioxidant measures (total radical antioxidant parameter and catalase levels) and lower values for oxidative stress parameters (TBARS, protein oxidation activity and superoxide anion levels) than control animals. In summary, our results reveal clear beneficial effects of EE in the absence of wheel running, both behaviourally and when subsequently testing oxidative stress parameters in the rat hippocampus.

Keywords: Environmental enrichment, Geometry learning, Landmark learning, Morris water maze, Thigmotaxis, Hippocampus of rats, Antioxidative measurements

P-14
SYNTHETIC SULFATIDE-LIKE SULFOGLYCOLIPIDS AS POTENTIAL NEUROPROTECTORS IN BRAIN DAMAGE

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Glycolipids are membrane lipids with an attached carbohydrate. Located predominantly at the outer leaflet of the plasma membrane, they contribute to cell recognition and membrane microdomain formation during differentiation, development, and immune reactions. Sulfation, phosphorylation and sialylation confer negative charge on glycolipids and such structures form a part of determinants for molecular interactions. We have synthesized sulfoglycolipid IG20 that has a structure very similar to sulfatides, a class of sulfated galactosylceramidase that are synthesized mainly in oligodendrocytes and belong to the great family of sphingosine derived sphingolipids. We have found that IG20 at 0.3–10 μM afforded neuroprotection in rat hippocampal slices stressed with veratridine, glutamate, or with oxygen plus glucose deprivation followed by reoxygenation (OGD/reox). The excess production of reactive oxygen species (ROS) elicited by those stressors was mitigated by IG20 that also restored the depressed levels of GSH and ATP and counteracted the augmented iNOS expression. Additionally, the

neuroprotecting effects of IG20 were prevented in the presence of inhibitors of the signaling pathways JAK2/STAT3, MEK/ERK 1/2, and PI3k/Akt, consistent with its ability to augment the phosphorylation of JAK2, ERK1/2, and Akt. It seems therefore that the activation of phase II response and the Nrf2/ARE pathway are mediating the antioxidant and anti-inflammatory as well as the ensuing neuroprotective actions of IG20. We are now testing how relevant is the presence of the sulfate residue of IG20 for its properties here reported. To achieve this we have synthesized other three sulfatide-like glycolipids lacking sulfate or sulfoglycolipid lacking a double bond.

P-15
HDAC5 AND SIRT2: EPIGENETIC TARGETS FOR ANTIDEPRESSANT THERAPY

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Background and purpose: Antidepressant action has been linked to increased synaptic plasticity in the prefrontal cortex (PFC), in which epigenetic mechanism could play a key role. Interestingly, we have recently identified the histone deacetylases enzymes HDAC5 and SIRT2 regulated by stress and antidepressant treatment. Here, we have studied comparatively, *in vivo* in the mouse PFC and *in vitro* in SH-SY5Y cells, the implication of these epigenetic targets in the molecular mechanisms of antidepressants.
Experimental approach: Mice (C57BL6, 8 weeks) were treated (21 days, i.p.) with imipramine (10 mg/kg), fluoxetine (15 mg/Kg) and reboxetine (15 mg/kg) or saline. In addition, SH-SY5Y cell cultures were incubated with these antidepressants (at 10 μM, 2 and 24 h) and with a novel selective HDAC5 inhibitor (MC3822, 5 μM, 24 h) presented here or a SIRT2 inhibitor (33i, 5 μM, 2 and 24 h). Epigenetic and synaptic plasticity markers where studied.
Key results: our study shows that antidepressants increased both *in vivo* and *in vitro* expression of the brain derived neurotrophic factor (BDNF), the vesicular glutamate transporter 1 (VGLUT1), and the acetylated histones 3 (AcH3) and 4 (AcH4). In addition, imipramine and reboxetine increased the phosphorylated form of HDAC5 (P-HDAC5), mediating cytoplasmic export of this enzyme. On the other hand, SIRT2 was downregulated by all antidepressants. Finally, specific inhibition of HDAC5 and SIRT2 with the compounds MC3822 and 33i respectively increased neuroplasticity markers *in vitro*.
Conclusion and implications: Here we present two epigenetic mechanisms, nucleocytoplasmic export of HDAC5 and SIRT2 downregulation, by which antidepressants could enhance synaptic plasticity markers linked to antidepressant action. Thus, it is highlighted the therapeutic potential of these epigenetic targets for major depression.

P-16
RESVERATROL PROTECTS C57BL/6J AGED MICE IN FRONT OF LPS ACUTE INFLAMMATORY INSULT

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Introduction: Neuroinflammation and oxidative stress (OS) are believed to play a pivotal role in development of cognitive impairment related with aging. Although there is no controversy that increase in OS in CNS is a negative event, the possible pleiotropic effect of proinflammatory cytokines such as IL-6 and TNF-α is unclear. There is evidence in both directions, positive and negative, because they have a pleiotropic action as neuroprotective factors modulating synaptic plasticity, cell death and excitotoxicity. Lipopolysaccharide (LPS) has been widely used to study the acute inflammatory and OS response in brain, inducing proinflammatory cytokines and oxidative stress markers increases. Resveratrol is a polyphenolic compound found in grapes and other plants; it exhibits antioxidant, anti-inflammatory, anti-aging and although the protective role of resveratrol in the CNS is well established, the mechanisms of these effects are not fully understood.
Aim: We evaluate the effects of resveratrol on the inflammatory response and antioxidant defences in aged mice after inflammatory insult by LPS.
Methods: Males C57BL/6J mice 22 months-old were distributed in four groups and following 8 weeks of treatment: Control, Resveratrol, LPS, and LPS + Resveratrol. Control and LPS mice had access to standard chow and mice with resveratrol diet (control and LPS) had access to standard chow diet enriched with the polyphenol (1 g/Kg, w/w). LPS mice were injected intraperitoneally (100 μg/Kg, i.p) 3 h before sacrifice. In order to evaluate the effect of LPS we performed Western blot and Real Time PCR. Specific antibodies and primers were used to determinate proteins and genes of interest.
Results and conclusions: The cytokines *IL-6*, *TNF*-α, *IL1*-β and *Cxcl10* hippocampal gene expression were significantly increased in LPS-RV group. In the same way, *iNOS*, *Hmox1*, *Aldh2* and *Cox2* gene expression, antioxidant markers, were increased in LPS-RV group in reference to other groups. On the basis of our results the conclusions point to a possible increase of resilience of aged brain induced by resveratrol against LPS insult that can be useful in infection or any other situation that requires an activation of inflammatory defenses to fight against a possible harmful stimuli.
Acknowledgments: This study was supported by the Ministerio de Economia y Competitividad and Fondo Europeo de Desarrollo Regional (MINECO-FEDER) (SAF2016-77703-C2-1-R). Verónica Palomera-Avalos PhD scholarship (V/2014/216) Universidad de Guadalajara. México.
Keywords: Resveratrol, inflammation, hippocampus, oxidative stress, aging

P-17
PHARMACOLOGICAL MODULATION OF CALCIUM SIGNALLING AND EXOCYTOSIS IN CHROMAFFIN CELLS FROM C57BL6/J MICE

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During cell activation, the variations in the concentrations of ionised Ca^{2+} in the cytosol ($[Ca^{2+}]_{c)}$ determine the efficacy of pre-exocytotic and exocytotic steps during the process of hormone and neurotransmitter release. In adrenal chromaffin cells those responses are configurated by a functional triad formed by voltage-activated calcium channels (VACCs), the endoplasmic reticulum (ER), and mitochondria. The transfer of Ca^{2+} between these three compartments fine tunes the $[Ca^{2+}]_c$ and catecholamine secretory responses during cell activation (Garcia et al., 2006, Physiol Rev. This Ca^{2+} circulation has been best studied in bovine and rat CCs. Scarce information is available in CCs from the mouse strain C57BL6/J (MCCs), that is being widely used as genetic carrier of mutations occurring in human disease. Therefore, here we have investigated how various drugs and manipulations that target the elements of the functional triad of MCCs, affect the quantal release of catecholamine monitored online at the single-cell level, with a carbon fibre microelectrode and amperometry. We have found that stimulation with physiological acetylcholine (ACh) triggers bursts of single-vesicle secretory spikes that have a kinetics considerable faster than the spikes evoked by other secretagogues. This could be due to differences in Ca^{2+} handling because when ACh was used in presence of mitochondrial protonophore FCCP that prevents the clearance of $[Ca^{2+}]_c$, the spike exhibited a slower kinetics. Also, the blockade by compound ITH1266 of the mitochondrial Na^{+}-Ca^{2+} exchanger (mNCX) potentiated the inactivated ACh secretory response.

Furthermore, ER Ca^{2+} depletors such as SERCA blockers thapsigargin and cyclopiazonic acid, augmented secretion elicited by repeated ACh brief pulses. Hence, we conclude that ER-mitochondria Ca^{2+} exchange contributes to the regulation of the fine kinetics of exocytosis. These data create a frame for the extrapolation of the functional triad concept to transgenic mouse model of disease. We are presently testing this concept in transgenic mouse models of Alzheimer's disease, amyotrophic lateral sclerosis, and Huntington's disease.

P-18
BLACK SWISS MICE: MOLECULAR ALTERATIONS RELATED TO MANIA-LIKE BEHAVIOUR

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Mania has long been recognized as aberrant behaviour indicative of mental illness which includes a variety of complex and multifaceted symptoms[1]. Even though the aetiology of the disease remains unwell defined, abnormal glutamatergic neurotransmission and dysregulation of GSK3β kinase, have been implicated in human mania[2]. Recent work described that the Black Swiss mice from Taconic Farms (BStac) recreate various features of that observed in mania[3]. Also and in contrast with a putative control, Black Swiss mice from Charles River (BScr), BStac mice did not developed depressive-like behaviours after exposure to multiple forms of stress[4]. The goal of the present study was explore the potential molecular alterations in the manic BStac comparing to BScr mice. To this end we evaluate the levels of *N*-methyl-D-aspartate receptor (NMDAR) subunits and expression and phosphorylation levels of the Akt/GSK3β signalling pathway. The molecular exploration shows that neither NMDAR subunits not total GSK3β levels in the frontal cortex differ in both strain of mice. However, phosphorylation analysis revealed marked differences in the regulation of GSK3β, Then, we observed that Akt-mediated inhibitory phosphorylation of GSK3β at serine 9 (P-S9) decreased in BStac in comparison to BScr mice. Also, the activating phosphorylation of GSK3β at tyrosine 216 (P-Y216), was increased in BStac. Thus, our results indicate that BStac mice have manic-like behaviours typical of increased GSK3β function and inhibition of GSK3β attenuated the expression of pro-manic mechanisms allowing the expression of depressive-like behaviours. (Supported by Plan Nacional de Drogas 2014-012 and MINECO, SAF-2015-65420R)

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P-19
COMPUTATIONAL APPROACHES FOR THE UNDERSTANDING OF AGONIST EFFICACY IN STRUCTURALLY AND FUNCTIONALLY COMPLEX GPCRs

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The knowledge of GPCR activation mechanisms and the concomitant concept of agonist efficacy have evolved in the last years to encompass the avalanche of new findings and increasing complexity in pharmacology. A giant conceptual leap has been made from the original description of efficacy, as the property of agonists that produces a pharmacologic effect by the formation of a binary ligand-receptor complex, to the present description, which includes multiple receptor pathways and multiple receptor binding sites. Moreover, the inherently complex properties of functional selectivity and allosterism are further complicated by the molecular interactions between the receptor and the diversity of molecules present in the membrane including water, ions, lipids and other receptors (homo and hetero-dimerization).

In an effort to explain and quantify agonist efficacy our group is performing a multiple approach to the understanding of receptor activation mechanisms which includes GPCR crystal structure analyses[1;2], molecular dynamics simulations[3–5] and mathematical modeling[6–8]. In addition to published data[1–8], new results on the stabilization of either the inactive or the active states of the β_2 adrenergic receptor depending on the charge distribution of membrane lipids, the relationship between functional selectivity and partial agonism for the $5HT_{2A}$ receptor and a new mathematical model for receptor heterodimerization are presented. Thus, we are fully convinced that the combination of molecular and mathematical approaches in an integrated framework may help to clarify the intricacies of the efficacy concept and to provide a mechanistic understanding of receptor function.

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Acknowledgements:

This work was supported by MINECO grants NEURON PCIN-2013-018-C03-02 and SAF2014-58396-R and Fundació La Marató de TV3 grant 110230.

P-20
α_{2A}- AND α_{2C}-ADRENOCEPTORS AS SIGNIFICANT TARGETS FOR DOPAMINE AND DOPAMINE RECEPTOR LIGANDS

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It has long been recognized that several brain areas show a mismatch between the innervation by the catecholamines norepinephrine and dopamine and the density of their canonical receptors. Particularly notorious is the low norepinephrine innervation and relative high density of Gi/o-coupled α_{2A} and α_{2C} adrenoceptors in the striatum, major target for the ascending dopaminergic system. This prompted the possibility that dopamine could provide an effective endogenous ligand for both adrenoceptors, but previous studies were inconclusive. In the present study, we addressed the same question by analyzing the ability of dopamine and several putative selective dopamine receptor ligands to bind and activate α_{2A} and α_{2C} adrenoceptors in transfected mammalian cells using sensitive bioluminescent resonance energy transfer-based techniques that detect ligand-dependent activation of specific G proteins or activation of their effector adenylyl cyclase. Furthermore, we also analyzed their ability to bind to α_2 adrenoceptors in cortical

tissue, which predominantly expresses α_{2A} adrenoceptors, and striatal tissue, which expresses both α_{2A} and α_{2C} adrenoceptors. Binding events were further studied with computer modeling of ligand docking. The results not only provide conclusive evidence for α_{2A} and α_{2C} adrenoceptors being both norepinephrine and dopamine receptors, but also for being common targets for compounds previously characterized as Gi/o-coupled dopamine receptor ligands. Furthermore, the present study demonstrates the Gαi/o protein subtype is fundamental in the determination of the unique pharmacological profile of Gi/o-coupled catecholamine receptor ligands.

P-21
INTERLEUKIN-1 RECEPTOR ANTAGONIST (IL-1RA) AND BRAIN ENDOCANNABINOIDS CROSSTALK TO CONTROL NEUROGENESIS

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Crosstalk between neuroimmune networks and the brain endocannabinoid (eCB) system contribute to the maintenance of neurogenesis. Moreover, the eCB system directs cell fate specification of neural stem cells (NSC) in the central nervous system (CNS). We have previously shown that the activation of CB1 and CB2 cannabinoid receptors suppressed chronic inflammatory responses through the attenuation of pro-inflammatory mediators such as interleukin-1β (IL-1β) by increasing the expression of IL-1 receptor antagonist (IL-1RA), an endogenous antagonist for the actions of IL-1 in the CNS. Endogenous IL-1RA mediates the neuroprotective and anti-inflammatory actions of CBs in primary neurons and glia. These effects appear to be mediated by both CB1 and CB2 receptors. CB-induced IL-RA release may negatively regulate IL-1 actions in the brain, via IL-1RA blocking the IL-1 receptor (IL-1RI), after inflammatory or excitotoxic insults. Interestingly, receptors for cannabinoids (CB1 and CB2 receptors) and interleukin-1 are co-expressed in NSC. In order to further explore the effects of IL-1RA on endocannabinoid signalling in NSC the levels of the endocannabinoids 2-arachidonoylglycerol (2-AG), 1-AG and anandamide (AEA) were detected using liquid chromatography-mass spectrometry (LC-MS) on a Waters Acquity H-Class UPLC coupled to TQSmicro triple quadrupole mass spectrometer following IL-1RA treatment. Treatment with IL-1RA caused marked increases in the levels of AEA (approximately three-fold) and 2-AG (approximately three-fold) in NSC nuclear extracts respectively, compared to the control group. Whereas, in supernatants the levels of 2-AG and 1-AG and AEA were similar to that obtained in the control group. In this study we show for the first time that acute administration with IL-1RA significantly increases levels of AEA and 2-AG in NSC. Thus it may be hypothesised that IL-1RA increases proliferation by increasing the levels of endocannabinoids, which acts via CB1 or CB2 receptors. These results provide crucial new insights into the effects of IL-1RA in regulating NSC proliferation and the pathways involved, and highlight the therapeutic potential of their interplay with eCB signalling in brain repair.

P-22
SEROTONIN 5-HT$_3$ RECEPTOR ANTAGONISM POTENTIATES THE ANTIDEPRESSANT ACTIVITY OF CITALOPRAM

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Selective serotonin reuptake inhibitors (SSRIs) are able to regulate the activity of different neurotransmission pathways involved in depression. The administration of the SSRI citalopram increases serotonin release in the locus coeruleus (LC), contributing to modulate the function of this noradrenergic nucleus. It has been shown that local 5-HT$_3$ receptor activation in LC decreases noradrenaline (NA) release in prefrontal cortex (PFC). In this context, the blockade of 5-HT$_3$ receptors in co-administration with SSRIs has been proposed to avoid countertherapeutic effects of SSRIs in the early stages of treatment.

In the present study, the role of 5-HT$_3$ receptors in the effect exerted by citalopram was investigated by dual-probe microdialysis in rats. Systemic administration of the 5-HT$_3$ agonist SR57227 (10 mg/kg i.p.) increased NA in LC (Emax = 200 ± 27%; $p < 0.0001$) and PFC (Emax = 133 ± 2%; $p < 0.05$). Such cortical NA increase was enhanced in the presence in LC of the 5-HT$_3$ antagonist Y25130 (50 μM) (Emax = 296 ± 41%; $p < 0.0001$). Local administration in PFC of SR57227 (1–100 μM) increased NA in the area (Emax = 815 ± 148%; $p < 0.0001$). This effect was attenuated by the cortical presence of Y25130 (Emax = 366 ± 58%; $p < 0.0001$), which is compatible with the existence of cortical 5-HT$_3$ receptors involved in NA release.

Systemic citalopram (10 mg/kg i.p.) increased NA in LC (Emax = 185 ± 11%; $p < 0.01$) and decreased it in PFC (Emax = -35 ± 7%; $p < 0.001$). In the presence in LC of the 5-HT$_3$ antagonist Y25130, both effects were blocked. When Y25130 (10 mg/kg i.p.) was pre-administered systemically with citalopram, the increment of NA in LC was blocked, whereas in PFC, NA switched from an inhibition (Emax = -40 ± 5%; $p < 0.001$) to an increase (Emax = 117 ± 8%; $p > 0.05$).

These results show that the addition of a 5-HT$_3$ receptor antagonist is able to reverse the decrease of NA release in PFC exerted by systemic citalopram. Therefore, co-administration of a 5-HT$_3$ receptor antagonist with SSRIs could represent a strategy to improve antidepressant response.

Key words: locus coeruleus; citalopram; noradrenaline; 5-HT3

P-23
SYNTHESIS OF NEW 1-SUBSTITUTED ISOQUINOLINES WITH POTENTIAL ANTI-PARKINSON ACTIVITY

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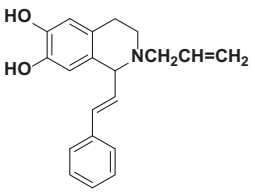
Introduction/Objectives: Dopaminergic ligands can bind to D$_1$-like (D$_1$ and D$_5$) and D$_2$-like (D$_2$, D$_3$ and D$_4$) dopamine receptors (DR) to "restore" the dopaminergic pathway. Agonists can be useful in the treatment of Parkinson's disease. Tetrahydroisoquinolines (THIQs) display important pharmacological activities including DR binding. Therefore, the aim of this study was to obtain new 1-substituted THIQs with dopaminergic activity.

Materials and Methods: (*E*)-1-Styryl-THIQs and (*E*)-1-(propenyl)-THIQs were synthesized *via* Bischler–Napieralski cyclization, and tested *in vitro* for their affinity towards DR in rat striatum. Functional assays to agonist activity was performed by measuring inhibition of forskolin-stimulated cyclic AMP production in CHO-K1 cells stably expressing human D$_2$ receptors. Cytotoxicity studies were carried out in both human neutrophils and HUVEC by MTT assay. Molecular modelling studies (MM) on DRs were performed to determine ligand/receptor complex interactions.

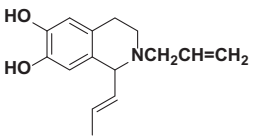
Results: 1-Substituted IQs were synthesized bearing 1-styryl or 1-propene substituent. Catecholic IQs displayed affinity towards D$_1$-DR and D$_2$-DR at μM and nM concentrations, respectively. *N*-methyl or *N*-allyl groups improved considerably the affinity towards D$_2$-like DR. The most active compounds, 1e and 2e, also showed high selectivity (K_i = 41 nM and 18 nM; K_i D$_1$/D$_2$ ratio = 147 and 95, respectively). The cAMP assays indicated that 1e and 2e behaved as full agonist (EC$_{50}$ = 500 nM and 555 nM, respectively) with maximal efficacy

values similar to quinpirole at 10 μM. None of these THIQs displayed relevant cytotoxicity in human cells. In agreement with the experimental data, MM studies on DRs revealed stronger molecular interactions with D_2-DR than with D_1-DR.

Conclusions: The catechol group and *N*-substitution at the IQ nucleus improved the affinity towards D_2-DR. Therefore, 1e and 2e, are potential candidates to be used in the treatment of PD.



1-(Styryl)-THIQ **1e**
(*series 1*)



1-(Prop-1-en-yl)-THIQ **2e**
(*series 2*)

Key words: Tetrahydroisoquinolines; synthesis; dopamine receptors; Parkinson disease

P-24
OPTICAL CONTROL OF PAIN IN VIVO WITH A PHOTOACTIVE MGLU$_5$ RECEPTOR NEGATIVE ALLOSTERIC MODULATOR

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Optopharmacology is a very promising approach to manipulate physiological processes with high spatiotemporal resolution. Indeed, the use of light-activatable drugs (i.e. caged-compounds) targeting G protein-coupled receptors (GPCRs) in space and time will provide extraordinary pharmacological precision. Interestingly, experimental evidences shown that the metabotropic glutamate type 5 receptor (mGlu$_5$ receptor) is widely expressed through the pain neuraxis, thus playing a key role in pain transmission. Importantly, the specific mGlu$_5$ receptor anatomical location within the pain neuraxis determines its pro- or antinociceptive action, thus the precise spatial manipulation of these receptors will determine the ultimate pain sensation. Thereby, selective mGlu$_5$ receptor negative allosteric modulators (NAMs) have consistently shown analgesic activity in experimental animal models of inflammatory pain. Accordingly, we synthesized a caged derivative compound, namely the JF-NP-26, using the chemical structure of raseglurant, an mGlu$_5$ receptor NAM, as a molecular scaffold. JF-NP-26 can be photo-activated by violet light (405 nm) irradiation to release active raseglurant. We evaluated the light-dependent ability of JF-NP-26 to preclude the mGlu$_5$ receptor function both in cultured cells and striatal neurons. Importantly, JF-NP-26 showed antinociceptive activity in an animal model of pain upon irradiation both at the peripheral (i.e. hind paw) and central level (i.e. thalamus) but did not show this specific effect when irradiated in unaffected areas (i.e. contralateral hind paw) or brain areas not involved in pain sensation (i.e. Striatum). Overall, these results demonstrated for the first time the usefulness of using light in combination with photoactive drugs for pain treatment.

Acknowledgements: This work was supported by MINECO/ISCIII (SAF2014-55700-P and PIE14/00034), the Catalan government (2014 SGR 1054), ICREA (ICREA Academia-2010), Fundació la Marató de TV3 (Grant 20152031) and IWT (SBO-140028).

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P-25
THE USE OF LICOCHALCONE A AS PRE-EMPTIVE PHARMACOLOGICAL INHIBITION OF JNK1, LEADS TO NEURONAL PROTECTION AGAINST EXCITOTOXIC INSULTS DERIVED OF KAINIC ACID

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The mitogen-activated protein kinase family (MAPK) is an important group of enzymes involved in cellular responses to diverse external stimuli. One of the members of this family is the c-Jun-N-terminal kinase (JNK). In particular, JNK1 is involved in neurodegenerative diseases like epilepsy and Alzheimer's disease. Kainic acid (KA) is a chemoconvulsant that mimics temporal lobule epilepsy (TLE) in humans. This molecule triggers neuronal death and glial reactivity, mechanisms modulated by the JNK1.

The root of *Glycyrrhiza inflata* is known to be a source for characteristic phenolic compounds that have various pharmacological applications. One of them, Licochalcone A (Lic-A), which inhibits the activity of the JNK1 isoform, is a suitable compound to prevent the neuronal degeneration induced by epileptic seizures. The aim of this study was to analyse the effect of Lic-A as a new approach for the treatment of epilepsy. Our results showed that an intraperitoneal pretreatment of Lic-A, previous to the KA administration caused a reduction in the convulsive pattern. Therefore, it was confirmed that Lic-A reduced the phosphorylation levels of the JNK. Also, several biomarkers were evaluated and it was observed that the neuronal degeneration associated with the KA administration completely disappeared, along with a significant reduction of apoptotic signals (BAX, BIM, calpain and caspase 3 activity), glial reactivity and cellular stress agents release like NO and TNFα. Additionally, the response of multiple proteins related to survival responses like AKT and CREB, was evaluated, and the data provided that Lic-A was able to reduce the activation of these pathways.

In conclusion, the effects of Lic-A give neuroprotection against the damage derived of KA administration. Therefore, this molecule could be a new potential drug for the treatment of epilepsy.

Keywords: Licochalcone A; epilepsy; kainic acid; JNK; apoptosis; neuroinflammation

P-26
IN VIVO PHOTOMODULATION OF GABA AND GLYCINE RECEPTOR CHANNELS

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The photochromic compound, UR-DW290, composed of diazepam and azobenzene groups, was studied *in vivo* and *in vitro*.
Whole-cell patch-clamp recordings were used to study ionic currents of GABA and glycine receptors and *Danio rerio* larvae (7 and 8 days postfertilization) swimming behaviour was studied and analysed using a ViewPoint ZebraLab system.
Under visible light, currents mediated by GABA receptors formed by $\alpha1/\beta2/\gamma2$ subunits were weakly inhibited by 50 μM UR-DW290 (on ≈15% and ≈5% at GABA 5 and 300 μM, respectively), while at UV illumination the inhibition was entirely absent. GlyRs were more effectively and subunit-specifically inhibited by UR-DW290. On mammalian or zebrafish α2 GlyRs, at visible light, UR-DW290 provoked inhibition of currents induced by 20 μM glycine on 38 ± 7% and 32 ± 3%, respectively. It caused even much stronger suppression upon UV illumination: on 73 ± 6% and 64 ± 2% for mammalian or zebrafish α2 GlyRs, respectively. Action on α1 GlyRs exhibited similar tendency, although with less efficiency. With increase of glycine concentration inhibitory power of UR-DW290 decreased, suggesting a competitive mechanism of the antagonist action. The behavioural analysis of zebrafish larvae (7 and 8 days post fertilization) showed an excitatory effect of UR-DW290. Animals treated with UR-DW290 (100 μM) experienced higher activity behaviour during the relaxation period (20 min in absence of light stimuli) in comparison to controls and vehicle animals. Notwithstanding their permanent excitation state, under 365 nm light animals treated with UR-DW290 evoked an overreaction to light changes which was reduced to control animal levels during blue light (455 nm) application.
We found significant differences for different behavioural parameters between UR-DW290 treated groups and controls including total animal activity and swimming distance, startle responses to light changing conditions and habituation time. We observed how in the presence of UR-DW290 animal responses to light stimuli can be tuned in a dose dependent way and how swimming behaviour, such as exploratory capacities, can be triggered.
Key words: photopharmacology; behaviour; GABA; glycine

P-27
THE MITOCHONDRIAL SUBTYPE-2 ANGIOTENSIN II RECEPTOR EXPRESSION DECLINES WITH AGEING, AND MODULATES THE CATECHOLAMINE SECRETION IN CHROMAFFIN CELLS

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Renin-Angiotensin-Aldosterone System regulates cardiovascular and renal function, where angiotensin II is the main final effector molecule. Renin is released from the juxtaglomerular cells in response to urine osmolarity. Moreover, adrenaline secreted in response to sympathetic stimulation from chromaffin cells (CCs) can also release renin throughout ß$_1$-adrenergic receptor. Angiotensin actions are mediated by angiotensin type-1 (AT_1) and -2 (AT_2) receptors, which are localized in cytoplasmic membrane of different cell types. In addition, the AT_2 receptors could be also located in mitochondrial membrane (mAT_2), releasing nitric oxide (NO), and regulating the mitochondrial respiration. Interestingly, the major expression of mAT_2 occurs during foetal life and it decline with ageing, throughout the maturation of the adrenal gland innervation by the splanchnic nerve. The importance of the absence of the innervation in foetal life is that the main physiological stimulus is the hypoxia. The sense is that the adrenaline released by hypoxic stimulus protects the newborn from the tissue damage by means of changes in vascular perfusion and pulmonary maturation. In spite of the importance, it remains already unexplored in CCs, and we have wanted to research it. In our study, have observed that the embryo-CCs shows with respect to adult: i) three-folds increased adrenaline secretion; ii) overall AT_1 expression does not change, but AT_2 receptor is three-folds decreased; iii) AT_2 receptor is localized in mitochondria, but not in adult; iv) mitochondrial depolarization induced by FCCP rose in six-folds the secretion; v) mAT_2 receptor stimulation is associated with NO-releasing in embryo-CCs. We conclude that mAT_2 receptor regulates adrenaline secretion in embryo-CCs by means of NO-pathway. These results are important to understanding the physiological or pathological hypoxic effect in either the foetus, newborn, or in the senescence, and could inspire new mechanism for anti-ageing drugs as well as for arterial hypertension based on angiotensin control.

P-28
UNRAVELLING THE CONTRIBUTION OF ADENOSINE A_{2A} RECEPTOR TO THE NEUROBIOLOGY OF SCHIZOPHRENIA

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Schizophrenia is a severe neuropsychiatric disorder with an unknown aetiology. Its pharmacotherapy mostly relies on restoring dysregulated striatal dopamine and prefrontal cortex glutamate neurotransmission. However, current treatments are often insufficient to coat all the symptomatology, thus the search for alternative and/or complementary neurotransmitter systems constitutes a big contest in psychiatry. Adenosine has been highlighted because of its relationship with both dopaminergic and glutamatergic neurotransmission. Thus, it has been postulated that restoring adenosine concentrations within schizophrenia-related brain areas might have beneficial antipsychotic properties. Here, we studied the relationship between adenosine A_{2A} receptor ($A_{2A}R$) and dopamine D_2 receptor (D_2R) expression, which may be related to the hyperdopaminergic hypothesis of schizophrenia. First, we used a pharmacological animal model of schizophrenia, using chronic administration of phencyclidine. Interestingly, while PCP treatment induced psychotic-like symptoms in mice, it also promoted a significant increase of D_2R expression in the striatum. On the other hand, as we recently demonstrated, the deletion of $A_{2A}R$ also promotes psychotic-like symptoms. And similar to PCP-treated mice, we observed an increased D_2R expression in KO-$A_{2A}R$ when compared to wild type (WT) littermates. Next, in an attempt to manipulate D_2R content in KO-$A_{2A}R$ mice, which may correlate with the psychotic-like symptoms described, we assessed the effects electroconvulsive therapy (ECT). ECT increased striatal D_2R expression in WT animals, while promoting a significant reduction in KO-$A_{2A}R$ mice. Importantly, this ECT-mediated D_2R reduction was followed by a concomitant improvement of the KO-$A_{2A}R$ mice's sensorimotor gating impairment, which was measured by the pre-pulse inhibition test. Finally, we used an adeno-associated virus to express $A_{2A}R$ in the striatum of KO-$A_{2A}R$ mice, in which we confirmed the relationship between $A_{2A}R$ presence/absence and the development of psychotic-like symptoms. Overall, a good correlation between striatal D_2R expression and psychotic like symptoms was established, in which it played a key role $A_{2A}R$ content.
Acknowledgements: This work was supported by MINECO/ISCIII (SAF2014-55700-P, PCIN-2013-019-C03-03 and PIE14/00034), the

Catalan government (2014 SGR 1054), ICREA (ICREA Academia-2010), Fundació la Marató de TV3 (Grant 20152031) and IWT (SBO-140028).

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P-29
COCAINE ADMINISTRATION DURING A WINDOW OF ADOLESCENT VULNERABILITY INDUCED LONG-TERM CONSEQUENCES ON NEGATIVE AFFECT IN ADULT RATS

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Many studies investigated vulnerability factors that predispose to cocaine addiction, but few have focused on adolescence—a critical period for neurobehavioral plasticity and drug use. Prior studies from our laboratory demonstrated a time-specific window during early-mid adolescence (postnatal day PND 33–39) in which cocaine administration induced immediate neurotoxic effects and long-term consequences on addiction-like behaviour. Contrarily, the long-term effects of adolescent cocaine exposure on inducing a pro-depressive phenotype were not clear and thus were the aim of this study. Adolescent male Sprague-Dawley rats were exposed to the forced swim test (FST, pre-test, PND 28 and test, PND 29: for baseline immobility levels). Then, rats were treated with saline (0.9% NaCl, 1 ml/kg, $n = 21$) or cocaine (5 mg/kg, $n = 23$) for 7 days (PND 33–39). Following prolonged withdrawal, rats were exposed to the FST (5 min test, PND 71) 45 min after a challenge dose of saline (saline-saline, $n = 9$; cocaine-saline, $n = 11$) or cocaine (saline-cocaine $n = 12$; cocaine-cocaine, $n = 12$). Rats were then exposed to a two-bottle choice test (1% sucrose preference, PND 76–77). The main results showed that cocaine administration during adolescence (PND 33–39) induced long-term consequences on negative affect in adulthood as measured by: (1) a pro-depressive phenotype (i.e., increased immobility time in the FST: $+63 \pm 17$ s, $p < 0.001$), and (2) a pro-anhedonic phenotype (i.e., decreased sucrose consumption on PND 77: -17 ± 7 g, $p < 0.05$) when comparing saline-cocaine vs. cocaine-cocaine groups. These results extend our prior data, which showed that cocaine administration at this window of adolescent vulnerability (PND 33–39) induced long-term effects on addiction-like behaviour, to also include long-term consequences on negative affect, and therefore confirming that early drug use leads to enduring behavioural negative consequences. Supported by 'Delegación del Gobierno para el Plan Nacional sobre Drogas' (MSSSI, Grants 2012/011, 2016/002) and 'Ramón y Cajal' Program to M.J.G.-F. (MINECO), and by RTA-RD16/0017/0010.

Key words: adolescence; cocaine; consequences; affect

P-30
DUAL-SPECIFICITY PHOSPHATASE DUSP6 REGULATES LONG-TERM MEMORY AND ERK/MAP-KINASE SIGNALING IN HIPPOCAMPUS

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Transient activation of the Erk/MAP-kinase pathway in hippocampal CA1 neurons is essential for contextual memory. However, little is known about the phosphatase(s) that directly regulate Erk1/2 during memory formation. Here we show that Dusp6 (a dual-specificity Y/T-phosphatase) is abundant in hippocampus, localizing in postsynaptic compartments of CA1 and CA3 pyramidal cells, including dendritic spines. Dusp6-KO mice showed marked deficits in contextual fear conditioning, object recognition, and reversal of spatial reference memory. Notably, expression of short hairpin RNAs against Dusp6 via adeno-associated vectors in area CA1 reproduced the memory deficits observed in global KO mice. Binding between Dusp6 and Erk1/2 transiently increased after contextual training, suggesting that Dusp6 regulates the time course of Erk1/2 activation. In keeping with this, the allosteric inhibitor of Dusp6, (E)-2-Benzylidene-3-(cyclohexylamino)-2,3-dihydro-1H-inden-1-one, prolonged increases in Erk1/2 and CREB phosphorylation in hippocampal neurons following synaptic stimulation. Furthermore, Dusp6-KO mice showed increased basal activation of Erk1/2 and downstream CREB, as well as impaired induction by training. Interestingly, Dusp6-KO mice also showed elevated levels of PSD-95, GluN2A and pY-GluN2B, indicating possible effectors underlying the memory deficits. Collectively, the results indicate that Dusp6 plays a critical role in hippocampus-dependent long-term memory, and underscore the importance of Dusp6 in Erk1/2 regulation in neurons.

Key words: MAP kinase; Dusp6; hippocampus; memory**:**

P-31
ACTIONS OF LITHIUM TREATMENT ON OXIDATIVE STRESS MARKERS IN MITOCHONDRIAL COMPLEX I AND COMPLEX III INHIBITION AND AFTER CO_2 EXPOSURE IN SH-SY5Y CELLS

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Bipolar disorder is a life-threatening psychiatric illness characterized by mood disturbances with recurrent periods of mania, hypomania and depression. Lithium is the classic mood stabilizer, but the basis of its therapeutic effect remains unclear. There is evidence that lithium exerts neuroprotective effects by increasing resistance to oxidative stress and it has been shown to stimulate cell proliferation. This study aims to evaluate the effects of lithium on resistance to oxidative stress and cell proliferation in the absence or presence of carbon dioxide (CO_2), rotenone (0.15 μM) an inhibitor of mitochondrial complex I or antimycin A (10 nM) an inhibitor of mitochondrial complex III. SH-SY5Y cells were treated for 72 h with lithium (0.5–5 mM). Superoxide anion (O_2^-) and hydrogen peroxide (H_2O_2) production were measured for fluorescence. All the experiments were carried out under standard conditions, after 4 h of CO_2 exposure or after 24 h of incubation with rotenone and antimycin A. At very low concentrations, lithium decreased O_2^- production under normoxic conditions and after exposure to CO_2 and rotenone. Lithium was not observed to produce any changes in H_2O_2 levels under standard conditions or after either the rotenone or antimycin A treatments; but changes were observed after CO_2 exposure. In addition, lithium increased cell proliferation. Our findings demonstrate the specific potential capacity of lithium at very low concentrations to protect SH-SY5Y cells against oxidative stress. The protection afforded by lithium might be due, at least in part, to its inhibition of O_2^- from the complex I; although it seems likely that multiple sites affected by lithium contribute to its antioxidant action. Also, lithium could favour neurogenesis and decrease the vulnerability of neuronal cells to cell injury.

Keywords: Lithium; SH-SY5Y; COX-2; oxidative stress; CO_2; rotenone; antimycin A

P-32
α_{2A}- AND α_{2C}-ADRENOCEPTOR SUBTYPES EXPRESSION IN POSTMORTEM PREFRONTAL CORTEX OF SUBJECTS WITH SCHIZOPHRENIA

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α_{2A}- and α_{2C}-adrenoceptor subtypes are present in the human prefrontal cortex (PFC) and could play a role in schizophrenia. Moreover,

α_2-adrenoceptors are targets for different antipsychotic drugs. In this context, the density of both α_2-adrenoceptor subtypes and their pre/postsynaptic expression has not been deeply evaluated in the PFC of schizophrenic subjects.

α_{2A}- and α_{2C}-adrenoceptor protein expression was determined by Western Blot in postmortem PFC of 24 subjects with an antemortem diagnosis of schizophrenia, and 24 controls. Both groups were matched by age, gender, and postmortem delay. Twelve of the schizophrenic subjects were taking antipsychotics at death (based on positive blood toxicological analysis), while the other 12 where antipsychotic-free (negative toxicology). α_{2A}- and α_{2C}-adrenoceptor expression was measured both in a preparation of synaptosomes and in postsynaptic membrane fractions, and was normalized for actin immunoreactivity as loading control.

α_{2A}-adrenoceptor protein expression in synaptosomes showed a non-significant trend to increase (+37%, $p = 0.114$) in schizophrenia subjects compared with controls. When subjects were divided regarding antipsychotic treatment, there was a significant increase in α_{2A}-adrenoceptor expression in antipsychotic-treated (+78%, $p = 0.025$) but not in antipsychotic-free subjects compared with controls. α_{2A}-adrenoceptor expression in postsynaptic fraction was significantly increased in schizophrenia subjects *vs* controls (+71%, $p = 0.026$). Again, the increase was significant in antipsychotic-treated subjects (+131%, $p = 0.014$) but not in antipsychotic-free subjects. α_{2C}-adrenoceptor protein expression was not significantly different between schizophrenia subjects and controls in synaptosomes and postsynaptic fraction, neither in antipsychotic-treated or antipsychotic-free subjects.

In conclusion, α_{2A}-adrenoceptor protein expression was increased in PFC of schizophrenia subjects receiving antipsychotic treatment. This increase was stronger in the postsynaptic fraction compared to synaptosomes (which include both pre- and postsynaptic membranes). These results might be a consequence of the α_{2A}-adrenoceptor antagonistic properties of some antipsychotic drugs.

Supported by grants from the Spanish *MINECO* (SAF2013-48586-R) and the Basque Government (IT616/13).

Keywords: schizophrenia, α_2 adrenoceptor, antipsychotics, human brain.

P-33
EFFECT OF FINGOLIMOD PHOSPHATE ON NEURONAL OXIDATIVE STRESS PRODUCED BY MENADIONE

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Introduction: Oxidative stress is considered to be an important component of various neurodegenerative diseases such as Parkinson's disease or multiple sclerosis, where the mitochondrial dysfunction is a major source of ROS production. Menadione induces toxic oxidant stress associated with tissue injury, mitochondrial damage and cell death.

Aim: To study the antioxidant effects of fingolimod phosphate (FP) on neuronal cell cultures against the oxidative damage produced by menadione.

Methods: SN4741 neuronal cells were grown and used as control (non-treated cells), or treated with 15 μM menadione alone or in presence of 50 nM FP during 6 h to study the following: cellular toxicity, intracellular levels of ROS production, levels of oxidative stress markers, and expression of antioxidant enzymes. Statistical differences were determined using one-way ANOVA. Statistical significance was set at $p < 0.05$.

Results: Menadione produces a 50% decrease in neuronal viability being FP capable of recovering half of the damage ($p < 0.05$). FP also reverted the effect of menadione on both intracellular and mitochondrial O2−• production measured via flow cytometry ($p < 0.05$). Studying oxidative stress markers, such as total thiol levels (TTL) or advanced oxidation protein products (AOPPs), menadione produces a decrease of 25% in TTL and an increase of 34% in AOPPS compared to control; these changes are reversed by co-incubation with FP ($p < 0.05$). The expression of mitochondrial antioxidant enzymes, Thioredoxin-2 (Trx2) and Superoxide dismutase-2 (SOD2) behaves in a similar way. Thus, menadione induces a decrease of 53% (Trx2 levels) and 42% (SOD2 levels) compare to control; these decreases are reduced to 8.5% (Trx2 levels) and 20% (SOD2 levels) in the presence of FP ($p < 0.05$).

Conclusion: FP exerts a modulating effect on the ROS production, oxidative stress markers levels and the expression of antioxidant enzymes that maintains the balance of neuronal oxidative status.

Key words: fingolimod phosphate; oxidative stress; antioxidant; neuroprotective.

P-34
ACUTE TREATMENT WITH 8-OH-DPAT IMPROVES COGNITION IN RATS BY CHANGING THE STRATEGY USED TO RESOLVE A MAZE AT DIFFERENT AGES

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Aging is a complex, heterogeneous, and multifactorial process in which a plasticity deficit in neuronal circuits such as in hippocampus is presented. Additionally serotonin (5-HT) is found to be involved in many physiological or pathophysiological processes including cognitive function. In this regard, while some studies reported enhanced memory following 8-OH-DPAT (a 5-HT_{1A} receptor agonist) administration others reported impaired memory functions. In this context, the present study evaluated whether acute treatment with a low dose of 8-OH-DPAT would improve cognitive function and whether this improvement would be conditioned by aging. To do so, male Sprague-Dawley rats were treated with 8-OH-DPAT (0.3 mg/kg, i.p.) or saline (0.9% NaCl, i.p.) at three different ages (3, 12 and 18 months old: $n = 10$ rats per age and treatment group) and cognitive function was evaluated in the Barnes maze 1 h later. The behavioural results showed that 8-OH-DPAT improved cognitive function by reducing the time spent (3 months: -61 ± 7%, $p < 0.05$; 12 months: -75 ± 4%, $p < 0.01$; 18 months: -31 ± 15%, $p > 0.05$) and the number of errors committed (3 months: -70 ± 11%, $p < 0.05$; 12 months: -88 ± 3%, $p < 0.05$; 18 months: -43 ± 19%, $p > 0.05$) to complete the maze as compared to vehicle-treated rats. Moreover, the strategy used to resolve the maze varied with age, fluctuating from a more serial/direct strategy towards a mixed one at later ages. Interestingly, 8-OH-DPAT improved cognition by decreasing mixed strategies while increasing either direct and/or serial strategies. Thus, the present results support a role for 8-OH-DPAT in improving cognition in rats at different ages.

Supported by SAF2014-55903-R and 'Ramón y Cajal' Program to M.J.G.-F (MINECO).

Key words: rat; age; cognition; 5-HT_{1A} receptor.

P-35
BEHAVIOURAL EFFECTS OF NOVEL MULTITARGET ANTICHOLINESTERASIC DERIVATIVES IN AGING AND ALZHEIMER'S DISEASE: STUDIES IN 3XTG-AD MICE

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Maintenance of cholinergic activity remains one of the primary objectives in cognitive-enhancing treatments for individuals with cognitive deficits associated to aging, mild cognitive impairment and Alzheimer's Disease (AD). Current drug development provides new AChEIs derivatives, hybrid molecules with multitarget actions aimed to result in a better clinical management outcome than the transitory and symptomatic therapy offered by classical AChEIs. Thus, they are expected to induce long-lasting amelioration of impaired neurotransmission and clinical symptoms but also to exert disease-modifying effects. Our multidisciplinary research consortium has synthesized and evaluated tacrine-huperzine A hybrids and their derivatives showing that Huprine X, a reversible AChE inhibitor hybrid of tacrine and huperzine A, affects the amyloidogenic process *in vitro*, and the AD-related neuropathology *in vivo* in mice models of AD. Besides, AVCRI104P3, a new donepezil-huprine heterodimer, exerts highly potent and selective inhibitory action on hAChE, hBuChE and BACE-1 activities as well as on the AChE-induced and self-induced Aβ aggregation, both *in vitro* and *ex vivo*. Here, we summarize their behavioural effects, both at cognitive (learning and memory) and neuropsychiatric-like (anxiety-like behaviours) levels and as assessed in three distinct *in vivo* biological scenarios: middle-age, cognitive deficits associated to aging, and AD-like phenotype. Furthermore, we provide new experimental data of the comparative assessment of chronic (i.p., 21 days) Huprine X (0.12 μmol/kg) and AVCRI104P3 (0.6 μmol/kg) treatment in 12 month-old (advanced stages) 3xTg-AD mice in two temporal circadian frames: after nocturnal activity or after 4 h of diurnal sleep. Besides the improvement of the hallmark cognitive symptomatology without inducing side effects, these drugs were able to modulate emotional and anxiety-like behaviours even in poor-performance circadian activity frames. Overall, the studies show that these novel multitarget AChEIs provide symptomatic and disease-modifying benefits of potential interest for the management of AD. Financial support: DGICYT-FEDER CTQ2011-22433, SAF2014-57094, ISC3-PI10/00283, 2014-SGR-52.

Key words: Huprine X, AVCRI104P3, cognition, learning and memory, anxiety, circadian activity, 3xTg-AD mice, Behaviour.

P-36
STUDY OF THE EFFECT OF EARLY POSTNATAL ADMINISTRATION OF A NMDA RECEPTOR ANTAGONIST IN MOUSE ON DIFFERENT FUNCTIONAL AND NEUROMORPHOLOGICAL SCHIZOPHRENIC PHENOTYPES

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Schizophrenia is a chronic and severe mental disorder. It is characterized by hallucinations and cognitive symptoms such as attention and memory deficits. Recent studies have pointed at a dysfunction of the glutamatergic neurotransmission as a possible source of cognitive deficits in schizophrenia. The decreased action of NMDA receptors (NMDAR) on GABAergic interneurons reduces the inhibitory control of excitatory pyramidal neurons leading to increased glutamate release. This situation results in excitotoxic damage and cognitive impairment. The stimulation of NMDAR affects the maturation of GABAergic interneurons during a critical period of development, for this reason, the blockade of these receptors during the early period implies changes similar to the neuropathophysiology of schizophrenia. The aim of this study was to characterize a mouse model of schizophrenia in the adult stage with early brain damage induced by ketamine. We assayed two experimental groups: a) C57BL/6 mice treated with ketamine (30 mg/kg, s. c.) on postnatal days 7, 9 and 11 and b) C57BL/6 mice treated with vehicle on postnatal days 7, 9 and 11. The experimental groups of mice were assessed in several cognitive and motor tasks in adulthood (PND 90–120). In addition, a neuromophological analysis of the parvalbumin interneurons cellularity in prefrontal cortex and hippocampus was performed by immunohistochemistry. Mice treated with early ketamine administration showed hypoactivity, an impaired recognition memory, deficits in their working memory and a decreased social memory in adulthood. Moreover, ketamine treatment reduced the parvalbumin cell number in prefrontal cortex and hippocampus. Our results provide new evidence for the alterations induced by early treatment with ketamine in mice.

Key words: Schizophrenia; mouse model; cognitive impairment; interneurons.

P-37
KETAMINE-INDUCED HYPNOSIS IN MICE REGULATES FADD AND MEK-ERK SIGNALING IN BRAIN CORTEX

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Ketamine (KET) is an antagonist at excitatory NMDA receptors that at low doses induces rapid antidepressant effects and at a higher dosage hypnosis in rodents. Recently, midazolam (MDZ, an agonist at inhibitory $GABA_A$ receptor)-induced hypnosis in mice was associated with upregulation of cortical p-FADD/FADD ratio and disruption of MEK (increased)-ERK (decreased) phosphorylation. This work assessed whether ketamine-induced hypnosis can regulate, similarly to MDZ, FADD and ERK signaling. Male CD1 Swiss mice were treated (i.p.) with KET (150 mg/kg, $n = 5–7$) or saline (2 ml/kg, $n = 5$). The sleeping time induced by KET was monitored according to the loss (LORR) or recovery (RORR) of the righting reflex. During KET-induced hypnosis and

after wake-up, subgroups of mice were sacrificed and the cerebral cortex prepared for protein analysis by Western blot. In KET-treated mice, compared with saline, cortical p-FADD, but not FADD, was increased ($p < 0.05$) in parallel with the hypnotic time course (at LORR: +16%; 20 min: +103%; RORR: +95% and 1 h post-RORR: +65%). Thus, KET increased cortical p-FADD/FADD ratios (LORR: +1.2-fold; 20 min: +2.7-fold; RORR: +2.3-fold and post-RORR: +2.1-fold). On the other hand, activated MEK1/2 (p-MEK/MEK ratio) was also increased ($p < 0.05$) following KET hypnotic time course (at LORR: +41%; 20 min: +75%; RORR: +58% and 1 h post-RORR: +81%). Paradoxically, the expected parallel activation of ERK1/2 (p-ERK/ERK ratio) was not observed but instead it was downregulated ($p < 0.05$) (LORR: no change; 20 min: -47%; RORR: -40% and 1 h after RORR: −8%).During KET-induced hypnosis in mice, p-FADD/FADD ratio (a survival index) was upregulated and the sequential activation of MEK to ERK was disrupted (ERK downregulation could lead to adverse effects including amnesia). The results also revealed the induction of very similar molecular mechanisms for excitatory KET and inhibitory MDZ mediating hypnosis in mice. Supported by SAF2014-55903-R and 'Ramón y Cajal' Program to M.J.G.-F. (MINECO).

P-38
EARLY EXPOSURE OF MICE TO MDPV INCREASES THE VULNERABILITY TO COCAINE IN ADULTHOOD

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3,4-Methylenedioxypyrovalerone (MDPV) is a synthetic cathinone which has recently emerged as a designer drug of abuse. It acts as a dopamine uptake blocker 10–50-fold more potent than cocaine, which suggests a high potential of abuse. Due to the rapid increase in the abuse of MDPV among the young population, the aim of this work was to investigate the influence of an early exposure to MDPV during adolescence on the psychostimulant, rewarding and reinforcing effects of cocaine in adulthood.

Adolescent (aged 41–44 days) male Swiss CD-1 mice were treated for 7 days with 1.5 mg/kg (s.c.) MDPV twice daily, followed by 21 days of withdrawal. Thereafter, when reached adulthood, they were tested to the psychostimulant, rewarding and reinforcing effects induced by cocaine assessing locomotor activity, conditioned place preference (CPP) and self-administration (SA). Moreover, the dopamine D_2 receptors density and the expression of c-Fos and ΔFosB in the striatum were determined. Differences between groups were compared using three-, two-way ANOVA or Student's *t*-test where appropriate. Subsequent Tukey's *post-hoc* tests were calculated when required. Breaking point was analysed using Mann-Whitney *U*-test.

After withdrawal, mice pretreated with MDPV showed a higher locomotor response and place conditioning to cocaine than those that had received saline. Acquisition of cocaine SA was similar in both groups. However, MDPV-treated animals showed a higher breaking point achieved under a progressive-ratio programme. MDPV pretreatment produced a significant decrease (20%) in D_2 receptor density and an increase in ΔFosB expression (3-fold), 24 h after treatment. The levels of ΔFosB declined during withdrawal but still remained elevated when measured 2 h after cocaine or saline challenge. Cocaine challenge induced an increase in c-Fos which was attenuated in MDPV-pretreated animals.

These results suggest that MDPV consumption during adolescence induces long-lasting adaptive changes leading to a higher vulnerability to cocaine abuse.

Key words: MDPV; cocaine; adolescence; self-administration.

P-39
IONOTROPIC GLUTAMATE RECEPTORS PHOSPHOPATTERN IS ALTERED IN SYNAPTOPATHY CONDITIONS

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The glutamatergic synapse is an exquisite dynamic structure involved in excitatory neurotransmission. The plasticity of the system is mediated, at least in part, by post-translational modifications. In particular, phosphorylation events control the subcellular location of glutamate receptors, a mechanism underlying synaptic strength and neuron survival. In line with this, our group has recently shown that DYRK1A – a protein kinase overexpressed in Down syndrome, DS and Alzheimer's disease, AD – directly phosphorylates GluN2A subunit of NMDA receptors (NMDARs), at serine residue 1048 (S1048). DYRK1A-mediated phosphorylation of GluN2A subunit increases the surface expression of NMDARs and alters NMDA evoked currents. Given the overexpression of DYRK1A kinase in synaptopathy conditions and the critical role of ionotropic glutamate receptors in cognitive performance, we hypothesized that the alterations on NMDARs phosphopattern might be underlying neuronal dysfunction, ultimately contributing to cognitive disorders. To address this hypothesis, we characterized the phosphoproteomic profile of synaptic and extrasynaptic NMDAR-associated protein complexes in the hippocampus of adult Ts65Dn mice (DS murine model) and in the entorhinal cortex and hippocampus of post-mortem AD biopsies. The subsynaptic fractions of the different brain regions were analyzed by Mass Spectrometry and further analysis revealed a specific subsynaptic and disease-associated NMDARs phosphopattern. Importantly, we have detected a significant increase of GluN2A (pS1048) in the postsynaptic density of hippocampus of adult Ts65Dn mice ($n = 8$ per condition; $^{**}p = 0.0011$; Student's t test), as well as in the entorhinal cortex of AD individuals ($n = 3$ controls and $n = 7$ AD; $^{*}p = 0.0167$; Student's t test). In agreement with this, our biochemical data supported the presence of Dyrk1A in the postsynaptic density, enabling the direct phosphorylation of GluN2A -containing NMDARs in a synaptic activity-dependent manner. Overall, our results demonstrate an altered phosphopattern in the pathological glutamatergic synapse, which can represent a therapeutic target.

This work has been supported by Grants PI16/00851PI13/00135 (ISCIII), La Marató (Project N. 20140210), PCIN-2014-105 (MINECO), AGAUR (SGR14-297) and Miguel Servet (CP10/00548) to XA.

Key words: Glutamate receptors; phosphorylation; DYRK1A; synaptopathy.

P-40
ASSOCIATION OF REGULATORY *TPH2* POLYMORPHISMS WITH HIGHER REDUCTION IN DEPRESSIVE SYMPTOMS IN CHILDREN AND ADOLESCENTS TREATED WITH FLUOXETINE

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Genetic variability related to the brain serotonergic system has a significant impact on both the susceptibility to psychiatric disorders, such as major depressive disorder, and the response to antidepressant drugs,

such as fluoxetine. *TPH2* is one of the most important serotonergic candidate genes in selective serotonin reuptake inhibitors (SSRIs) pharmacogenetic studies. The aim of the present study was to evaluate the influence of regulatory polymorphisms that are specifically located in human *TPH2* transcription factor binding sites (TFBSs), and therefore could be functional by altering gene expression, on clinical improvement in paediatric population treated with fluoxetine for the first time. The selection of SNPs was also based on their linkage disequilibrium with *TPH2* rs4570625, a polymorphism with questionable functionality, which was previously associated with clinical response in our population. Eighty-three children and adolescents were diagnosed 12 weeks after initiating fluoxetine treatment. Clinical improvement was assessed by reduction in Children's Depression Inventory (CDI) scale score. To estimate the independent contribution of each SNP to clinical improvement, general linear models were used. Three polymorphisms were, for the first time, significantly associated (applying Bonferroni correction) with clinical improvement. Higher reductions in CDI scale scores were found in minor allele homozygotes for rs34517220 (GG: 20.7 ± 3.1 vs AA+AG: 7.6 ± 0.8; $p = 0.0000007$) and for rs60032326 (AA: 21.7 ± 1.2 vs GG+GA: 8.4 ± 0.9; $p = 0.006$, and also in minor allele carriers for rs11179002 (TT: 9.4 ± 1.1 vs CT: 16.8 ± 3.9 vs CC: 6.4 ± 1.2; $p = 0.00059$). Interestingly, rs34517220 is located in a *TPH2* TFBS for two relevant transcription factors in the serotoninergic neurons, Foxa1 and Foxa2, which together with the high level of significance found for this SNP, could indicate that it is in fact the crucial functional polymorphism related to the fluoxetine response. These results provide new evidence for the role of regulatory genetic variants that could modulate *TPH2* expression in the SSRI antidepressant response.

Keywords: Fluoxetine; Pharmacogenetics; Children; *TPH2*; Polymorphism; rs34517220

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P-41
LONG-TERM CONSEQUENCES OF TREATING RATS WITH METHAMPHETAMINE AT A WINDOW OF VULNERABILITY DURING YOUNG ADULTHOOD ON HIPPOCAMPAL CELL DAMAGE

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A recent study from our laboratory demonstrated certain degree of hippocampal cell damage (i.e., impaired neurogenesis) exerted by methamphetamine when administered specifically at a window of vulnerability during young adulthood (postnatal day, PND 54–57) as measured 24 h after the last injection (PND 58). As a follow up, the present study aimed at evaluating the long-term consequences to drug-induced brain changes of treating rats with methamphetamine at this period of vulnerability. To do so, male Sprague-Dawley rats were pretreated with BrdU (2 × 50 mg/kg, i.p., 3 days) which marks new born cells (PND 48–50). Then, rats were treated following a binge paradigm (3 pulses per day, i.p., every 3 h, for 4 days) with saline (0.9% NaCl, 1 ml/kg, $n = 12$) or methamphetamine (5 mg/kg, $n = 14$) (PND 54–57). Following prolonged withdrawal (PND 91), rats were killed 45 min after a challenge dose of saline (saline-saline, $n = 6$; methamphetamine-saline, $n = 7$) or 5 mg/kg of methamphetamine (saline-methamphetamine, $n = 6$; methamphetamine-methamphetamine, $n = 7$). The left hippocampus was cryostat cut and slide-mounted for evaluation of Ki-67 + cell proliferation by immunohistochemistry. The right hippocampus was dissected to study the cell fate regulator BDNF (i.e., pro- and mature-BDNF forms) by western blot analysis. The main results showed that methamphetamine (PND 54–57) induced enduring hippocampal cell damage on cell proliferation (Ki-67+ cells/sections: -21 ± 5%, $p < 0.05$) and mature-BDNF (-24 ± 6%, $p < 0.01$), but not pro-BDNF (-8 ± 5%, $p > 0.05$), as observed when comparing control-saline vs. methamphetamine-saline groups. The main results showed that a history of methamphetamine during a window of vulnerability in young adulthood (PND 54–57) induced enduring hippocampal cell damage that persisted in time (PND 91) as observed by decreases on recent cell proliferation and on the cell fate marker mature-BDNF, which is associated with neuronal survival, growth and differentiation. Supported by 'Fundación Alicia Koplowitz' and 'Ramón y Cajal' Program to M.J.G.-F. (MINECO), and by RTA-RD16/0017/0010.

Key words: methamphetamine; withdrawal; consequences; hippocampal neurogenesis.

P-42
THE FIFTH SUBUNIT MODULATES THE FUNCTION OF CANONICAL AGONIST SITES IN α4β2 NICOTINIC RECEPTORS

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Nicotinic receptors (nAChR) containing the α4 and β2 subunits are the most prevalent type of nAChR in the brain, where they modulate an assortment of physiological functions such as cognition, mood, reward and analgesia. The α4β2 receptors assemble in two functional forms, $(\alpha4\beta2)_2\alpha4$ and $(\alpha4\beta2)_2\beta2$. These two receptors have different pharmacological properties, which is partly accounted for by the presence of an additional agonist site at the signature α4/α4 interface of the $(\alpha4\beta2)_2\alpha4$ nAChR. Previous studies found that the canonical agonist sites of these receptors function asymmetrically, even though they are structurally equivalent. These findings suggested that the fifth subunit (α4 in the $(\alpha4\beta2)_2\alpha4$ and a β2 in the $(\alpha4\beta2)_2\beta2$ may asymmetrically modulate the agonist sites.

We study the impact of the fifth subunit on receptor function by using two-electrode voltage-clamp electrophysiology, along with subunit-targeted mutagenesis and the substituted cysteine scanning method applied to fully linked $(\alpha4\beta2)_2\beta2$ receptors.

By incorporating a cysteine residue in the fifth subunit and measuring its rate of derivatization by a thiol-reactive reagent (MTS) in the presence or absence of ACh, we found that the fifth subunit increases ACh efficacy. This effect is dependent on the presence of ACh and impairment of the agonist sites perturbs the rate of MTS-modification of the fifth subunit asymmetrically. For the $(\alpha4\beta2)_2\alpha4$ receptors, we examined the effects of mutations introduced in the fifth subunit or flanking subunits on Zn^{2+} potentiation of $(\alpha4\beta2)_2\alpha4$ receptors. Zn^{2+} potentiation of the agonist responses of the $(\alpha4\beta2)_2\alpha4$ receptors is mediated by a site located on the fifth subunit (α4). We found that Zn^{2+} potentiation is inhibited by alanine substitutions of amino acids linking the fifth subunit to neighbouring agonist sites.

Overall, we propose that the fifth subunit allosterically communicates with adjacent subunits to modulate agonist binding site function.

Key words: Nicotinic receptor, α4β2 receptors, Cys-loop receptors.

P-43
SELENOSUGARS ARE A PROMISING CLASS OF SELENIUM DERIVATIVES WITH VASCULO-PROTECTIVE EFFECTS

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Peroxynitrite ($ONOO^-$) is crucially involved in the initiation and progression of human cardiovascular disease. Selenium is an essential micronutrient that plays a key role in redox regulation and antioxidant defence. Synthetic organoselenium compounds such as ebselen can mimic the function of endogenous antioxidant selenoenzymes, but

ebselen can be toxic to cells at high concentrations. In this study, we aimed to analyse the antioxidant properties of two different reactivity profile selenosugars, βGlcSeMe and $\beta GlcSe_2$, that we hypothesize they have vasculo-protective effects. The parallel artificial lipid membrane permeation assay was used to predict passive membrane permeability. $ONOO^-$ scavenging activity was evaluated by measuring the consumption of Pyrogallol red. The effect of preincubation with selenosugars on contractile responses to phenylephrine (1 nM–100 μM) was evaluated by wire myography in aortic rings from Oncins France 1 mice (34.2 ± 0.1 g) in the absence or presence of $ONOO^-$ (50 μM; 45 min). Superoxide anion ($O_2^{\cdot-}$) formation, and inducible nitric oxide synthase (iNOS) or protein tyrosine nitrosylation levels were determined by ethidium fluorescence and immunofluorescence, respectively, in mice aorta or human cultured microvascular endothelial cells (HMVEC) submitted to hypoxia (overnight)/reoxygenation (24 h). Either ebselen or both selenosugars showed high predictive lipid membrane permeability. Both selenosugars exhibited lower $ONOO^-$ scavenging activity than ebselen, and $\beta GlcSe_2$ was a more potent ($p < 0.001$) $ONOO^-$ scavenger than βGlcSeMe. Ebselen (3 μM) failed to ameliorate $ONOO^-$-induced impairment of phenylephrine contractions and rather decreased ($p < 0.05$) maximal contractions. In contrast, $\beta GlcSe_2$ (10 μM) but not βGlcSeMe (10 μM) attenuated ($p < 0.05$) aortic contractile dysfunction triggered by $ONOO^-$. Furthermore, $\beta GlcSe_2$ reduced ($p < 0.05$) the $ONOO^-$-mediated increase in aortic $O_2^{\cdot-}$ and iNOS expression levels. Hypoxia/reoxygenation in HMVEC augmented O_2^- ($p < 0.05$) and nitrosylation ($p < 0.01$) levels, an effect prevented ($p < 0.001$) by $\beta GlcSe_2$. In conclusion, we suggest that selenosugar $\beta GlcSe_2$ can mitigate the $ONOO^-$-induced detrimental effects on the vasculature.

Key words: selenosugars; antioxidant; peroxynitrite; aortic reactivity.

P-44
INVOLVEMENT OF ENDOPLASMIC RETICULUM STRESS AND MITOCHONDRIAL DYSFUNCTION IN ABDOMINAL AORTIC ANEURYSM DISEASE

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Aim: Clinical management of abdominal aortic aneurysm (AAA) is limited to elective surgical repair while an effective pharmacotherapy is awaited. We aimed to elucidate the involvement of endoplasmic reticulum (ER) and mitochondrial stress in vascular wall degeneration during the development of AAA.

Methods and results: We determined the expression of ER stress, mitochondrial dysfunction and oxidative stress markers in a cohort of AAA samples from 100 patients and 20 healthy donors.

The mRNA and protein levels of ER stress markers such as ATF6, IRE-1, XBP-1 and CHOP were up-regulated in AAA samples compared with donors and this was accompanied by an exacerbated apoptosis measured by TUNEL and by cleaved caspase-3. Higher expression of NOX2, p22phox and NRF2 and an enhanced superoxide anion production, measured by dihydroethidium staining, were also found in AAA. Furthermore, mitochondrial biogenesis measured by the ratio between the expression of cytochromes b/c and Beta-actin was decreased by 50% in AAA. However, the expression of mitochondrial dysfunction markers (NRF1, PGC1-alpha and TFAM) remained unchanged between patients and donors. Additionally, in abdominal aorta of a mouse model of AAA ($ApoE^{-/-}$ infused with angiotensin II), the expression of ATF4, GRP78 and IRE-1 was increased while no differences were found in ATF6 or CHOP between AAA and control mice. Similarly to human, mitochondrial biogenesis was decreased by 60% in murine AAA.

Conclusion: Our results provide evidence supporting a role for chronic ER stress and mitochondrial stress in the development of AAA and identify them as potential therapeutic targets.

P-45
CHANGES IN THE EXPRESSION OF THE NT-3/TRKC PATHWAY IN VESSELS AND LEFT VENTRICLE DURING CULTURE

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Background: The expression of neurotrophin-3 (NT-3), a regulator of survival, development and function of sympathetic neurons during embryonic development, decreases in the adult central nervous system (1) but remains constant in the cardiovascular system from development to adulthood (2).

Aims: To quantify mRNA levels for NT-3 and its receptor TrkC in aorta (Ao), left ventricle (LV) and different cardiovascular cell types (freshly isolated or cultured) from human origin or Wistar rats.

Methods: Human Ao, LV, aortic smooth muscle cells (SMC), cardiac or aortic fibroblasts (FB), cardiomyocytes, endothelial cells (EC) from aorta, coronary artery and coronary microvasculature, were used. SMC and EC from Ao and cardiac FB from Wistar rats were isolated by incubation with collagenase (fresh cells) or cultured until 80% confluence (cultured cells). Some rat Aos were maintained in culture medium for 7 days (quiescent Ao). mRNAs encoding NT-3, TrkC and GAPDH (as a housekeeping gene) were quantified by RT-PCR as previously described (3).

Results: Human Ao and LV expressed significant levels of NT-3 and TrkC genes. This expression was observed in lower proportion in all the cultured human cell types. Rat Ao and LV express similar levels of NT-3 and TrkC than human tissues. This expression dramatically decreased in quiescent Ao and cultured vs freshly isolated cells. The decrease was more marked for TrkC gene which almost disappears in some cells.

Conclusions: The significant decrease in the expression of NT-3 and TrkC in cultured tissues and cells suggests a role for the NT-3/TrkC pathway in cell homeostasis that would become unnecessary when "ideal" culture conditions were established.

1. Physiol Rev, 89: 279–308, 2009:
2. Life Sci 81, 385–392, 2007:
3. J Cardiovasc Pharmacol 68, 230–40, 2016: Founded by Ministerio de Economía y Competitividad (SAF2013-45362-R). AZ received a fellowship from the Santiago Grisolía Foundation.

Key words: neurotrophin-3, trkC, endothelial cells, smooth muscle cells, fibroblasts, aorta, left ventricle.

P-46
CHONDROITIN SULFATE AND GLUCOSAMINE COMBINATION TREATMENT FOR MUSCULOSKELETAL DISEASES AND OSTEOARTHRITIS: A CHANCE TO KILL TWO BIRDS WITH ONE STONE? RESULTS IN A RAT-INJURY MODEL

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Background: Skeletal muscle injuries and osteoarthritis (OA) are prevalent in middle-aged active individuals. The similarities between

cartilage and muscle tissue may have clinical implications. There is an acute need to develop effective therapeutic strategies on sports-related muscle lesions.
Objective: Our aim was to evaluate the beneficial effects of chondroitin sulfate (CS) and glucosamine hydrochloride (GLU) administration (both compounds used for the symptomatic OA treatment) on muscle healing in a recently established rat model of skeletal muscle injury which reproduces the lesions seen in human athletes.
Methods: Male 8-weeks-old Wistar rats with a medial gastrocnemius injury received daily treatment for 3 weeks with CS + GLU by oral gavage administration (140 and 175 mg/kg of CS and GLU, respectively) or intraperitoneal injection (400 and 500 mg/kg, respectively). Healthy and untreated animals were used as controls.
Results: CS + GLU administration stimulated the growth of newly formed regenerating myofibers (1.35- and 1.77-fold increase after oral ($p < 0.01$) and i.p. ($p < 0.001$) administration, respectively), that was accompanied by 1.22- and 1.28-fold ($p < 0.01$) of improvement in gastrocnemius muscle force. Both treatments showed a clear anti-fibrotic effect by reducing 21% (oral, $p < 0.05$) and 28% (i.p., $p < 0.01$) the intramuscular collagen-I deposition. Oral ($p < 0.01$) and i.p. ($p < 0.05$) administration induced an increase of more than 30% in CS intramuscular deposition. Treated animals also revealed a tendency in accelerating the muscle regeneration process by showing a decrease of approximately 15% of developmental Myosin Heavy Chain (dMHC)-positive regenerating fibers.
Conclusions: CS and GLU administration improves muscle healing and force recovery of the injured skeletal muscle in rats, thus suggesting an important role of these products as potential new therapies for the treatment of muscle injuries in sports medicine. These results could help clinicians to account effects of comorbidities on musculoskeletal diseases and OA, as well as to consider new treatment options that show potential in both conditions.
Key words: osteoarthritis; skeletal muscle; chondroitin sulfate; glucosamine.

P-47
ENDOTHELIUM-DERIVED HYPERPOLARIZATION (EDH)-INDUCED RELAXATION OF RENAL ARTERIES: ROLE OF H_2O_2

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Background and purpose: The endothelium mediates vasodilation through endothelium-derived hyperpolarization (EDH) and hydrogen peroxide (H_2O_2) appears to play a pivotal role. Thus, we sought to investigate whether H_2O_2 is involved in the EDH-mediated relaxations of rat renal arteries.
Material and methods: Renal interlobar arteries of male Wistar rats were used. Changes in isometric wall tension of the vessels were also studied using wire myography. We studied the relaxant effect of H_2O_2 under conditions of NOS- and COX-blockade. Moreover, electrophysiological recording of K^+ currents were made in freshly isolated endothelial cells (EC) under the whole cell configuration of the patch clamp technique (E. Pinilla, et al., J. Physiol. Biochem. 72 (2016) S48).
Key results: Under conditions of NOS and COX blockade, relaxations to ACh were largely inhibited by catalase (200 UI/ml) in renal arteries suggesting the involvement of endothelium-derived H_2O_2. Furthermore, ACh-stimulated H_2O_2 generation measured by Amplex Red fluorescence assay as well, and exogenous H_2O_2 elicited relaxations that were accompanied by simultaneous decreases in VSM $[Ca^{2+}]i$ thus mimicking catalase-sensitive renal vasodilation. Under voltage-clamp conditions we observed a marked change in the shape of the currents in response to H_2O_2, reflecting selective activation of K^+ channels. The SERCA inhibitor cyclopiazonic acid (CPA) evoked a change in the shape of the outward currents similar to that evoked by H_2O_2. However, in the presence of CPA H_2O_2 did not further modify the amplitude of these currents.
Conclusions and implications: The results show that H_2O_2 is involved in the EDH relaxant responses of intrarenal arteries. H_2O_2 induced a slow, pronounced and long-lasting activation of outward KCa currents in EC, which indicates that H_2O_2 released by endothelial stimulation hyperpolarizes EC and may thus initiate an EDH vasodilator response in renal arteries.
Keywords: Renal arteries, EDH, H_2O_2, K_{Ca} channels.
Supported by: MINECO SAF2016-77526:

P-48
GUT MICROBIOTA CONTROLS ARTERIAL BLOOD PRESSURE IN RATS BY ALTERING GUT-BRAIN-BONE MARROW INTERACTION

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The aim of this study was to investigate whether changes in gut microbiota alter brain-gut-bone marrow (BM) interaction inducing changes in vascular tone and SBP. Fecal contents were collected and pooled from 20 week old spontaneously hypertensive rats (SHR) and normotensive Wistar-Kyoto (WKY) rats. Recipient WKY and SHR (25 weeks old) were orally gavaged with donor fecal contents for 3 consecutive days, and weekly during 4 weeks. Four groups were involved in this study: WKY with WKY microbiota (W-W), WKY with SHR (W-S), SHR with SHR (S-S) and SHR with WKY (S-W). Fecal microbiota transplantation (FMT) from WKY rats to SHR rats reduced basal SBP. Similarly, FMT from SHR to WKY increased basal SBP. The higher reduction on SBP after ganglionic blockade and plasma noradrenaline levels found in S-S group as compared to W-W group were reduced in S-W group, whereas in W-S these variables were also higher than that in W-W group. In S-S group mRNA levels of gap junction proteins and pro-inflammatory cytokines in the proximal colon were reduced and increased, respectively, as compared to W-W group and these changes were inhibited in S-W group. Relaxation induced by acetylcholine was impaired in aortic rings from S-S as compared to W-W and improved in S-W. S-W restored imbalance between Th17/Treg found S-S in mesenteric lymph nodes (MLN) and BM. Relaxation induced by acetylcholine was higher in aortic rings from Wistar rats after incubation with the condition media of lymphocytes from MLN of S-W group, as compared with the condition media from S-S. In conclusion, gut dysbiosis disrupts gut barrier function and increases sympathetic outflow. The central and endothelial effects, that increase vascular tone and SBP, seem to be associated with T cell infiltration, possibly as a result of a change in T cells polarization in MLN and BM.
Key words: bone marrow, endothelial dysfunction, fecal microbiota transplantation, gut dysbiosis, hypertension, T cells.

P-49

HIV TRANSGENE EXPRESSION INDUCES ENDOTHELIAL DYSFUNCTION AND KV7 CHANNEL IMPAIRMENT IN THE PULMONARY VASCULATURE

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Introduction: Pulmonary arterial hypertension (PAH) is a severe disease that results in increased pulmonary vascular resistance, right heart failure and death (1). The vascular dysfunction that leads to PAH is likely to be multifactorial, but endothelial dysfunction and downregulation of K^+ channels are considered common abnormalities in most forms of PAH. Human immunodeficiency virus (HIV) infection is an established risk factor for PAH, however the pathogenesis of HIV-related PAH remains unclear. Our aim was to analyse if the expression of HIV proteins is associated with impairment of endothelial or K^+ channel function in the pulmonary circulation.
Methods: HIV transgenic mice (Tg26) expressing seven of the nine HIV viral proteins and wild type (Wt) mice were used in this study. Right ventricular systolic pressure and systolic, diastolic and mean pulmonary arterial pressures were measured in open chest mice. Vascular reactivity was studied in endothelium-intact pulmonary arteries mounted in a wire myograph. K^+ currents were recorded in freshly isolated pulmonary artery smooth muscle cells using the patch-clamp technique. K^+ channel gene expression was assessed using rt-PCR. Data are expressed as mean ± sem.
Results: Right ventricular systolic pressure and systolic, diastolic and mean pulmonary arterial pressures were similar in Tg26 and Wt mice. Interestingly, pulmonary arteries from Tg26 mice showed impaired endothelium relaxation (11 ± 5% vs 33 ± 5% relaxation to Acetylcholine 1 μM; $n = 6$ $p < 0.05$). Likewise, pulmonary arteries and pulmonary artery smooth muscle cells derived from Tg26 mice had decreased Kv7 channel activity assessed by vascular reactivity and patch-clamp experimental approaches. This was associated with reduced expression of Kv7.1 and Kv7.4 channels, which have recently emerged as protective in the pulmonary circulation (2).
Conclusion: Our data indicate that HIV proteins induce endothelial dysfunction and Kv7 channel impairment, which could contribute to the vascular dysfunction in HIV-associated PAH.
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Supported by SAF 2014-55399 and SAF2016-77222-R.
Key Words:
Pulmonary arterial hypertension, Kv7 channels, endothelial dysfunction.

P-50

PGE_2 DERIVED FROM MPGES-1 FACILITATES EXCESSIVE ALDOSTERONE PRODUCTION FROM ADIPOSE TISSUE IN OBESITY. ROLE IN VASCULAR FUNCTION

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Adipocytes are more than fat storage cells since they release a number of factors contributing to energy homeostasis and vascular tone and structure. We demonstrated that adipocytes are a source of aldosterone in response to Ang II and that this is facilitated by mPGES-1-derived Prostaglandin E_2 (PGE_2) (unpublished). However, whether this pathway is activated in obesity is unknown. Mineralocorticoid-Receptor (MR), is involved in the stiffness and the endothelial dysfunction observed in obesity. We determined if mPGES-1 participates in aldosterone production from adipocytes in obesity and whether this is involved in endothelial dysfunction and vascular stiffness observed in this pathology.
Epididymal fat from DBA mPGES-$1^{+/+}$ and mPGES-$1^{-/-}$ mice fed with normal or high fat diet (HFD) was analyzed. CYP11B2 and MR expression was studied by qRT-PCR. Changes in vascular function and stiffness were studied using wire and pressure myographs. 3T3-L1 adipocytes were stimulated with 16,16-Dimethyl Prostaglandin E_2 ($DPGE_2$). Furthermore, visceral fat was obtained from patients and gene data were correlated with parameters of vascular stiffness. We found that CYP11B2 mRNA expression is augmented in the adipose tissue of the mPGES-$1^{+/+}$ HFD mice, but not in mPGES-$1^{-/-}$. However, we did not find differences in the MR. Moreover, $DPGE_2$ increases CYP11B2 mRNA expression in 3T3-L1 adipocytes. HFD provokes endothelial dysfunction in both genotypes, which is prevented by eplerenone. HFD induces vascular stiffness in mPGES-$1^{+/+}$ but not in mPGES-$1^{-/-}$mice. Preliminary data in patients show positive correlations between mPGES-1, CYP11B2 and MR gene expression. Moreover, there is a positive correlation between pulse wave velocity and CYP11B2 gene expression.
Our study suggests that mPGES-1-derived PGE_2 is involved in the excessive aldosterone synthase expression observed in adipose tissue in obesity and this might have a role in the vascular damage observed in this pathology.
Key words: mPGES-1; aldosterone; obesity; vascular function.
Support: ISCIII-Fondo Europeo de Desarrollo Regional (FEDER): PI13/01488; RD12/0042/0024 and MINECO:SAF 2016-80305-P; Roche-IDIPAZ; CIBERCV, COST-ADMIRE:BM1301.

P-51

NADPH OXIDASE-DERIVED H_2O_2 IS INVOLVED IN THE ENDOTHELIUM-DEPENDENT RELAXATIONS OF INTRARENAL ARTERIES

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The role of reactive oxygen species (ROS) in kidney vascular function has extensively been investigated in the harmful context of oxidative stress in hypertension and diabetes and obesity-associated kidney disease (Sharma, *Diabetes* 64:663, 2015). We have recently demonstrated that H_2O_2 derived from endothelial CYP epoxygenases is involved in the non-nitric oxide (NO) non-prostanoid EDH relaxations of rat intrarenal arteries (Muñoz et al., *Free Radic Biol Med* 106:168, 2017). The present study was sought to investigate whether NADPH oxidases (Nox) may be functional sources of vasodilator H_2O_2 and to assess their role in the endothelium-dependent relaxations of intrarenal arteries. Renal interlobar arteries isolated from the kidney of Wistar rats were mounted in microvascular myographs to assess function. H_2O_2 production was measured by Amplex Red fluorescence and Nox2 and Nox4 enzymes were detected by Western blotting and by immunohistochemistry. Under conditions of cyclooxygenase and NO synthase blockade, acetylcholine (ACh) induced catalase sensitive endothelium-dependent relaxations that were blunted by the NADPH oxidase inhibitor apocynin, by the Nox2 inhibitor gp91ds-tat and by the dual Nox1-Nox4 inhibitor GKT136901. Acetylcholine (10^{-5} M) stimulated H_2O_2 production that was reduced by p91ds-tat and GKT 136901. Nox2 and Nox4 proteins were expressed in renal interlobar arteries and cortex,

levels of expression of both enzymes being higher in the cortex. Nox4 was co-localized with eNOS in the endothelium of renal arteries and glomeruli. These results suggest that both Nox2 and Nox4 are physiologically relevant renal endothelial sources of ROS generation and that Nox-derived H_2O_2 is involved in the endothelium-dependent relaxations of renal arteries.

This work was supported by grant SAF2016-77526 from MINECO (Spain):

Key words: H_2O_2, renal endothelium, Nox2, Nox4.

*Equal contribution.

P-52
EFFECT OF SWIMMING TRAINING ON NEUROGENIC CONTRACTION AND STOCK OF INTRACELLULAR CALCIUM CONCENTRATION IN SPONTANEOUSLY HYPERTENSIVE RATS

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Introduction: Calcium (Ca^{2+}) is an important second messenger cell; Changes in Ca^{2+} concentrations in the intracellular stocks were linked to the pathogenesis of hypertension. Studies have shown that exercise training has beneficial effects in both humans and animals, such as lowering blood pressure (BP), decreased sympathetic activity, decreased peripheral vascular resistance, and it is known that Ca^{2+} participates in these functions. However it is not known whether the exercise is able to modify the Ca^{2+} concentration of intracellular stores as sarcoplasmic reticulum (SR) and mitochondria (MIT) before the onset of the disease, thus leading to an improvement in blood pressure levels in SHR young and adults.

Aims: To study the effect of swimming training (ST) on BP, neurogenic contraction in vas deferens (VD) and the concentration basal of calcium in adrenal gland SHR youth and adults.

Materials and Methods: All experimental procedures were approved by Ethical Committee of Federal University of São Paulo, Brazil (7866020315). The ST consisted of swimming sessions of 60 min, 5 days per week, for 8 weeks. Using animals NWR and SHR Young (12 weeks) and adults (20 weeks), $n = 8$; We study: 1) BP Measurement. 2)Neurogenic Contraction through electrical stimulation (0.2 Hz, 60 V) of the sympathetic nerves of the vas deferens (VD); 2a) Using CRT mixture of caffeine 10^{-2} M, Ryanodine 3×10^{-6} M and thapsigargin 10^{-6} M to depletion of Ca^{2+} RE. 2b) Neurogenic Contraction using CCCP 10^{-6} M to depletion of Ca^{2+} MIT. 3) Fluorescence microscopy with Fura-2AM, fluorescent probe to measure intracellular Ca^{2+} basal unstimulated, in adrenal gland slices in trained and sedentary animals.

Results and Conclusion: Compared to the sedentary animals trained animals both NWR and SHR youth and adults showed: 1) Decreased BP (mmHg): Young = NWR (s) 145.5 ± 1.0; NWR (t) 128.6 ± 1.2 and SHR (s) 199.5 ± 1.3; SHR (t) 141.2 ± 1.5; Adults = NWR (s) 151.4 ± 0.56; NWR (t) 144.4 ± 1.1 and SHR (s) 228.5 ± 2.0; SHR (t) 173.5 ± 1.9 2)) Decrease in neurogenic contraction in training groups; 2a) Decrease in neurogenic contraction (expressed in g of tension / g of tissue) using CRT: Young = NWR (s) 33.9 ± 2.1; NWR (t) 25.17 ± 2.1 * and SHR (s) 43.57 ± 0.8; SHR (t) 26.9 ± 1.3 ***; Adults = NWR (s) 28.5 ± 3.7; NWR (t) 18.41 ± 0.7 * and SHR (s) 48.06 ± 4.2; SHR (t) 26.96 ± 1.3. 2b) Decrease in neurogenic contraction using CCCP those from group.3) Decreased basal intracellular Ca^{2+} expressed in [Ca2+]i basal (UAF): Young = NWR (s) 0.95 ± 0.08; NWR (t) 0.89 ± 0.11; SHR (s) 1.3 ± 0.1; SHR (t): 1.0 ± 0.2. Adults = NWR (s) 1.3 ± 0.12; NWR (t) 0.99 ± 0.23; SHR (s) 2.4 ± 0.19; SHR (t): 1.99 ± 035. With these results we can infer that the ST was able to improve the condition of these animals by reducing all parameters studied before the development of hypertension in young animals as well as in adults, showing the importance of physical activity, because it leads to improved quality and the life expectancy of animals, results could be extrapolated to humans.

Financial Support: FAPESP, CAPES, CNPq.

P-53
SCLERODERMA FIBROBLASTS SUPPRESS ANGIOGENESIS VIA TGF-β/CAVEOLIN–DEPENDENT SECRETION OF PIGMENTED EPITHELIUM DERIVED FACTOR: TGF-β RECEPTOR INHIBITORS AS A NOVEL THERAPEUTIC TARGET FOR THE TREATMENT OF SCLERODERMA

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Systemic Sclerosis (SSc) or Scleroderma is an autoimmune condition characterised by vasculopathy and tissue fibrosis of the skin and multiple internal organs, with heterogeneous severity and clinical outcome. Transforming Growth Factor-beta (TGF-β) has been shown to play a major role in the pathogenesis of tissue fibrosis in Scleroderma, whereas its role in the pathogenesis of vasculopathy and defective angiogenesis is less clearly elucidated. SSc fibroblasts secrete Pigmented Epithelium Derived Factor (PEDF), a major antiangiogenic protein implicated in the vascular homoeostasis of the retina and pigmented epithelium. By means of immunohistochemistry we show that PEDF expression is increased in SSc skin biopsies whilst caveolin-1 (Cav-1) expression is downregulated. Importantly we show that 10 ng/ml TGF-β increases PEDF in both healthy and SSc fibroblasts and this effect is associated with downregulation of Cav-1. Additionally, we show that PEDF has a strong anti-angiogenic effect in an organotypic fibroblast/endothelial co-culture angiogenesis model, and demonstrate that fibroblasts lacking Cav-1 suppress angiogenesis through PEDF, without affecting endothelial cell proliferation. Concordantly *in vivo*, mice with transgenic hyperactivation of TGF-β receptor display reduced Cav-1 expression and increased expression of PEDF in the skin, which is accompanied by a reduced capillary density. Altogether, our data show that TGF-β plays an important role in the pathogenesis of vasculopathy in SSc through downregulation of Cav-1 in tissue fibroblasts, and corresponding increased expression of PEDF leading to suppression of angiogenesis. Consequently, mice with fibroblast specific activation of the TGF-β pathway may be a useful pre-clinical model to study the contribution of TGF-β in vasculopathy of Scleroderma. Our studies establish the molecular basis for the therapeutic use of TGF-β receptor inhibitors (currently in phase 2/3) in the treatment of vasculopathy associated with fibrotic diseases.

Key words: Systemic Sclerosis; vasculopathy; Transforming Growth Factor β; Pigmented Epithelium Derived Factor.

P-54
HIGH-FAT FEEDING PROMOTES DYSFUNCTION OF PERIVASCULAR ADIPOSE TISSUE

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Perivascular adipose tissue (PVAT) releases multiple vasoactive substances and regulates vascular tone. AMPK-activated protein kinase is present in PVAT and involved in the anticontractile effect of PVAT (1). In mice, high fat feeding increases expression of pro-inflammatory genes within the PVAT which may induce dysfunction (2). In this

study we investigated the effect of up to 12 weeks fat feeding on PVAT function and whether the effects were altered in mice lacking AMPK.
Wild-type (WT) and knock-out (KO) mice were fed chow or high fat diet (HFD) and blood pressure was monitored every 4 week by tail-cuff plethysmography. At 12 weeks, vascular function in endothelium-denuded rings of thoracic aorta with or without attached PVAT was studied by small artery wire myography. Macrophage markers in PVAT were studied using RT-PCR and release of adiponectin quantified by ELISA. AMPK activity in PVAT was assessed by Western blotting.
HFD caused a significantly greater weight gain in WT and KO mice compared to chow diet but had no effect on blood pressure in either strain. Relaxation to cromakalim was significantly attenuated in PVAT-containing aortic rings from WT mice fed HFD but not KO mice. HFD did not affect relaxation in aortic rings without PVAT in either strain. Fat feeding increased IL-1β and iNOS expression in WT PVAT and significantly reduced adiponectin secretion but this was not observed in KO PVAT. In WT PVAT, the level of phospho-AMPK was significantly greater compared to KO mice. HFD significantly reduced total and phospho-AMPK in WT but not KO mice.
In conclusion, 12 weeks of HFD causes PVAT to lose its anticontractile effect. This is likely due to reduced adiponectin secretion, possibly through alterations in AMPK activity in the PVAT.
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Keywords: AMPK; perivascular adipose tissue; fat-feeding; adiponectin

P-55
NEUROTROPHIN-3 AND TRKC EXPRESSION IN BLOOD VESSELS AND LEFT VENTRICLE OF ADULT RODENTS

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Neurotrophin-3 (NT-3), an established neurotrophic factor that participates in embryonic heart development (1), has also been detected in heart and blood vessels of adult animals (2, 3).
In the present study the expression pattern of NT-3 and its receptor TrkC has been determined in left ventricle (LV) and blood vessels (aorta and tail artery) as well as in cerebral cortex of adult Wistar rats by real time quantitative RT-PCR and Western blot analysis. In addition, mouse expressing the lacZ reporter gene, encoding the enzyme beta-galactosidase, from the NT-3 locus ($Ntf3^{+/lacZneo}$) were used for studying by immunohistochemistry, the distribution of the NT-3 gene in these tissues.
The results obtained shown that mRNA and protein expression levels of NT-3 were detected in blood vessels in a major proportion than in LV and cerebral cortex. TrkC was expressed at higher levels in blood vessels and cerebral cortex compared to LV. 5-bromo-4-chloro-3-indolyl-beta-D-galactopyranoside (X-Gal) staining in $Ntf3^{+/lacZneo}$ mice reveals that NT-3 appears in blood vessels of LV and cerebral cortex and is largely observed in the aorta and tail artery wall.
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Keyword: neurotrophin-3, TrkC, blood vessels, heart.
Funded by Ministerio de Economía y Competitividad (SAF2013-45362-R). AZ received a fellowship from the Santiago Grisolía Foundation.

P-56
LACTOBACILLUS FERMENTUM CECT5716 CONSUMPTION PROTECTS KIDNEY AND IMPROVES ENDOTHELIAL DYSFUNCTION IN MURINE LUPUS

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Systemic lupus erythematosus (SLE) is a multisystemic chronic autoimmune inflammatory disorder that is associated with a high risk for the development of renal and cardiovascular diseases associated with intestine dysbiosis. *Lactobacillus fermentum* CECT5716 (LC40) is a probiotic bacteria that modulates the human immune system. The aim of this study was to examine whether LC40 ameliorates disease activity and cardiovascular complications in a female mouse model of lupus. Eighteen-week-old NZBWF1 (lupus) and NZW/LacJ (control) mice were treated with LC40 ($5x10^8$ CFU day^{-1}) during 15 weeks. Blood pressure, plasmatic double-stranded DNA autoantibodies, lipopolysaccharides (LPS) and cytokines, nephritis, spleen and mesenteric lymph nodes lymphocytic populations, endothelial function, and vascular oxidative stress were compared in treated and untreated mice. LC40 treatment reduced lupus disease activity, blood pressure, cardiac and renal hypertrophy, splenomegaly, albuminuria, and renal injury in lupus mice. LC40 reduced the elevated T, B, Treg and Th1 cells in spleen and in mesenteric lymph nodes from lupus mice. LC40 lowered the higher plasma concentration of proinflammatory cytokines observed in lupus mice. Aortae from lupus mice showed reduced endothelium-dependent vasodilator responses to acetylcholine. Endothelial dysfunction induced by SLE is related to both increased NADPH oxidase driven-superoxide production and Rho-kinase mediated eNOS inhibition which were normalized by LC40. In addition, LC40 treatment reduced hypertension, endothelial dysfunction, and organ damage in severe lupus mice, which was associated with reduced plasma antidouble-stranded DNA autoantibodies and anti-inflammatory and antioxidant effects in target tissues. Probiotic administration to lupus mice reduced the metabolic endotoxemia since it decreased the LPS plasmatic levels, which were related to a significant improvement of the gut barrier disruption. In conclusion, our findings identify this gut microbiota manipulation as a promising target for an alternative approach in the treatment of SLE and its associated vascular damage.
Keywords: probiotics, lupus, endothelial dysfunction, kidney, T cell, nitric oxide, reactive oxygen species
*These authors contributed equally as first author.

P-57
INCREASED CONTRACTILE RESPONSES TO PHENYLEPHRINE IN THE ASCENDING AORTA OF A MURINE MODEL OF MARFAN SYNDROME REVEAL REGIONAL AND SEX DIFFERENCES

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Marfan syndrome (MFS) is a hereditary disorder of the connective tissue that results from mutations in the gene for fibrillin 1 (*Fbn1*) that cause life-threatening aortic aneurysm. MFS is equally prevalent in

men and women, but epidemiologic evidence suggests that men are at higher risk for aortic complications. Aneurysmal expansion initiates at the aortic root and can progress into the ascending portion. Nevertheless, neither the mechanisms involved in sexual dimorphism nor the ascending aortic reactivity have been fully investigated. We aimed to study the influence of sex on aortic function in 6-month-old MFS (*Fbn1*$^{C1039G/+}$) mice. Ascending and descending thoracic aorta reactivity was evaluated by wire myography and elastic fiber integrity was assessed by Verhoeff-Van Gieson histological staining. Contractions to phenylephrine (Phe) were only altered in ascending aorta of males with MFS, where higher ($P < 0.05$) contractions than wild-type (WT) mice were observed. In contrast, MFS descending thoracic aorta only showed a trend ($P = 0.0599$) toward decreased contraction in males. In ascending aorta, incubation with indomethacin (10 μM), a non-selective cyclooxygenase inhibitor, removed the MFS-induced enhancement of Phe contractions in males. Although endothelium-dependent acetylcholine (ACh)-induced relaxations were similar, the presence of Nω-nitro-l-arginine methyl ester (300 μM) plus indomethacin revealed augmented ($P < 0.05$) endothelium dependent hyperpolarization (EDH)-type relaxations in MFS regardless of sex. Consistently, subsequent inhibition of EDH-type relaxations with small (apamin; 100 nM) and intermediate (charybdotoxin; 100 nM) calcium-activated potassium channel inhibitors abolished MFS-related differences on EDH-type responses. As expected, elastic fiber ruptures increased in MFS compared to WT ascending aortas, but no sex differences were observed. These results suggest that *Fbn1*$^{C1039G/+}$ mice show regional and sex differences in aortic contractility. Increased α_1-adrenoceptor-dependent vasoconstriction and enhanced EDH-type relaxations could be functional adaptations to counteract excessive aortic enlargement in males with MFS. The potential long-term impact of these alterations during aortic disease development deserves attention.

Keywords: Marfan syndrome; sex differences; ascending thoracic aorta reactivity; aneurysm

P-58
PROTECTIVE EFFECTS OF PPARβ/Δ ACTIVATION ON ENDOTHELIAL DYSFUNCTION INDUCED BY PLASMA FROM LUPUS PATIENTS: ROLE OF ENDOPLASMIC RETICULUM STRESS

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Abstract: We tested whether GW0742, a peroxisome proliferator-activated receptor beta/delta (PPARβ/δ) agonist, improves endothelial dysfunction induced by plasma from systemic lupus erythematosus (SLE) patients involving the inhibition of endoplasmic reticulum (ER) stress. 12 lupus and 5 healthy (control) nonpregnant women participated in the study. Cytokines and double-stranded DNA autoantibodies (anti-dsDNA) levels were tested in plasma samples. Endothelial cells, isolated from human umbilical cord veins, (HUVECs) were used to measure nitric oxide (NO), and intracellular reactive oxygen species (ROS) production, NADPH oxidase activity, and ER stress markers. Interferon-γ, interleukin-6, and interleukin-12 levels were significantly increased in plasma from SLE patients with active nephritis (AN), as compared to both SLE patients with inactive nephritis (IN), and control group. The NO production stimulated by both the calcium ionophore A23187 and insulin, was significantly reduced in HUVECs incubated with plasma from AN-SLE patients as compared with control group. Plasma from IN-SLE patients did not modify A23187-stimulated NO production. Increased ROS production and NADPH oxidase activity were found in HUVECs incubated with plasma from AN-SLE patients, which were suppressed by the ER stress inhibitor 4-PBA and the NADPH oxidase inhibitor apocynin. GW0742 incubation restored the impaired NO production, the increased ROS levels, and the increased ER stress markers induced by plasma from AN-SLE patients. These protective effects were abolished by the PPARβ/δ antagonist GSK0660 and by silencing PPARβ/δ. In conclusion, PPARβ/δ activation may be an important target to control endothelial dysfunction in SLE patients.

Keywords: Endothelial dysfunction, PPPARβ/δ, Systemic lupus erythematosus, Nitric oxide, ROS, HUVECs, Endoplasmic reticulum

P-59
ELECTROPHYSIOLOGICAL CHARACTERISATION OF TWO NOVEL KCNK3 (TASK-1) MUTATIONS INDICATED IN PULMONARY ARTERIAL HYPERTENSION

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Introduction: Pulmonary arterial hypertension (PAH) affects approximately 15–50 people per million population (1). KCNK3, the gene that encodes the two pore domain potassium channel TASK-1 (K2P3.1), has been identified as a possible disease-causing gene within familial PAH (2). Recently two new mutations have been identified in KCNK3, G106R and L214R in PAH patients (3). The aim of this study is to assess the electrophysiological profile of these mutated KCNK3 channels and understand how this might contribute to PAH.

Method: Currents through wild-type (WT) and mutated human KCNK3 channels transiently expressed in tsA201 cells were measured using whole-cell patch-clamp electrophysiology. Data are given as mean ± SEM (n=cells) of the current measured between -40 and -80 mV and statistical analysis used one-way ANOVA with post-hoc Dunnett's multiple comparison test.

Results: At pH 8.4, where KCNK3 channels are not inhibited by H ions, G106R and L214R mutant KCNK3 channels had a significantly reduced current amplitude compared to WT channels (WT: 374 ± 59 pA, $n = 6$; G106R 145 ± 35 pA $n = 6$; L214R 197 ± 44 pA, $n = 6$; $P < 0.05$). Similarly, in the presence of the phospholipase A2 inhibitor ONO-RS-082 (10 μM), a compound known to increase KCNK3 current (2), mutant channel current was significantly smaller compared to WT channels (WT: 336 ± 110 pA, $n = 5$; G106R: 40 ± 7 pA, $n = 7$; L214R, 106 ± 20 pA $n = 7$; $P < 0.05$).

Conclusion: The KCNK3 mutants G106R and L214R have been indicated in the pathogenesis of PAH (3). Evidence from this study suggests these mutant channels carry less current than WT channels and, in contrast to other KCNK3 mutations (2) this activity is not recovered by application of ONO-RS-082.

Keywords: Pulmonary arterial hypertension, KCNK3 (TASK-1), Electrophysiology, Potassium channel

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P-60
OPTICAL CONTROL OF MUSCARINIC ACETYLCHOLINE RECEPTORS USING PHOTOSWITCHABLE BITOPIC LIGANDS

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Muscarinic acetylcholine receptors (mAChRs) are class A GPCRs characterized by a widespread tissue distribution and involved in the control of numerous central and peripheral physiological responses. The high sequence homology of the different subtypes (M1–M5) in the transmembrane region hampers the development of subtype selective orthosteric agonists. On the other hand, the allosteric site, located in the extracellular loop, is less conserved, thus muscarinic allosteric agents are commonly endowed with a more pronounced subtype-selectivity. Recently, a new strategy was developed towards the selective modulation of mAChRs, i.e. the development of dualsteric ligands, which are molecules that can bind simultaneously to both the orthosteric and the allosteric sites of such receptors. The most interesting bitopic ligands emerging from this investigation were hybrid derivatives incorporating a) iperoxo, an oxotremorine-related unselective orthosteric superagonist, b) a polymethylene spacer, and c) a moiety targeting the allosteric site.[1]
Inspired by this strategy, in the course of our ongoing development of photoswitchable ligands for the optical control of (neuro)biological functions,[2] we designed and synthesized a new set of light-regulated muscarinic bitopic ligands by replacing the polymethylene spacer chain with an azobenzene linker to serve as molecular photoswitch. This modification enabled the remote control of the mutual position between the two pharmacophoric moieties with light, thus potentially modulating affinity and efficacy of our compounds as a function of their photo isomerization state. One of our ligands (P-Azo-Iper) turned out to be a potent activator of M2 receptors under UV illumination (*cis* isomer), but inactive after relaxation in the dark or under illumination with blue light or white light (*trans* isomer). All compounds were investigated in binding and enzymatic experiments. Their cellular responses were evaluated *in vitro* and *in vivo* in the Xenopus tropicalis heart. The design, synthesis and pharmacological profile of these new photopharmacological tools will be discussed.
Keywords: photopharmacology; muscarinic receptors; dualsteric ligands; GPCRs
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P-61
SENESCENCE AND LATE-ONSET ESTROGEN THERAPY PROMOTE DELETERIOUS EFFECTS IN COMMON CAROTID ARTERY OF FEMALE MICE: INFLUENCE OF EPIGENETIC REGULATION

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The incidence of vascular disease increases with aging at alarming rates in women. Yet, the risk-benefits of estrogen (E_2) therapy in postmenopausal women are still under debate. Controversies may be explained by age of postmenopausal women and timing to start E_2 therapy. We aimed to determine the effects of early (E_2E) and late-(E_2L) onset E_2 therapy in carotid of senescent (SAMP8) and non-senescent (SAMR1) female mice. Six-month-old SAMP8 and SAMR1 were ovariectomized (OVX) and treated with E_2 (5 μg/kg) starting at the first day of OVX (E_2E) or 6 weeks after OVX (E_2L). Concentration-response curves to phenylephrine (Phe1 nM-100 μM) were performed in isolated carotid rings in the absence or presence of the non-selective cyclooxygenase inhibitor, indomethacin (1 μM). Prostaglandin I_2 (PGI_2) and thromboxane A_2 (TXA_2) were measured by ELISA, estrogen receptor (ER) α and β expression by immunofluorescence and the degree of DNA methylation of genes encoding ER by methylation-sensitive qPCR. In OVX-SAMR1, both E_2E and E_2L decreased vasoconstriction. Indomethacin reduced vasoconstriction in non-treated OVX-SAMR1, but was without effect in E_2-treated mice. PGI_2 levels were greater after both E_2 treatments, while TXA_2 was reduced in E_2E vs. OVX-SAMR1. In SAMP8, E_2L, but not E_2E, enlarged vasoconstriction compared to OVX. Indomethacin incubation did not affect vasoconstriction in either SAMP8 group. E_2-treatment in OVX-SAMP8 did not modify PGI_2 release, but increased TXA_2 levels in E_2L mice. Ratio of ERα/ERβ expression was higher in SAMR1 than SAMP8 after E_2E and E_2L treatments. These results are in association with a higher degree of DNA methylation at exon 8 of ERα in SAMP8. In conclusion, senescence abolishes the beneficial effects of E_2 in carotid arteries. While E_2E has no effect on vascular function, E_2L worsen vasoconstriction. These effects are associated with senescence-mediated modifications on DNA-methylation status of ERα, which may influence its pattern of expression.
Keywords: estrogen; carotid artery; senescence; DNA methylation
Financial support: FAPESP (2015/26690-9), CAPES (269/12), Programa Hispano-Brasileño de Cooperación Interuniversitaria (HBP-2011-0054_PC); Red de Investigación Cardiovascular – HERACLES (RD12/0042/0006).

P-62
INFLUENCE OF LYCOPENE ON METABOLIC SYNDROME INDUCED BY FRUCTOSE IN WISTAR RATS

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Overconsumption of fructose results in dyslipidemia, hypertension, and impaired glucose tolerance, which characterizes the metabolic syndrome (MS). Thus, the aim of this study was to investigate the effects of a diet supplemented with lycopene on MS induced by fructose in rats.
Methods: Wistar rats were divided into 3 groups: control **(C)** fed with standard diet, group **(F)** receiving a supplementation of 20% fructose and group **(F+L)** fed with a diet supplemented with 20% fructose plus 0.01% of lycopene, for 8 weeks. Blood pressure was monitored by the tail cuff method. Glucose tolerance test and plasmatic triglycerides levels were determined. The liver hypertrophy index was determined using liver weight/body weight ratio.
The endothelial function was evaluated on aortic rings. Concentration-response curves to acetylcholine and phenylephrine were performed in rings with or without perivascular adipocyte tissue.
Results: High fructose diet caused a significant increase on blood pressure (from 108 ± 1 to 134 ± 2 mmHg), plasma triglycerides levels, oral glucose tolerance, and liver weight. The endothelium function in presence of perivascular fat was also impaired.
Lycopene supplementation prevented the increase of blood pressure and improved the oral glucose tolerance compared to group (F). Lycopene treatment was able to reduce the triglycerides levels in plasma increased by fructose ingestion. The liver hypertrophy index was

higher in group (**F**) than group **(F+L)** and control group (29.3 ± 1.8 vs. 26.3 ± 1.6 and 27.1 ± 1.6 mg/g respectively, $P < 0.05$). In addition, lycopene treatment ameliorated the endothelial function in presence of perivascular adipocyte tissue, recovering the impairment of vasorelaxation and vasoconstriction.

Conclusion: Our results indicate that a diet supplemented with lycopene may be a beneficial therapeutic approach for MS.

Lycopene was kindly supply by DSM NUTRITIONAL PRODUCTS (México).

Keywords: Lycopene; metabolic syndrome; fructose

P-63
EFFECT OF THE PROBIOTIC *LACTOBACILLUS FERMENTUM* CECT5716 IN HYPERTENSIVE RAT INDUCED BY CHRONIC NITRIC OXIDE BLOCKADE

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Lactobacillus fermentum CECT5716 (LC40) reduced blood pressure in spontaneously hypertensive rats. We tested whether the probiotic Lactobacillus fermentum CECT5716 ameliorates hypertension in rats with chronic nitric oxide synthase inhibition. Rats were randomly divided into four different treatment groups for 4 weeks: a) vehicle (control, 1 ml of tap water once daily), b) vehicle plus L-NAME (50 mg 100 ml^{-1} in drinking water), c) LC40 (10^9 colony-forming units/day by gavage), and d) LC40 plus L-NAME. The evolution in systolic blood pressure, and morphological variables, proteinuria, lymphocytes populations in mesenteric nodes and vascular NADPH oxidase activity and vascular reactivity at the end of experiment were analysed. LC40 did not inhibit the development of L-NAME-induced hypertension, the increase in the left ventricular hypertrophy, and proteinuria. However, this probiotic partially prevents the impaired endothelium- and NO-mediated relaxation to acetylcholine in aorta, being without effects in small mesenteric arteries. This improvement was suppressed in presence of apocynin in the bath and was accompanied by reduced NADPH oxidase activity in both arterial beds from LC40 plus L-NAME group as compared with L-NAME group. In mesenteric nodes L-NAME increases Th17 cells and reduces Treg cells. These changes were prevented by LC40 treatment. In most cases these effects were not observed in normotensive animals. In conclusion, this study confirms the critical role of NO in the antihypertensive and end-organ protective effects of the probiotic LC40 in animal models of hypertension.

Keywords: L-NAME-induced hypertension, Probiotics, LC40, NADPH oxidase, Nitric oxide, immune response

P-64
POMACE OLIVE OIL ENRICHED IN OLEANOLIC ACID RESTORES VASCULAR DYSFUNCTION AND METABOLIC PARAMETERS IN MICE FED A HIGH FAT DIET

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Background and objective: Despite the amount of research on the effects of virgin olive oil and its components in cardiovascular disease, little attention has been paid to the effects of Pomace Olive Oil, an subproduct traditionally used in Spain, which is an important source of triterpenic acids such as oleanolic acid. Our aim was to evaluate the potential effects of a Pomace Olive Oil enriched in oleanolic acid (POMO) on blood biochemical parameters and vascular function related to obesity.

Methods and results: Male C57BL/6J mice were fed a high fat diet (HFD) for 10 weeks. Mice were randomized into 2 groups: 1) HFD; 2) HFD supplemented with POMO (17% w/w) for 10 weeks. In parallel, another group was fed a standard diet (SD). Body weight, food and water intake were weekly evaluated. After treatment, functional studies were performed in aortic rings in isometric myograph and serum glucose and total cholesterol (TC) were determined. Introduction of POMO in the diet reduced body weight gain ($P < 0.0001$ vs. HFD). Oral glucose tolerance test revealed significant differences between SD and HFD animals ($P < 0.01$ vs. SD) that were restored by POMO administration ($P < 0.05$ vs. HDF). Serum glucose and TC levels were also attenuated in POMO-fed mice ($P < 0.001$ vs. HFD). POMO also restored endothelial dysfunction in aorta compared to HFD group. In aortas from POMO group, the ACh relaxation was not completely abolished by L-NAME, suggesting the possible involvement of a NO-independent mechanism.

Conclusion: These findings suggest that pomace olive oil enriched in oleanolic acid reduces body weight, improves glucose tolerance and hypercholesterolemia in obese mice and ameliorates vascular dysfunction associated to HFD-induced obesity.

Keywords: Pomace Olive Oil, Vascular function, Glucose tolerance, High-fat diet

P-65
MARCKS REGULATES VASCULAR CONTRACTILITY

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Myristoylated alanine-rich C-Kinase (MARCKS) is expressed in vascular smooth muscle cells (VSMCs), but its function is unclear. The present work investigates the role of MARCKS in regulating vascular tone.

Experiments were carried out using freshly isolated VSMCs and tissue lysates from rat and mice mesenteric arteries. Expression and interactions between MARCKS, voltage-dependent Ca^{2+} channels (VDCCs), and PIP_2, were studied using western blot, immunocytochemical, co-immunoprecipitation, and dot-blot methods. The cell-permeable MARCKS inhibitor, MANS peptide, and MARCKS knockdown were examined on contractility using isometric tension recordings. The result of MANS peptide on VDCC activity was measured using whole-cell patch clamp techniques.

MARCKS was expressed in mouse and rat mesenteric arteries, predominantly at the plasma membrane. MANS peptide evoked concentration-dependent increases in vascular contractility, which were abolished by VDCC blockers. Knockdown of MARCKS significantly reduced contractions evoked by MANS peptide. In un-stimulated tissue, MARCKS interacted with the L-type VDCC protein, $CaV_{1.2}$, and PIP_2. MANS peptide released PIP_2 from MARCKS and increased interactions between $CaV_{1.2}$ and PIP_2. MANS peptide induced an increase in the peak amplitude, and a negative shift in activation and inactivation of whole-cell VDCC currents. Depletion of PIP_2, with wortmannin, abolished excitatory effects of MANS peptide on whole-cell VDCC activity.

This is the first study to demonstrate that MARCKS is involved in regulating vascular contractility. We propose that in un-stimulated vessels, MARCKS prevents opening of VDCCs by acting as a PIP_2 buffer, but following MARCKS inhibition PIP_2 is released leading to channel gating and contractility.

Keywords: MARCKS; PIP_2, VDCCs, Vascular Contractility

P-66
LUPALBIGENIN, A PRENYLATED ISOFLAVONE IN *DERRIS SCANDEN* EXTRACTS, LACKS VASODILATOR ACTIVITY IN THE PORCINE ISOLATED SPLENIC ARTERY

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Extracts of the isoflavone-rich plant *Derris scandens* are used in S.E Asia for musculoskeletal pain instead of NSAIDs[1] and the isoflavone genistein has been advocated for the treatment of rheumatoid arthritis because it possesses anti-inflammatory activity[2]. Any benefit of genistein in this condition may be offset by the well-known, oestrogen-like vasodilator effect of isoflavones[3]. A prenylated metabolite of genistein, lupalbigenin, is a key component of *Derris scandens*, and we compared the vascular activity of this isoflavone with that of genistein, daidzein and 17-ß-oestradiol in the porcine isolated splenic artery.

Porcine isolated splenic artery segments (4 mm) were prepared for isometric tension as previously described[4]. After establishment of stable submaximal contractions to the thromboxane-mimetic U46619, cumulatively increasing concentrations of the isoflavones were added. Effects of drugs have been expressed as negative logarithm of the concentration required to reduce contractions by 50% (pIC_{50}).

Genistein (pIC_{50} 5.30 ± 0.08, n = 7), daidzein (pIC_{50} 5.03 ± 0.15, n = 7) and 17-ß-oestradiol (pIC_{50} 4.63 ± 0.14, n = 10) caused slow, concentration-dependent inhibition of U46619-induced contractions and abolished tone. In contrast, lupalbigenin (3–30 μM) induced small rhythmic contractions (approx' 30% of U46619 tone) without affecting basal tone. Neither the vasodilator activity of genistein (pIC_{50} 5.19 ± 0.19), nor the maximum response, were affected by the presence of 30 μM lupalbigenin (genistein pIC_{50} 5.07 ± 0.20, n = 7).

Genistein, and daidzein inhibited vascular tone of the porcine isolated splenic artery in a similar manner to 17-ß-oestradiol, while the prenylated isoflavone, lupalbigenin, facilitated contractile activity. Future experiments will establish whether lupalbigenin possesses anti-inflammatory activity, like other isoflavones[2], and can account for the beneficial effects attributed to *Derris scandens* extracts in musculoskeletal conditions.

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Keywords: Genistein, Lupalbigenin, porcine splenic artery, oestrogen

P-67
ROLE OF MITOGEN-ACTIVATED PROTEIN KINASE (MAPK) IN THE REGULATION OF L-TYPE CHANNEL CALCIUM ENTRY IN RESISTANCE ARTERIES

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Ca^{2+} entry through L-type channels plays a key role in arterial vasoconstriction but also in Ca^{2+}-dependent gene transcription of vascular smooth muscle (VSM) (Kudryavtseva et al, *FEBS J* 280: 5488, 2013). Extracellular signal regulated (ERK) mitogen activated protein kinase (MAPK) phosphorylates and activates the cardiac L-type Ca^{2+} channel and modulates agonist-induced L-type Ca^{2+} entry in cardiac myocytes (Takashi et al., *Circ Res* 94:1242, 2004). In the present study, we sought to assess whether MAPK plays a role in the regulation of Ca^{2+} entry in resistance arteries. Simultaneous measurements of $[Ca^{2+}]_i$ and tension were performed by Fura-2M ratio fluorometry in 3rd order branches of mesenteric arteries from Wistar rats mounted in a microvascular myograph. Activation of α-1 adrenoceptors with phenylephrine (Phe, 10 μM) induced a pronounced vasoconstriction largely inhibited by blockade of L-type Ca^{2+} channels with nifedipine. Phe stimulated Ca^{2+} entry along with contraction and removal of extracellular Ca^{2+} markedly reduced Phe responses to small transients in $[Ca^{2+}]_i$ and tension (8 ± 3 and 5 ± 1% of control, n = 5, respectively). Readdition of Ca^{2+} to the medium concentration-dependently restored Phe-induced increases in Ca^{2+} and contraction indicating that α_1-mediated vasoconstriction is largely dependent on Ca^{2+} influx to VSM. Inhibition of the ERK-MAPK with PD98059 (3 μM) reduced by 37 ± 8% ($P < 0.01$, n = 5, vs. control) the increase in $[Ca^{2+}]_i$ elicited by Phe without altering vasoconstriction. Moreover, PD98059 inhibited by 41 ± 9% ($P < 0.01$, n = 4, vs. control) Ca^{2+} entry induced by depolarization of the arteries with a high K^+ solution. The present results provide evidence for an involvement of the ERK-MAPK pathway in the L-type channel Ca^{2+} entry not coupled to vasoconstriction in resistance arteries.

Keywords: L-type calcium channel, ERK-MAP kinase, resistance arteries

This work was supported by grant SAF 2012-31631 from MINECO, Spain.

P-68
PATAGONIA CALAFATE (*BERBERIS MICROPHYLLA*) BERRY EXTRACTS HAS A POTENT CELLULAR ANTIOXIDANT PROFILE AND ELICITS ENDOTHELIUM-DEPENDENT VASCULAR RELAXATION

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Patagonian calafate (Berberis microphylla) is a native dark purple berry reported with the highest antioxidant profile from a collection of endemic southern chilean fruits; the pharmacology of this fruit has been scarcely investigated. Chemically, the berry has a high ascorbic acid and polyphenolic content which includes anthocyanins, flavonols and phenolic acid derivatives. Since red berries cultured in southern Chile have a beneficial effect in chronic vascular diseases, we proposed that extracts of calafate berries might have be vasoactive and present a favorable cellular antioxidant profile.

To this aim, ethanol or acetone calafate extracts (1–300 μg/ml), were evaluated as mesenteric bed vasodilators previously contracted with 50 μM noradrenaline and assessed whether the effect involves endothelial nitric oxide (NO) production and release. Cellular antioxidant activity was evaluated in isolated endothelial cells.

Calafate extracts elicited a concentration-dependent antioxidant activity comparable to that of quercetin standards. Extracts elicited a concentration-dependent vasodilator response with an EC_{50} of 3.5 μg/ml; the maximal response was reached with 10–30 μg/mL (which caused a 75–80% vasodilatation in noradrenaline precontracted preparations). The vasodilatation was significantly reduced by either endothelium removal (after a 90 sec 0.1% saponin perfusion) or eNOS inhibition with 150 μM N-nitro-L-arginine. Moreover, relaxation significantly correlated with the concentration-dependent extract NO production (R^2 =0.8, $P < 0.05$). The chemical polyphenol extract profile (derived from HPLC-ESI-MS/MS analysis), identified delphinidin, petunidin and malvidin as 3-glucosides as the main extract polyphenols. We are in the process of assessing whether these molecules could partially explain the extracts induced-vascular responses.

In conclusion, the calafate berry extracts elicited a potent endothelium-dependent vascular relaxation coupled NO production, a vascular response likely linked to its cellular antioxidant properties.

Keywords: Vascular relaxation, cellular antioxidant activity, nitric oxide and anthocyanins of calafate

Funding sources FONDECYT 114-1132 and Conicyt doctoral grant 21141226.

P-69
PIOGLITAZONE ALTERS THE VASCULAR CONTRACTILITY IN HYPERTENSION BY INTERFERENCE WITH ET-1 SYSTEM

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Introduction: Hypertension is an inflammatory disease associated with high cytokine levels and proinflammatory mediators such as cyclooxygenase-2 (COX-2) and reactive oxygen species (ROS), which contribute to develop the hypertension-associated vascular alterations. The endothelin (ET) system is an important modulator of vascular tone; alterations in this system contribute to hypertension. Peroxisome proliferator-activated receptors-γ (PPARγ) have significant antiinflammatory actions by transrepression mechanisms. Its activation has cardioprotective effects by decreasing cytokines and ROS production.
Aim: To analyse the modulation of the vascular ET-1 system by PPARγ in hypertension. For this, we have used: (1) vascular segments from Wistar-Kyoto (WKY) and spontaneously hypertensive rats (SHR) treated or not with the PPARγ agonist pioglitazone (2.5 $mg \cdot kg^{-1} \cdot day^{-1}$, 28 days), and (2) vascular smooth muscle cells (VSMC) from both strains stimulated with ET-1 incubated or not with pioglitazone.
Results: Pioglitazone reduces the increased vascular COX-2, pre-pro-ET-1 and ET_AR expressions observed in hypertension, while increases the ET_BR levels. In SHR, but not in WKY, ET-1, through ET_AR, potentiates phenylephrine-induced contraction. TP receptors activation and NO bioavailability reduction, associated to the increased ROS, participate in this effect. In arteries from pioglitazone-treated SHR, ET-1 reduces phenylephrine contraction through ET_BR by increasing NO bioavailability associated to oxidative stress reduction. In VSMC from SHR, ET-1 induces COX-2 expression through ET_AR; activation of AP-1 and NF-κB and the subsequent ROS production plays a role in this effect. Pioglitazone, by interfering with AP-1 and NF-κB and the subsequent ROS production, reduces this COX-2 expression
Conclusion: Pioglitazone, by reducing the ET-1 effect, diminishes the increased vascular COX-2 expression in hypertension; in addition, pioglitazone shifts the ET_A/ET_B ratio expression. In this situation, ET-1 induces an opposing effect on phenylephrine-induced contraction by increasing NO bioavailability. The antioxidant activity contributes to these vascular effects.
Supported by MINECO (SAF2015-69294-R) and ISCIII (CB16/11/00286).
Keywords: ET-1; hypertension; vascular responses; PPARγ

P-70
DECREASED EXPRESSION OF ETS-2 IN EPCS AS AN EARLY MARKER OF CARDIOVASCULAR INSTABILITY IN CABG PATIENTS, BENEFICIAL EFFECTS OF SITAGLIPTIN

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In patients with cardiovascular disease (CVD), the endothelial progenitor cells (EPCs) play a key role in endothelial repair processes. It has been hypothesized that Ets2 transcription factor could be involved in the instability of CVD/hyperlipidaemia. Our objective was to determine the degree of expression of transcription factor Ets2 and the endothelial repair factors Endoglin and CXCR4 in EPCs from patients undergoing coronary artery-bypass grafting (CABG), and to prove a possible beneficial effect of Sitagliptin.

Methods: seventy patients undergoing CABG from the cardiac surgery service were selected for the study and categorized into five CVD stages. The Expression of Ets-2, CXCR4, and Endoglin were measured by Western blot. Sitagliptin effect on EPCs and on the expression of different factors was studied by Wb, ELISA and immunofluorescence.
Results: An increase in the expression of the transcription factor Ets-2 in patients, without CV predictor risk factor associated with early stages of CV instability was observed (GI: 2.16 ± 1.4). Ets-2 expression decreased in patients with longer evolution of CVD (GII: 1.16 ± 0.8; GIII1.06 ± 0.5; GIV: 0.91 ± 0.3; GV: 0.56 ± 0.3). A direct association of expression of Ets-2 with age ($P = 0.04$) and Endoglin expression ($P = 0.008$), and indirectly with the evolution of CVD ($P = 0.008$) and hyperlipidaemia ($P = 0.03$) was found. A direct effect of Sitagliptin 1 μM on the colony formation of EPCs at 6 and 12 hours ($P = 0.0077$) and on expression of CXCR at 24 hours ($P = 0.0201$) and SDF1 at 6 and 24 hours ($P = 0.0085$) was observed.
Conclusions: The transcription factor Ets-2 could be an early marker of cardiovascular instability. Our data suggest that a poor functionality of circulating EPCs could be associated with decreased expression of the transcription factor Ets-2 in advanced stages of cardiovascular disease. Sitagliptin treatment of cultured EPCs, could help to revert EPCs deficient functionality. Therefore, the activatory response might depend on the SDF1-CXCR4 axis.
Keywords: cardiovascular disease (CVD), the endothelial progenitor cells (EPCs), Ets2 transcription factor, Endoglin, CXCR4, Sitagliptin

P-71
HYPOXIC PRECONDITIONING INCREASES THE POTENTIAL OF EXOSOMES DERIVED FROM MESENCHYMAL STEM CELLS TO LIMIT PULMONARY VASCULAR DYSFUNCTION INDUCED BY LIPOPOLYSACCHARIDE (LPS)

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Preclinical studies have demonstrated promising results using mesenchymal stem cells (MSCs) for the treatment of inflammatory lung diseases, including acute respiratory distress syndrome (ARDS), pulmonary arterial hypertension (PAH) or bronchopulmonary dysplasia (BPD). However, recent evidences suggest that their therapeutic activity is mediated by paracrine mediators, most notably exosomes, rather than cell engraftment. Hypoxic preconditioning is thought to enhance the therapeutic potential of MSCs. In this work, we aimed to analyse whether MSCs-derived exosomes are able to prevent the pulmonary vascular dysfunction induced by lipopolysaccharide (LPS) and to enhance their effectiveness by the use of hypoxia-preconditioning.
Exosomes released by umbilical cord blood (UCB)-derived MSCs cultured under normoxic (21% O_2) or hypoxic (3% O_2) conditions were obtained by differential ultracentrifugation. Rat pulmonary artery (PA) smooth muscle cells (PASMCs) were treated with 1 μg/ml of LPS for 48 hours in the absence or presence of MSC-derived exosomes (0.5–5 μg/ml). IL-6 levels were determined by ELISA. Contractile responses were analysed in rat PA mounted in a wire myograph.
Exposure to LPS significantly increased IL-6 release by rat PASMCs (Ctrl = 2.56 ± 0.24; LPS = 75.46 ± 4.16 ng/ml), inhibited hypoxic pulmonary vasoconstriction (HPV), induced endothelial dysfunction and potentiated the contractile effects induced by serotonin in isolated PA. Treatment with normoxic MSC-derived exosomes had no effect in these responses. In contrast, hypoxia-preconditioned MSCs-derived

exosomes significantly prevented the impairment of HPV, the hyperresponsiveness to serotonin and attenuated the reduction of the relaxant responses to acetylcholine. Neither exosome preparation was able to decrease the IL-6 release induced by LPS.
Our data show that hypoxia-preconditioned MSCs-derived exosomes prevent the development of pulmonary vascular dysfunction induced by LPS in isolated PA. These findings suggest that hypoxic preconditioning increases the therapeutic potential of MSCs-derived exosomes and may represent a new therapeutic approach in pulmonary vascular diseases associated with inflammation.
Supported by ISCIII (CP12/03304 and PI15/01100).
Key words: Exosomes, inflammation, pulmonary vascular dysfunction.

P-72
PHARMACOLOGICAL AND GENETIC DISSECTION OF THE ROLE OF TRPA1 AND TRPM8 CHANNELS IN INTRINSIC VASCULAR RESPONSES TO COLD

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It is well-known that cold induces vasoconstriction in skin blood vessels as a protective response against heat loss. This phenomenon is thought to be mediated by an efferent reflex to the activation of cold-sensitive afferent nerves in the skin. In contrast to this view, we found in *ex vivo* myography experiments that cooling to 10°C induced a 28 ± 3.01% contraction in endothelium-denuded plantar arteries dissected from wild type (WT) mice. Cold produced a significantly smaller vasoconstriction in the presence of the TRPA1 inhibitor HC030031 (9 ± 2.23%), and in arteries dissected from *Trpa1* (11.04 ± 2.78%) or *Trpm8* (10 ± 1.54%) knockout (KO) animals. Application of HC030031 virtually abolished the responses to cold in arteries from *Trpm8* KO (2 ± 1.13%). Neither TRPA1 nor TRPM8 channels could be detected in vascular smooth muscle cells, suggesting that the effects of cold are mediated by activation of these channels in perivascular sensory and/or sympathetic nerves. Cold-induced vasoconstriction was potentiated in the presence of the CGRP receptor inhibitor BIBN 4096 (40 ± 4.26%) and reduced after depletion of catecholamines from the sympathetic nerve terminals with guanethidine (9 ± 2.6%). Cold had no effect in arteries were incubated with both BIBN 4096 and guanethidine. We detected mRNA of both TRPA1 and TRPM8 channels in sympathetic ganglia isolated from WT mice and we could confirm the presence of perivascular sympathetic nerves in confocal images of plantar arteries labelled with an anti-TH antibody. Taken together, our data demonstrates that cold has a dual action, mediated by both vasodilatory and a vasoconstrictor responses via the activation of TRPA1 and TRPM8 channels. To our knowledge, our results represent the first evidence for an intrinsic response to cold in cutaneous arteries and for the presence and the functional role of sensory TRP channels in efferent nerve fibers.
Keywords: TRPA1, cold, TRPM8, vascular response

P-73
DYNAMIC MASS REDISTRIBUTION PHENOTYPIC ASSAY FOR IDENTIFYING LIGANDS ACTIVE AT GPR35 RECEPTORS

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Introduction: GPR35 is an orphan receptor reported to be involved in inflammatory disorders (HU H. 2012), CNS disfunction (Shore DM 2015), pain (Alkondon M 2015), diabetes (Tanoguchi Y 2006) and immunological diseases such as asthma (Yenkins L 2010). Kynurenic acid (HU H. 2012), 2-oleoyl lysophosphatidic acid and recently chemokine CXCL17 (Berlinguer-Palmini R 2013) have been described as potential endogenous ligands but without confirmation; showing differences in efficacy between human and rat orthologues (Yenkins L 2010). Phenotypic assays based on Dynamic Mass Redistribution (DMR) revealed as powerful tool screening libraries in orphan receptors which signalling pathways and biology remain unknown. We aimed to develop a miniaturized phenotypic assay based on a DMR label-free technology that enables the detection of new ligands for human and rat GPR35 receptors.
Material and Methods: HT-29 cell line and IEC-6 cell line were seeded in LFC-384 well microplates (PerkinElmer 6057408). 24 hours after seeding, the culture medium was replaced with medium or HBSS buffer to optimize the assay conditions. Plates were incubated before reading a base line and later standard compounds were added. Measurements were done using an EnSpire (PerkinElmer) reader with Corning ® Epic® Label-free technology.
Results: A miniaturized phenotypic assay based on a DMR was developed to find new ligands for hGPR35 and rGPR35. For both cell lines, 15000 cells per well were selected as a suitable concentration. McCoy′s 5A medium supplemented with 25 mM HEPES pH=7.4 was selected as Buffer Assay for HT-29 whereas HBSS buffer was selected for IEC-6. To validate the feasibility of the method, we obtained concentration-response curves of a synthetic agonist of GPR35 (Taniguchi Y 2008), Zaprinast, with values of EC_{50}=0.50 ± 0.25 μM for human GPR35 and values of EC50 = 4.2 ± 0.30 μM for rat GPR35.
Conclusions: We have developed a miniaturized phenotypic assay based on a DMR technology to measure the activity of compounds in human and rat cell lines expressing the orphan receptor GPR35.

P-74
FUNCTIONAL μ-OPIOID-GALANIN RECEPTOR HETEROMERS IN THE VENTRAL TEGMENTAL AREA

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The neuropeptide galanin has been shown to interact with the opioid system. More specifically, galanin counteracts the behavioral effects of the systemic administration of μ-opioid receptor (MOR) agonists. Yet the mechanism responsible for this galanin– opioid interaction has remained elusive. Using biophysical techniques in mammalian transfected cells, we found evidence for selective heteromerization of MOR and the galanin receptor subtype Gal1 (Gal1R). Also in transfected cells, a synthetic peptide selectively disrupted MOR–Gal1R heteromerization as well as specific interactions between MOR and Gal1R ligands: a negative cross talk, by which galanin counteracted MAPK activation induced by the endogenous MOR agonist endomorphin-1, and a cross-antagonism, by which a MOR antagonist counteracted MAPK activation induced by galanin. These specific interactions, which represented biochemical properties of the MOR-Gal1R heteromer, could then be identified *in situ* in slices of rat ventral tegmental area (VTA) with MAPK activation and two additional cell signaling pathways, AKT and CREB phosphorylation. Furthermore, *in vivo* microdialysis experiments showed that the disruptive peptide selectively counteracted the ability of galanin to block the dendritic dopamine release in the rat VTA induced by local infusion of endomorphin-1, demonstrating a key role of MOR-Gal1R heteromers localized in the VTA in the direct control of dopamine cell function and their ability to mediate antagonistic interactions between MOR and

Gal1R ligands. The results also indicate that MOR-Gal1R heteromers should be viewed as targets for the treatment of opioid use disorders.
Keywords: dopamine; galanin receptor; MAPK; opioid receptor; receptor heteromer; ventral tegmental area

P-75
CHARACTERIZATION OF THE ACTIONS OF THE NOVEL $P2Y_2$ RECEPTOR ANTAGONIST, AR-C118925XX

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Introduction: The endogenous nucleotides, adenosine 5′-triphosphate (ATP), uridine 5′-triphosphate (UTP), and uridine 5′-diphosphate (UDP) act via P2X and P2Y receptors. Due to the limited selectivity of most antagonists, the functions of many individual P2 subtypes are poorly characterised. A putative $P2Y_2$ antagonist, AR-C118925XX, has recently become available, so the aims were to quantify the action of AR-C118925XX at recombinant $P2Y_2$ receptors and then to determine the contribution of $P2Y_2$ receptors to nucleotide-evoked vasoconstriction.
Methods: Recombinant human $P2Y_1$, $P2Y_2$, $P2Y_4$ and $P2Y_{11}$ receptors, stably expressed in 1321N1 cells, were grown on glass coverslips, which were placed in a fluorimeter, and intracellular Ca^{2+} measured using the Ca^{2+}-sensitive dye, Cal-520AM. Concentration-response curves (CRC) were constructed by superfusing cells with agonists ($P2Y_1$-ADP, $P2Y_2$-UTP, $P2Y_4$ and $P2Y_{11}$-ATP), in the absence and presence of AR-C118925XX. The Hill equation was fitted to the data and a Schild plot generated using the EC_{50} values. 5 mm rings of rat intrapulmonary artery (rIPA) were mounted under isometric conditions *in vitro* at 37°C. Contractions were elicited by addition of P2Y agonists to the bath.
Results: In 1321N1 cells expressing $hP2Y_2$ receptors, UTP (10 nM-3 μM) evoked a concentration-dependent rise in intracellular Ca^{2+} (EC_{50}=54 nM, 95% cl. 43–67 nM, n = 5). Increasing concentrations of AR-C118925XX (10 nM-1 μM), produced a progressive rightward shift in the UTP CRC, with no decrease in maximum response (n = 6 each). These inhibitory effects were fully reversible. Schild analysis produced a pA_2=8.30 and slope=0.985. In contrast, AR-C118925XX (1 μM) had no effect at human $P2Y_1$, $P2Y_4$ and $P2Y_{11}$ receptors (n = 5 each). In rIPA UTP, ATP and UDP (300 μM) evoked reproducible contractions that were unaffected by AR-C118925XX (1 μM) (n = 4 each).
Conclusion: AR-C118925XX is a potent, competitive and reversible $P2Y_2$ antagonist, making the identification of the physiological functions of $P2Y_2$ receptors possible. $P2Y_2$ receptors do not appear to play a role in nucleotide-evoked contractions of rIPA.
Keywords: P2Y receptors AR-C117825XX pulmonary artery

P-76
SIGNALING OF THE FRACTALKINE RECEPTOR CX_3CR1 AND ITS NATURAL GENETIC VARIANTS: IMPACT OF RECEPTOR NON-SYNONYMOUS SINGLE NUCLEOTIDE POLYMORPHISMS

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The chemokine receptor CX_3CR1 is a $G_{i/o}$ G protein-coupled receptor (GPCR) expressed in monocytes, NK cells, T lymphocytes, astrocytes and microglia, among other cells, and it plays an important role in inflammation and immunity as well as in neuron-microglia communication in the Central Nervous System [1]. Its known ligand is fractalkine (CX_3CL1), the sole member of the CX_3C chemokine subfamily. Genomic studies have identified non-synonymous single nucleotide polymorphisms (nsSNPs) in the *CX_3CR1* gene. Specifically, the common receptor genetic variant CX_3CR1-V249I/T280M has been associated with faster progression to disease in HIV-infected patients, cardiovascular atheroprotection, increased risk of age-related macular degeneration, and obesity [2]. We aimed to investigate the possible functional impact of the currently identified nsSNPs of CX_3CR1 on the pharmacology of this receptor.
Receptor interaction with G protein-coupled receptor kinase 2 (GRK2) and beta-arrestins were investigated in transfected HEK293 cells by bioluminescence resonance energy transfer (BRET)-based assays. Our results indicate that the CX_3CR1-V249I/T280M receptor variant interacts with more efficacy than the wild type receptor with beta-arrestins 1 and 2 (Emax 146% and 204% of wild type, respectively) and GRK2 (Emax 310% of wild type) in response to fractalkine. The functional impact of this observation on the dynamics and compartmentalization of the receptor signaling is being further investigated. Being GRKs and beta-arrestins crucial regulators of G protein-dependent and -independent signaling of GPCRs [3], our findings expand our current knowledge on the signaling pathways modulated by CX_3CR1 and their possible implications in physiological and pathological condition.
Acknowledgements: This work is funded by the Spanish Ministry of Economy and Competitiveness (MINECO) (SAF2014-57138-C2-1-R).
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P-77
GLIBENCLAMIDE INHIBITS ATP-SENSITIVE K CURRENTS IN THE CANTU SYNDROME

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(*KCNJ11*) and β-subunits SUR2A (*ABCC9*). Cantu syndrome (CS) is a rare genetic disorder, which leads to multiple alterations including hypotension and cardiac abnormalities. CS is caused by mutations in *KCNJ8* and *ABCC9* that reduce channel sensitivity to ATP-blockade, increasing K_{ATP} channel activity. Glibenclamide, a K_{ATP} blocker, has been proposed as a potential treatment for CS. However, it is unknown whether CS-associated mutations are sensitive to K_{ATP} modulating drugs. We analyzed the effects of glibenclamide on pinacidil-activated K_{ATP} currents (I_{KATP}) generated by channels with Kir6.1 (V65M and G343D) and SUR2A (H60Y, S1020P, and S1054Y) CS-associated mutations. I_{KATP} were recorded in Chinese Hamster Ovary (CHO) cells transiently transfected with the cDNA encoding WT or mutant Kir6.x and SUR2A subunits by whole-cell patch-clamping. Pinacidil increased I_{KATP} generated by Kir6.1WT+SUR2AWT and Kir6.2WT+-SUR2AWT channels in a concentration-dependent manner, the effective concentration 50 (EC_{50}) being 1.9 ± 0.03 and 4.9 ± 1.1 μM, respectively. The EC_{50} values for pinacidil were significantly increased

in the channels composed by V65M (5.5 ± 0.03 μM) and G343D (8.4 ± 0.04 μM) Kir6.1 + SUR2AWT subunits. Pinacidil-induced increase of Kir6.2WT+SUR2A H60Y and Kir6.2WT+SUR2A S1020P was similar to that of Kir6.2WT+SUR2AWT channels. Unexpectedly, pinacidil failed to increase I_{KATP} generated by Kir6.2WT+SUR2A S1054Y channels. Glibenclamide inhibited currents generated by Kir6.1WT+SUR2AWT and Kir6.2WT+SUR2AWT in a similar extent, yielding inhibitory concentration 50 (IC_{50}) values (0.3 ± 0.1 and 0.22 ± 0.06 μM, respectively) within the range of therapeutic concentrations of glibenclamide. The presence of the V65M mutation in Kir6.1 significantly reduced the glibenclamide potency (IC_{50}=1.6 ± 0.04 μM), while the rest of the mutations in Kir6.1 (G343D) or SUR2A (H60Y and S1020P) did not affect the glibenclamide-induced block. These results demonstrate that most of the CS-associated mutations reduced channel response to pinacidil, whereas only the V65M mutation in Kir6.1 affected channel sensitivity to glibenclamide.

Keywords: Kir6.x, SUR2A, glibenclamide, Cantu Syndrome, cardiac

P-78
THE PARKINSON'S DISEASE-ASSOCIATED GPR37 RECEPTOR IS AN ADENOSINE A_{2A} RECEPTOR REPRESSOR IN MICE

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GPR37, also known as parkin-associated endothelin-like receptor (Pael-R), is an orphan G protein-coupled receptor that has been related with Parkinson's disease (PD) neuropathology. Interestingly, the genetic blockade of GPR37 enhances cell surface expression of striatal dopamine transporter (DAT), resulting in reduced dopamine content in the striatum. In addition, it has been shown that deletion of GPR37 triggers anxiolytic-like effects and sensitizes mice to adenosine A_{2A} receptors ($A_{2A}R$)-mediated signaling. Here we report that GPR37 and $A_{2A}R$ physically and functionally interact both in living cells and in native tissue. Thus, by using biochemical techniques (i.e. co-immunoprecipitation and proximity ligation assay) we demonstrated a physical interaction between these two receptors in the striatum. Also, by means of post-*embedding immunogold-electron microscopy* (EM) *techniques* detection it was demonstrated a close proximity at the postsynaptic level of striatal synapses. On the other hand, GPR37 deletion promoted striatal $A_{2A}R$ cell surface expression, which correlated well with an increased $A_{2A}R$ agonist-mediate cAMP accumulation both in primary striatal neurons and synaptosomes from striatum. Furthermore, GPR37-KO mice showed enhanced catalepsy induced by an $A_{2A}R$ agonist and an increased response to $A_{2A}R$ antagonist-mediated locomotor activity. Overall, these results demonstrate for the first time an important role for GPR37 controlling $A_{2A}R$ expression and function in the striatum, which may be of interest for the treatment of PD.

Acknowledgements: This work was supported by MINECO/ISCIII (SAF2014-55700-P and PIE14/00034), the Catalan government (2014 SGR 1054), ICREA (ICREA Academia-2010), Fundació la Marató de TV3 (Grant 20152031) and IWT (SBO-140028). Also supported by the PhD fellowship FPI (BES-2012-052722).

P-79
CELLULAR BIOLOGY OF HUMAN CARDIAC NAV1.5-KIR2.1 CHANNEL COMPLEXES

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Cardiac Kir2.1 and Nav1.5 channels generate the inward rectifier K^+ (I_{K1}) and the Na^+ (I_{Na}) currents, respectively. Nav1.5 and Kir2.1 channels exhibit positive reciprocal modulation and the augmented presence of Nav1.5 and Kir2.1 channels at the membrane could be the result of an increase in the protein synthesis and forward trafficking and/or to a decrease in channel internalization. Here we compared the differential characteristics of these processes when Nav1.5 and Kir2.1 channels are expressed together or separately. The proximity ligation assays demonstrated that Nav1.5 and Kir2.1 proteins are in close proximity to each other (<40 nm apart) in the membrane of ventricular myocytes, suggesting that they form complexes. Patch-clamp experiments in heterologous transfection systems demonstrated that the inhibition of endoplasmic reticulum (ER) to Golgi transport with Brefeldin A did not abolish the positive reciprocal modulation between Kir2.1 and Nav1.5 channels and that the internalization time constants of Kir2.1 (5.1 ± 0.5 h) and Nav1.5 (4.9 ± 0.4 h) channels were not modify when they were coexpressed with Nav1.5 and Kir2.1 channels, respectively. Either when they were coexpressed or not, the inhibition of dynamin-dependent endocytosis similarly reduced the internalization of Nav1.5 and Kir2.1 channels. Inhibition of the dynein/dynactin motor suggested that it is involved in the backward and forward traffic of Kir2.1 and Nav1.5, respectively. Conversely, Nav1.5-Kir2.1 complexes were forwarded by the dynein/dynactin motor. Nav1.5 but not Kir2.1 were ubiquitinated by Nedd4-2 ubiquitin-protein ligase and degraded by the proteasome. Nav1.5-Kir2.1 complexes were also degraded following this route as demonstrated by the overexpression of Nedd4-2 and the inhibition of the proteasome with MG132. We concluded that there is a pool of Kir2.1 and Nav1.5 channels that form complexes whose biology is similar to that of the Nav1.5 channels.

Keywords: Nav1.5 channel, Kir2.1 channel, cardiac, intracellular traffic

P-80
A METHOD FOR QUANTIFYING BIASED SIGNALLING OF INVERSE AGONISTS

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Biased agonism, the ability of an agonist to selectively trigger specific signalling pathways when they act at a given receptor, is an increasingly frequently described pharmacological phenomenon. Several methods to quantify the bias of agonists have been proposed, for example the ΔΔlog(τ/Ka) parameter of Kenakin and co-workers (Kenakin et al, 2011, ACS Chem. Neurosci. 3, 193–203). However, all of the methods used for the analysis of biased signalling focus on the analysis of agonists as they are based on the use of the Operational model. However, the classical Operational model does not account for constitutive receptor signalling and cannot be used to analyse the properties of inverse agonists. Here we report a method for the analysis of ligand bias based on the Operational model of Slack & Hall (Slack & Hall, 2012, Br. J. Pharmacol. 166, 1774–1792) which accounts for receptor constitutive activity and hence can be used to analyse biased signalling in response to inverse agonists as well as agonists. This method also has the advantage of providing a measure of absolute rather relative bias since it is derived from estimates of ligand intrinsic

efficacy and is not, therefore, influenced by the efficiency of the signal transduction cascades involved.
Keywords: biased agonism, constitutive activity, inverse agonism, Operational model

P-81
A MUTATION IN THE TBX5 TRANSCRIPTION FACTOR DECREASES THE HUMAN CARDIAC SODIUM CURRENT AND IS ASSOCIATED WITH THE BRUGADA SYNDROME

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In a proband diagnosed with Brugada syndrome (BrS), in whom screening for mutations in all described BrS genes was negative, we found a missense mutation in the Tbx5 transcription factor (p.F206L) that was predicted as pathogenic. It has been described that Tbx5, besides its effects on cardiac development, drives the expression of Nav1.5 channels in the adult mouse heart. Since BrS is associated to loss-of-function Nav1.5 mutations, here we analyzed the effects of p.F206L Tbx5 on the cardiac sodium current (I_{Na}) generated by Nav1.5 channels to unravel whether the mutation can underlie the BrS. Human native (WT) and mutated Tbx5 tagged with GFP were transfected in HL-1 cells or included in lentiviral particles for infecting human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CM). Tbx5 WT doubled the peak I_{Na} density recorded in HL-1 cells (from -37.5 ± 5.1 to -62.6 ± 8.2 pA/pF, $n \geq 6$, $P < 0.05$), whereas p.F206L Tbx5 strongly reduced the peak I_{Na} density (-6.7 ± 0.2 pA/pF; $n = 6$; $P < 0.01$). Importantly, in hiPSC-CM Tbx5 WT and p.F206L significantly increased (-27.6 ± 1.9 pA/pF; $n = 7$) and decreased (-9.5 ± 1.9 pA/pF), respectively, the I_{Na} peak density compared to non-infected cells (-19.4 ± 2.8 pA/pF; $n = 10$; $P < 0.05$). Both in HL-1 cells and hiPSC-CM neither WT nor mutated Tbx5 modified the voltage dependence of Nav1.5 channels activation and inactivation. Luciferase reporter assays using the human minimal promoter of the gene encoding Nav1.5 channels (*SCN5A*) demonstrated that p.F206L Tbx5 completely abolished the Tbx5 pro-transcriptional activity produced by WT Tbx5. We concluded that the p.F206L mutation markedly decreases the I_{Na} density by suppressing the remarkable Tbx5 pro-transcriptional activity over the human *SCN5A* gene and thus, it could be associated with the BrS.
Keywords: sodium current, Tbx5, Brugada Syndrome, cardiac

P-82
THE LARGE CONDUCTANCE, CA^{2+}-ACTIVATED K^+ CHANNEL OPENER VSN16R MODULATES HIPPOCAMPAL CA1 PYRAMIDAL NEURON FIRING

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Ion channels are critical regulators of neuronal excitability and are also implicated in a variety of pathologies such as epilepsy. Large conductance, Ca^{2+}-activated K^+ (BK_{Ca}) channels play an important functionally modulate neuronal firing and have a known role in seizure aetiology. BK_{Ca} are widely expressed in the central nervous system, regulate action potential duration, firing frequency and consequential neurotransmitter release, and are activated by membrane depolarization and increased intracellular Ca^{2+}. The unique coupling of Ca^{2+} signalling to membrane depolarization associated with BK_{Ca} plays a crucial role in controlling neuronal hyperexcitability, since K^+ efflux via BK_{Ca} causes neuronal hyperpolarisation. Moreover, loss-of-function mutations to, or reduced expression of, BK_{Ca} contribute to neuronal hyperexcitability that has been associated with temporal lobe epilepsy, tonic-clonic seizures and alcohol withdrawal-induced seizures. Conversely, there is evidence to show that that BK_{Ca} can facilitate high-frequency neuronal firing in some neuronal populations (e.g. hippocampal CA1 pyramidal neurons) and some gain of function mutations to BK_{Ca} subunits have been associated with the development of idiopathic epilepsy (primarily absence epilepsy). Thus, both loss of function and gain of function of BK_{Ca} channels can differentially subserve modulation of seizure phenotypes such as temporal lobe seizures and absence seizures, respectively.
Here, we examined the effects of the BK_{ca} channel opener, VSN16R (50 μM), on the firing properties of hippocampal CA1 pyramidal neurons in acute hippocampal slices obtained from healthy male C57BL/6 mice (P30–P40) using whole-cell current clamp electrophysiological recording. Neuronal outputs in response to a variety of hyperpolarizing and depolarizing stimulation protocols were assessed. Modulate of hippocampal CA1 pyramidal neuron firing via VSN16R effects on BK_{Ca} channels results will be presented and discussed.
Keywords: BK_{ca} channels; Hippocampal pyramidal neurons

P-83
MODELING HUMAN BIPOLAR DISORDER: THE HINT1-DEFICIENT MICE

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Human bipolar disorder (BPD) is a chronic and debilitating illness with alternating periods of mania and depression. Several currently available animal models reproduce select behavioral facets of human mania and depression and can be used to reliably detect novel drugs. However, in the case of BPD, there is no single valid animal model for reproducing the fluctuating moods of affected patients. Mice with histidine triad nucleotide-binding protein 1 (HINT1) deletion exhibit manic-like symptoms that evolve into depressive-like behavior in response to stressful paradigms. Recent studies have indicated that HINT1−/− mice exhibit molecular changes similar to those found in BPD patients, such as increased PKC, PKA, and GSK3β activities, as well as glutamate N-methyl-D-aspartate receptor (NMDAR)/α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic receptor (AMPAR) and NR2B/NR2A subunit ratios[1,2]. Pharmacological interventions stabilized their behavior. GSK3β inhibitors and valproate directly attenuated the expression of the manic-like symptoms, whereas PKC inhibition, lamotrigine, or risperidone promoted NMDAR-mediated depressive-like behaviors that counterbalanced the preexisting manic-like symptoms. Naïve HINT1−/− mice exposed to stressful paradigms rapidly manifested depressive-like behaviors in subsequent stressful situations that persisted for a couple of weeks thereafter. During the depressive-like phase, citalopram, amitriptyline and MK801 precipitated manic-like behaviors in stressed HINT1−/− mice. Notably, as observed in BPD patients, the antagonism of NMDARs prevented HINT1−/− mice from alternating behaviors in response to stress[3]. In HINT1−/− mice, PKC supports manic like symptoms and reduces the expression of depressive-like behaviors via activation of GSK3β and regulation of NR2B-enriched NMDARs. Our data show that HINT1−/− mice represent a suitable model for studying human BPD and may facilitate the identification of novel targets and drugs to treat this mental disorder.(Supported by Plan Nacional de Drogas 2014-012 and MINECO, SAF-2015-65420R)
1-Hahn et al., 1999. *Bipolar disord.* **1**: 81–86.
2-Naughton et al., 2014 *J. Affetc. Disord.* **156**: 24–35.
3-Ghasemi et al., 2014 *Neurosci. Biobehav. Rev.* **47**: 336–58.

P-84

THE P.P888L SAP97 POLYMORPHISM SHORTENS THE CARDIAC ACTION POTENTIAL DURATION AND THE QT INTERVAL

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The *DLG1* gene encodes for SAP97, an scaffolding protein that interacts with several cardiac ion channels including those underlying the fast Na (I_{Na}) and the transient outward (I_{to}) currents. By next generation sequencing we identified a common *DLG1* polymorphism (p.P888L) in a man and two sisters diagnosed with Brugada Syndrome (BrS). This work aimed to determine the electrocardiographic and the cellular electrophysiological consequences of the SAP97 polymorphism.

Native (WT) and p.P888L SAP97 tagged with ds-red were cotransfected or not together with the cDNA encoding the alpha and beta subunits underlying human I_{Na} and I_{to} in Chinese hamster ovary (CHO) cells. Two cardiac–specific transgenic-like mouse models on the basis of adeno-associated virus gene transfer were created expressing WT and p.P888L SAP97, respectively.

Co-expression of WT SAP97 significantly increased the I_{Na} and I_{to} recorded using patch-clamp in CHO cells and in ventricular myocytes from SAP97 overexpressing mice. The SAP97 polymorphism increased the I_{Na} similarly as WT SAP97 did, both in CHO cells and in transgenic-like mouse myocytes. Conversely, p.P888L SAP97 further increased the time constant of current inactivation and, thus, the I_{to} charge density in both CHO cells (from 18.6 ± 2.3 to 42.2 ± 6.1 pC/pF at +50 mV, $n \geq 8$, $P < 0.05$) and mouse myocytes (from 0.4 ± 0.04 to 0.7 ± 0.06 pC/pF at +50 mV, $n \geq 15$, $P < 0.05$). As a consequence, in p.P888L SAP97 mouse myocytes the AP duration (APD) measured at 20% and 50% of repolarization was significantly shortened. Furthermore, in transgenic-like mice p.P888L overexpression significantly shortened the QT interval compared with WT SAP97 (from 61.5 ± 2.7 to 52.5 ± 2.1 ms, $n = 6$, $P < 0.05$).

The SAP97 p.P888L polymorphism shortens the QT interval and the APD as a consequence of a marked increase of the Ito charge. Therefore, this polymorphism could contribute to the phenotypic manifestations of the BrS.

Keywords: Sodium current, transient outward potassium current, SAP97, cardiac

P-85

PHARMACOLOGICAL AND BIOPHYSICAL PROPERTIES OF PLASMA MEMBRANE PORES INDUCED BY SILICA NANOPARTICLES

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KU Leuven

Amorphous silica nanoparticles (SiNPs) are extensively used for their beneficial properties in cosmetics and food industry as an anti-caking, densifying and hydrophobic agent. However, the high levels of exposure have raised concerns about health hazards, since SiNPs can penetrate tissues and cells resulting in health problems. In this study we first evaluated the effects of commercial 9 nm SiNPs (Ludox®) on intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) in HEK293, CHO and mouse airway epithelial cells. We found that extracellular application of SiNPs at 25 °C increased $[Ca^{2+}]_i$ when cells were co-stimulated with arachidonic acid or LPS. These effects were not observed when Ca^{2+} was omitted in the extracellular solution, indicating that SiNPs induce a Ca^{2+} entry pathway through the plasma membrane. Similar results were obtained when cells were exposed to SiNPs and heating from 25 to 35 °C, suggesting that SiNPs are sufficient to induce the Ca^{2+} entry pathway at physiological temperatures. Whole-cell patch-clamp experiments revealed that SiNPs combined with arachidonic acid trigger large currents that could be blocked by the cation channel blocker ruthenium red in a voltage-dependent manner. Analysis of the selectivity properties of this current showed that they are mainly carried by cations. Taken together, our results demonstrate that SiNPs induce Ca^{2+}-permeable pores in the plasma membrane, and that this phenomenon is enhanced by factors that increase membrane fluidity. We propose that this Ca^{2+} entry pathway may be relevant for the toxicological properties of SiNPs.

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RETT-LIKE SEVERE ENCEPHALOPATHY CAUSED BY A *DE NOVO GRIN2B* MUTATION IS ATTENUATED BY D-SERINE DIETARY SUPPLEMENT

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N-Methyl-D-aspartic acid subfamily of glutamate ionotropic receptors (NMDARs) are activated during fast excitatory transmission and they have been proved to be key elements in synaptic plasticity, synaptogenesis and neuron survival. Several genetic studies have identified *de novo* NMDAR mutations in patients with neurodevelopmental diseases (including severe encephalopathies, autism, intellectual disability) as well as psychiatric disorders. In this work we report a case study of a 4 years-old Rett-like patient with a severe encephalopathy. The genetic studies of this patient (WES and Sanger sequencing) showed the presence of a missense *de novo* mutation of GRIN2B(p.P553T) coding for the GluN2B subunit of NMDARs.

Given the key role of GluN2B subunit in the very early stages of synaptogenesis, we hypothesized that this mutation could be leading to neuronal dysfunction and, subsequently, its normalization would potentially ameliorate the patient's symptomatology. In heterologous expression systems, GluN2B(P553T) mutant construct do neither affected NMDAR oligomerization nor their surface expression in primary neuronal cultures. However, electrophysiological studies showed that although functional, the mutant receptor displayed a significantly reduced channel conductance concomitant with a strong reduction of NMDA-evoked current density. These data are in agreement with our structural molecular model, and strongly suggest the hypo-functionality of mutant NMDARs that, potentially, could be rescued throughout the enhancement of their activity. In accordance with this hypothesis, *in vitro* administration of D-serine, a physiological NMDAR co-agonist, displayed a significant increase of NMDA-evoked currents of mutant receptors. Next, a clinical trial with dietary supplement of D-serine was performed. Importantly, after fourteen-months dietary supplement of D-serine, the patient showed an increase of serine plasma levels, together with a noteworthy clinical improvement. Our results show the possibility to enhance the hypofunctionality of glutamatergic transmission as a therapeutic approach to attenuate cognitive and motor impairment in early childhood.

This work has been supported by Grants PI16/00851PI13/00135 (ISCIII), La Marató (Project N. 20140210), PCIN-2014-105 (MINECO) and Miguel Servet (CP10/00548) to XA; BFU2014-57562-

P (MICINN) to DS; PI15/01082 (ISCIII) to AGC; BFU2012-34398 and BFU2015-69717-P (MINECO), Career Integration Grant (ref. 304111), Marie Curie Intra-European Fellowship (ref. 221540), Ramón y Cajal Fellowship (RYC-2011-08391p) to AB; SAF2012-40102 (MINECO/FEDER), FP7 Marie Curie CIG grant (#631035) and Ramón y Cajal (RYC-2011-08026) to CS. CA received a FPI contract (MINECO). We thank the 'Medicinal Computational Laboratory' from Universitat Autònoma de Barcelona for providing computing facilities. XA, CS and AB also benefit from the financial support of AGAUR (SGR14-297).

P-87

HISTOCHEMICAL AND FUNCTIONAL EVIDENCE OF METABOTROPIC GLUTAMATE 5 RECEPTOR EXISTENCE IN BRAIN DOPAMINERGIC NEURONS, WITH MODELING OF RECEPTOR INTERACTIONS

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Glutamatergic neurotransmission is involved in schizophrenia, a chronic disorder currently treated with dopamine D2 receptor antagonists. Metabotropic glutamate 5 receptors (mGlu5R) are class C heptahelical receptors naturally arranged as homodimers, although heteromerization with dopamine D2 receptors has been reported[4]. The majority of brain striatal mGlu5R are expressed postsynaptically in medium spiny projection neurons. Although presynaptic dopaminergic terminals have been reported to be devoid of mGlu5R immunoreactivity[5], here we present histochemical evidence of mGlu5R immunoreactivity colocalization with tyrosine hydroxylase-labelled terminals in rat and human brains. Dopamine synthesis, a specific functional property of catecholaminergic terminals, was explored in search for the relevance of mGlu5R expression in this type of nerve endings. Two different mGlu5R negative allosteric modulators (MTEP and fenobam) were able to modify D2 autoreceptor inhibition of dopamine synthesis in tissue minces obtained from rat brain striatum, which lends support to the hypothesis of functional mGlu5R expression in dopaminergic nerve endings. However, the precise role of mGlu5R in these neurons needs further clarification: MTEP and fenobam blocked the decrease of dopamine synthesis elicited by D2 partial agonists aripiprazole and (-)PPP, but not that of full agonist quinpirole. A classical allosteric model can be used to interpret this result, where D2 and mGlu5R interact in the membrane as a result of heteromerization, leading to cooperativity between both receptor sites. *In silico* long-timescale molecular dynamics simulations showed stable direct receptor-receptor interactions. Thus, the simplest explanation for our functional results would be a direct D2/mGlu5R interaction in the membranes of dopaminergic nerve endings. These results could be relevant for the treatment of neuropsychiatric disorders, where D2 receptors are important targets of pharmacotherapy.

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Funded by MINECO grant SAF2014-58396.
Keywords: GPCR; operational model; psychosis; intrinsic efficacy

P-88

OPTICAL CONTROL OF LOCOMOTOR ACTIVITY IN A MOUSE MODEL OF PARKINSONISM USING A PHOTOACTIVE ADENOSINE A_{2A} RECEPTOR ANTAGONIST

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G protein-coupled adenosine receptors are promising therapeutic targets for a wide range of pathological conditions, such as Parkinson's disease. However, the ubiquity of adenosine receptors and eventual lack of selectivity of most of adenosine-based drugs have frequently diminished their therapeutic potential. Optopharmacology is a novel approach that may help sorting out this issue, since it allows the spatiotemporal control of receptor functioning. We have developed the first generation of light-sensitive caged adenosine A_{2A} receptor ($A_{2A}R$) ligands: MRS7145 is a SCH442416 (an $A_{2A}R$ antagonist) derivative that is coumarin-blocked at the 5-amino position. First, MRS7145 was photochemically characterized by *in vitro* spectroscopy, monitoring SCH442416 release upon violet light illumination (405 nm). Next, the light-dependent pharmacological profile of MRS7145 was assessed in living cells (HEK-293T cells permanently expressing the receptor). Thus, upon photoactivation, MRS7145 precluded $A_{2A}R$ ligand binding and agonist-induced cAMP accumulation. Thereafter, the ability of MRS7145 to block $A_{2A}R$ in a light dependent manner was assessed *in vivo*. To this end, $A_{2A}R$ antagonist-mediated locomotor activity potentiation was evaluated in brain (striatum) fiber-optic implanted mice. Upon light irradiation (405 nm) of the dorsal striatum, MRS7145 induced significant hyperlocomotion. Finally, it was evaluated the efficacy of MRS7145 reversing motor impairments in an animal model of movement disorders, namely the hemiparkinsonian 6-OHDA lesioned mouse. Thus, photo-activated MRS7145 was able to potentiate the number of contralateral rotations induced by L-3,4-dihydroxyphenylalanine (L-DOPA). Overall, MRS7145 is a new light-operated $A_{2A}R$ antagonist with potential utility for the treatment of Parkinson's disease.

Acknowledgements: This work was supported by MINECO/ISCIII (SAF2014-55700-P and PIE14/00034), the Catalan government (2014 SGR 1054), ICREA (ICREA Academia-2010), Fundació la Marató de TV3 (Grant 20152031) and IWT (SBO-140028).

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$CA_V2.2$ (N-TYPE) VOLTAGE-GATED CALCIUM CHANNELS ARE ACTIVATED BY SUMO-1 PROTEIN

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SUMOylation is an important post-translational modification process involving covalent attachment of SUMO (Small Ubiquitin-like MOdifier) protein to target proteins, including plasma membrane ion channels and receptors (Silveirinha *et al*., 2013). Here, we tested the hypothesis that SUMO-1 can modulate function of the $Ca_V2.2$ (N-type) voltage-gated calcium channel (VGCC), a protein vital for presynaptic

neurotransmitter release and implicated as a therapeutic target in diseases such as chronic pain. $Ca_V2.2$ function was investigated using *in vitro* electrophysiology in recombinant HEK cells (patch clamp recording) and native superior cervical ganglion (SCG) neurons (dual microelectrode recording). Co-expression of SUMO-1 with its conjugating enzyme Ubc9 caused an increase in maximal conductance (G_{max}) in current-voltage relationships, accompanied by a hyperpolarizing shift in the midpoint of activation ($V_{1/2}$), an effect not seen in control, conjugation deficient mutant SUMO-1ΔGG (Table 1). In synaptically-coupled SCG neurons, SUMO-1 protein was distributed throughout the cell body, axons and dendrites and presumptive presynaptic terminals, whilst SUMO-1ΔGG protein was largely confined to the cell body. SUMO-1, in comparison to SUMO-1ΔGG, increased paired excitatory postsynaptic potential ratio at short (20 -120 ms) inter-stimuli intervals. Together, these data demonstrate activation of $Ca_V2.2$ (N-type) VGCCs by SUMO-1 protein; in native neurons, data are consistent with $Ca_V2.2$ SUMOylation causing an increase in residual presynaptic Ca^{2+} current and an increase in release probability of synaptic vesicles.

References:

Silveirinha *et al*., 2013, *J Neurochem.* 127:580–591.

Table 1. Effects of SUMO proteins on $Ca_V2.2$ biophysical properties.

	$Ca_V2.2$ (n = 16)	$Ca_V2.2$/ SUMO-1 (n = 16)	$Ca_V2.2$/ SUMO-1/ Ubc9 (n = 13)	$Ca_V2.2$ (n = 16)	$Ca_V2.2$/ SUMO-1 ΔGG (n = 14)	$Ca_V2.2$/ SUMO-1 ΔGG/ Ubc9 (n = 10)
Gmax (nS)	7.7 ± 0.9	13.4 ± 2.0	25.8 ± 4.0**	7.7 ± 0.9	6.5 ± 0.8	9.4 ± 1.3
$V_{1/2}$ (mV)	1.2 ± 1.0	-0.5 ± 1.0	-3.5 ± 0.9*	0.3 ± 0.9	3.5 ± 1.9	0.0 ± 2.0
k (mV)	4.4 ± 0.4	3.9 ± 0.3	3.7 ± 0.4	4.2 ± 0.4	5.6 ± 0.4	4.0 ± 0.8

* = $P < 0.05$, ** = $P < 0.01$ vs. $Ca_V2.2$, ANOVA with Bonferroni *post-hoc* test.

P-90
CONTACTIN-ASSOCIATED PROTEIN 1 INTERACTS WITH METABOTROPIC GLUTAMATE RECEPTOR TYPE 5 AND MODULATES ITS FUNCTION IN THE HIPPOCAMPUS

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Metabotropic glutamate receptor type 5 ($mGlu_5$) receptors are G protein-coupled receptors that regulate the excitability of hippocampal CA1 pyramidal neurons and thus being implicated in multiple forms of neuronal plasticity. Accordingly, the pharmacological manipulation of these receptors became valuable for the management of neurological disorders affecting the hippocampus. Here, by means of a modified Membrane Yeast Two-Hybrid (MYTH) approach, we identified contactin-associated protein 1 (CASPR1), a type I transmembrane protein member of the neurexin family, as new $mGlu_5$ receptor partner. CASPR1 is expressed on both axons and dendrites of neurons and it is believed to play a key role in the targeting of contactin, Na^+/K^+ channels and AMPA receptors to appropriate sites within the neuronal membrane. Thus, CASPR1 participates in key processes such as myelination and excitatory neurotransmission.

We show that $mGlu_5$ receptor and CASPR1 co-distribute and co-assemble both in heterologous expression systems and in rat brain. Interestingly, down regulation of CASPR1 in rat hippocampal primary cultures decreased intracellular Ca^{2+} accumulation in response to $mGlu_5$ receptor agonists. In addition, silencing $mGlu_5$ receptor expression in the hippocampus by means of an AAV10 shCASPR1 impaired receptor-dependent spatial memory. Overall, these results demonstrate that CASPR1 plays a key role controlling the functionality of hippocampal $mGlu_5$ receptors.

Acknowledgements: This work was supported by MINECO/ISCIII (SAF2014-55700-P and PIE14/00034), the Catalan government (2014 SGR 1054), ICREA (ICREA Academia-2010), Fundació la Marató de TV3 (Grant 20152031) and IWT (SBO-140028). Also supported by the PhD fellowship FPI (BES-2012-052722).

P-91
EFFECT OF *SIDERITIS HYSSOPIFOLIA* ON APOPTOSIS IN HUMAN PROSTATE CANCER LNCAP CELLS

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Introduction: Prostate cancer is the second major cause of cancer-related deaths and the most common cancer diagnosed in men in Europe. The main treatment for most cancers is chemotherapy but it has important adverse effects. In recent years, medicinal plant compounds are being studied and used to treat cancer. Considering the antioxidant properties of *Sideritis hyssopifolia*, the aim of the study was to determine the effects of the ether, methanol and chloroform extracts obtained from the aerial parts of this plant on apoptosis in LNCaP cells of human prostate cancer.

Material and Methods: The analysis of apoptosis was conducted by flow cytometry. Apoptosis was measured by using a specific kit (FITC-Annexin V Apoptosis Kit) following the manufacturer's instructions. Briefly, LNCaP cells were seeded ($2x10^5$ cells/well) in 2 ml of culture medium. After 24 h, the cells were treated with the extracts and incubated at 37°C for 72 h. Cells harvested by trypsinization were collected and washed twice with ice-cold PBS. After centrifugation, cells were resuspended in 100 μL of binding buffer, and 5 μL of FITC-Annexin V and 1 μL of propidium iodide (1 mg/ml) were added. After 15 minutes of incubation, 400 μL of binding buffer were added and cells were analyzed by flow cytometry using Summit 4.3 software. Data were processed using Flowing Software 2.5.1.

Results and Conclusions: The results obtained indicated that all extracts induced apoptosis of LNCaP cells. In the untreated cells (control group), the majority of cells were viable (87%). In contrast, when samples were treated with extracts, the percentage of viable cells were: 17.79, 13.69 and 13.36% (ether, methanol and chloroform extracts, respectively). The highest number of cells in early apoptosis was found for methanol extract (56.17%) and in late apoptosis or necrosis for ether extract (42.52%).

Keywords: LNCaP; *Sideritis hyssopifolia*; flow cytometry; apoptosis

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EFFECT OF *SIDERITIS HYSSOPIFOLIA* ON CELL CYCLE DISTRIBUTION IN HUMAN PROSTATE CANCER LNCAP CELLS

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Introduction: *Sideritis hyssopifolia* is a little woody plant belonging to *Lamiaceae* family with antioxidant properties that contains polyphenols. These compounds possess potentially health-promoting properties

like anticarcinogenic and chemopreventive activities. The aim of the study was to evaluate the effects of the ether, methanol and chloroform extracts, obtained from the aerial parts of this plant on cell cycle in human prostate cancer LNCaP cells.
Material and Methods: LNCaP cell line were seeded ($2x10^5$ cells/well) in 2 ml of culture medium. After 24 h, the cells were treated with the extracts and incubated at 37°C for 72 h. Cells harvested by trypsinization were collected, centrifuged and resuspended in cold PBS. To determine cell cycle distribution, cells were incubated for 15 min in the dark with Igepal CA-630 (1%), RNase (10 mg/ml) dissolved in sodium acetate (0.01 M at pH 5.2) and propidium iodide (1 mg/ml). Cells were quantified by flow cytometry using Summit 4.3 software, followed by data analysis using Flowing Software 2.5.1.
Results and Conclusions: The results were expressed as the percentage of cells in subG0/G1 (apoptotic cells), G0/G1, S and G2/M phases of the cell cycle. The treatment with the extracts induced cell accumulation in the subG0/G1 phase: 2.04% in untreated cells (control group), 12.20% after ether extract treatment, 14.74% with methanol extract and 27.25% with chloroform extract. These results showed the presence of apoptotic cells. It was also observed a reduction in the number of cells in the other phases, being the percentages obtained (G0/G1, S and G2/M phases, respectively): 74.40, 9.43 and 12.67% in the control group; 74.63, 6.65 and 5.01% with ether extract; 67.49, 9.81 and 5.76% with methanol extract and 56.05, 7.54 and 5.62% with chloroform extract.

P-93
OPTICAL CONTROL OF ENDOGENOUS RECEPTORS AND CELLULAR EXCITABILITY USING TARGETED COVALENT PHOTOSWITCHES

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Light-regulated drugs allow remotely photoswitching biological activity and enable plausible therapies based on small molecules. However, only freely diffusible photochromic ligands have been shown to work directly in endogenous receptors and methods for covalent attachment depend on genetic manipulation. Here we introduce a chemical strategy to covalently conjugate and photoswitch the activity of endogenous proteins and demonstrate its application to the kainate receptor channel GluK1. The approach is based on photoswitchable ligands containing a short-lived, highly reactive anchoring group that is targeted at the protein of interest by ligand affinity. These targeted covalent photoswitches (TCPs) constitute a new class of light-regulated drugs and act as prosthetic molecules that photocontrol the activity of GluK1-expressing neurons, and restore photoresponses in degenerated retina. The modularity of TCPs enables the application to different ligands and opens the way to new therapeutic opportunities.

P-94
SYNTHESIS AND BIOLOGICAL EVALUATION OF NOVEL INHIBITORS OF SIGNAL TRANSDUCTOR AND ACTIVATOR OF TRANSCRIPTION (STAT) PROTEINS IN HEMATOLOGICAL MALIGNANCES

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Background: Aberrant activation of JAK/STAT signaling pathway has been related to several hematological malignances, including Chronic Myelogenous Leukemia (CML). Naphthoquinones (NFQ)-based derivatives have been shown to suppress STAT signaling pathway in non-cancer, inflammatory, and cancer cells. Now, the Ihf-c6, a novel NFQ-based derivative, has been identified as anti-leukemic drug by using a phenotyping screening of chemical library.
Methods: Novel NFQ-based derivatives structures were obtained by using multicomponent reactions. The mitochondrial metabolization of the tetrazolium salt 3-(4,5-methylthiazol-2yl-)-2,5diphenyl-tetrazolium bromide] (MTT) was used as indicator of cell viability. Cytostatic and cytotoxic effects were evaluated by using a Live-Cell Imaging System (Incucyte Zoom HD). Phosphorylated and total levels of STAT, MAPK and leukemic-relevant proteins were determined by immunoblotting. Cytokine-stimulated pY-STAT3/5 and STAT binding to DNA were also analyzed in adenocarcinoma breast (T47D) cells.
Results: Ihf-c6 showed antitumoral effects *in vitro*. Ihf-c6 was highly effective in inhibiting human promyelocytic leukemia HL60 cells ($IC50 = 0.3 \pm 0.1$ μM), human erythroleukemia HEL cells ($IC50 = 1.3 \pm 0.6$ μM), and human CML K562 cells ($IC50 = 1.4 \pm 0.7$ μM) whereas non-blood tumor cells (e.g., MCF7 and SKBR3) and non-tumor cells (MRC.5) were higher resistant to Ihf-c6. Ihf-c6 reduced the proliferation of K562 cells in a time and dose-dependent manner. Ihf-c6 (3 μM for 6 h) caused 50% and 30% inhibition of constitutive pY-STAT5 and pY-Bcr-Abl in K562 cells, respectively. Moreover, Ihf-c6 (5 μM for 6–12 h) reduced constitutive pY-JAK2, pY-STAT3, and pY-STAT5 in HEL cells. GH-induced pY-STAT5 or IL-6-induced pY-STAT3, as well as cytokine-induced STAT-DNA interactions, were inhibited by Ihf-c6 in T47D cells in a time (maximal at 30 min)- and dose-dependent (maximal at 3 μM) manner.
Conclusions: Ihf-c6 is an effective inhibitor of constitutive as well as cytokine-induced pY-STAT5/3. NFQ-based derivatives might be potential therapeutic agents for STAT5/3-dependent blood cancer.
Sociedad Española Farmacologia 2017, Barcelona, Spain, June 2017.

P-95
IDENTIFICATION OF DIFFERENTIAL KINASE OFF-TARGETS AMONG CLINICAL PARP INHIBITORS: NEW OPPORTUNITIES FOR PRECISION ONCOLOGY?

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The on-going development of a more comprehensive and systems-based approach to pharmacology is uncovering a far more promiscuous interaction between drugs and the human proteome than was previously anticipated, a behaviour termed polypharmacology with clinical implications that are still not well understood.[1,2] While many off-targets of available drugs still remain to be identified, computational

methods that exploit publicly available knowledge bases are becoming a cost-effective approach to uncover unknown polypharmacology.[2,3] PARP inhibitors are a new class of targeted small-molecule cancer therapeutics that have shown unexplained differential effects in cellular models and clinical trials.[4] With two PARP inhibitors recently approved by the FDA for BRCA-mutated ovarian cancer and several more in late-stage clinical trials, it is essential to fully characterize their mechanism of action to help oncologists' decide which PARP inhibitor should be prescribed in each case. Here, we use computational target prediction and *in vitro* validation to identify new targets of PARP inhibitors that may explain their differential clinical effects. What appeared as a single robust class of PARP inhibitors with similar pharmacological properties should now be regarded as a set of PARP inhibitors with a different pharmacological profile that makes them essentially unique and thus expands largely their potential therapeutic opportunities in the framework of precision oncology.

Keywords: Systems pharmacology, precision oncology, PARP inhibitors, off-targets, polypharmacology, computational methods

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P-96
ACTINIC KERATOSIS: STANDARD COMPOUNDS VALIDATION IN BOTH *IN VITRO* AND *IN VIVO* MODELS

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Actinic keratosis (AK) is the most common premalignant skin tumor, affecting as many as 25% of adults in the northern hemisphere. It is due principally to long-term sun exposure and affects 50% of predisposed individuals more than 60 years of age in Europe. Without treatment, AK lesions may progress to squamous cell carcinoma (SCC) and invade the dermis. Depending on the nature of the growth and the patient's age and health, various treatment options are available. Isolated or widespread actinic keratosis lesions may be treated with topical formulations. In this regard, there are four topical active ingredients currently approved: 5-fluorouracil (5FU), diclofenac, ingenol mebutate and imiquimod, which represent distinct therapeutic classes. The goal of this work was to study the mechanism of action of these compounds in a battery of relevant cutaneous *in vitro* and *in vivo* assays which could provide measures of the anti-proliferative, cytotoxic or immunostimulant activity of those drugs. Different *in vitro* tests using HaCaT cells, normal human keratinocytes or human skin explants were used. In addition, the effect of the topical formulations was studied on mouse skin *in vivo* and on an isograft tumor model. AK reference standards show distinct behaviors in all the selected models, with 5-FU showing a marked antiproliferative activity and ingenol mebutate showing the strongest pro-inflammatory profile. Understanding these differences is key for the selection of new therapies for AK.

Keywords: Actinic keratosis; 5FU; ingenol; imiquimod; diclofenac; *in vitro* models; *in vivo* models

P-97
THE ROLE OF RECEPTOR PROTEIN TYROSINE PHOSPHATASE BETA/ZETA IN CELL MIGRATION

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Receptor protein tyrosine phosphatase beta/zeta (RPTPβ/ζ) is highly expressed in human induced pluripotent and embryonic stem cells, while in adult humans, RPTPβ/ζ is mostly expressed in the brain, skin, stomach and testis. Interestingly, it is significantly over-expressed in the brain and lung of corresponding cancer patients compared with normal controls, suggesting that it may play a role in glioblastomas and/or lung cancer. We have previously shown that RPTPβ/ζ is a receptor for pleiotrophin (PTN) and vascular endothelial growth factor A (VEGF-A), mediating their stimulatory effect on human umbilical vein endothelial cell migration through interaction with $\alpha_v\beta_3$ integrin. In the present work, we studied the role of RPTPβ/ζ in the effect of PTN and VEGF on the migration of microvascular endothelial cells, as well as glioblastoma cell lines that do not express $\alpha_v\beta_3$. In all cases, RPTPβ/ζ has an effect on cell migration which is either stimulatory or inhibitory in a cell-dependent manner. In all cases, RPTPβ/ζ mediates the effect of PTN on cell migration, while the effect of VEGF-A seems to be affected in endothelial but not glioblastoma cells. Expression of $\alpha_v\beta_3$ seems to explain, at least partly, the differential effect of RPTPβ/ζ on cell migration, although it seems that other factors may be also involved and are going to be discussed. Collectively, our data suggest that RPTPβ/ζ significantly affects cell migration and $\alpha_v\beta_3$ is a key molecule in RPTPβ/ζ signaling. This knowledge, together with data on cancer cell characteristics and cancer angiogenesis, can lead to new therapeutic approaches in controlling brain and lung cancer development.

Acknowledgements: This research was partly supported by a Marie Curie Intra European Fellowship within the 7th European Community Framework Programme (ALTangioTARGET, grant agreement No 626057). Penelope Castana is a recipient of a PhD fellowship from the Hellenic State Scholarships Foundation (IKY).

Keywords: angiogenesis, cancer, tyrosine phosphatases, signaling

P-98
NIMOTUZUMAB AND RADIOTHERAPY IN THE TREATMENT OF PEDIATRIC PATIENTS WITH DIFFUSE INTRINSIC BRAIN ASTROCYTOMAS

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A multicenter, phase II clinical trial to evaluate safety and efficacy of Nimotuzumab, a monoclonal antibody against epidermal growth factor receptor added to standard therapy for Brain Tumors in Pediatric Patients. Forty-five patients analyzed, 23 were included in Brazil and 22 patients in Cuba, since 2007 - 2012. There was a discontinuation of treatment in 38 patients, the progression of the disease being 68.4%. The efficacy analyzed in those patients who had received at least 12 doses of Nimotuzumab and 54 Gy or more of RT. A response observe in 20 patients (44.4%). The median progression-free survival and overall survival was equivalent to 9.03 and 15.1 months. There were no differences in overall or progression-free survival among patients treated in Cuba or Brazil. Progression-free survival at the sixth month was 72.9%. Five hundred sixty-nine, adverse events was reported, 178 of them (31.3%) in the neurological system. Gastrointestinal events

ranked second and constituted 26.7% of all events. The rest of the reported AEs do not exceed 10% each separately (general manifestations, 7.6%, respiratory system 6.5%, infection 6.3%, dermatological and pain, 5.6%, hematological, 4.2%). In conclusions, the treatment with, Nimotuzumab in combination with radiotherapy in pediatric patients with diffuse intrinsic brain stem astrocyte tumors, demonstrated an increase in the rate of progression-free survival (PFS) (72.9%) at 6 months treatment in relation to the historical rate (50%). The combination of radiotherapy and Nimotuzumab.

P-99

THE PROGNOSTIC VALUE OF THE MELATONERGIC SYSTEM-BASED ASMT:CYP1B1 INDEX IN SOLID TUMORS

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Melatonin synthesized and released by the pineal gland at night is responsible for translating the environmental dark phase to the organism. The production of melatonin has also been detected in many extra-pineal tissues, including retina, gastrointestinal tract and brain. Generally, extra-pineal melatonin is poorly released into the circulation, acting locally in an autocrine and paracrine manner. Currently, it is well established that melatonin exerts oncostatic effects across a wide variety of tumors, impairing tumor growth, invasion and angiogenesis. In this sense, we have recently demonstrated that the ability of gliomas to synthesize/accumulate melatonin negatively correlates with their overall malignancy (Kinker *et al.*, 2016). Using the analysis of The Cancer Genome Atlas (TCGA) glioma RNAseq data, we designed a predictive model of the content of melatonin in the tumor microenvironment, the ASMT:CYP1B1 index, combining the gene expression levels of melatonin synthesis and metabolism enzymes. The ASMT:CYP1B1 index negatively correlates with glioma malignancy grade and is a grade- and histological type-independent prognostic factor. Additionally, the analysis of TCGA RNAseq data of 7,125 samples from 17 solid tumor types (including gliomas) demonstrated that the ASMT:CYP1B1 index negatively correlates with the expression of genes involved in the control of tumor growth, migration and invasion. Interestingly, many of these genes are known to have their expression modulated by melatonin, including *COL1A1*, *ITGAV*, *ACTA2*, *IGF1*, *STAT3*, *HIF1A* and *SPHK1*. More importantly, as observed for gliomas, a low ASMT:CYP1B1 index value, suggestive of reduced melatonin, is associated with poor survival in bladder urothelial carcinomas, cervical/endocervical cancers, colorectal adenocarcinomas, lung squamous cells carcinomas, pancreatic adenocarcinomas, paragangliomas/pheochromocytomas, stomach adenocarcinomas, and endometrial cancer. Overall, our data reveal the prognostic value of the melatonergic system in solid tumors and support further investigations of the biological relevance of melatonin synthesized by malignant cells. Financial support: FAPESP (2010/52687-1, 2013/13691-1, 2014/27287-0), CNPq (480097/2013-5, 162670/2014-1).
Keywords: solid tumors, melatonin, ASMT, CYP1B1, molecular markers

P-100

PHARMACOGENETIC STUDY FOCUSED ON FLUOXETINE PHARMACODYNAMICS IN CHILDREN AND ADOLESCENT PATIENTS: IMPACT OF THE SEROTONIN PATHWAY

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Background: Although fluoxetine has been shown to be effective in children and adolescents, there is great inter-individual variability in clinical response. Pharmacogenetic studies of fluoxetine in pediatric patients are scarce. After reporting the effect of genetic variants in genes related to the fluoxetine pharmacokinetics on clinical response in a pediatric population, we now evaluate the impact of genetic markers involved in its pharmacodynamics.
Methods: Eighty-three children and adolescents aged between 10 and 17 years of age receiving fluoxetine treatment were recruited. Clinical improvement after 12 weeks of fluoxetine treatment was assessed by score changes in different scales including CDI, OCI-CV, SCARED, GAF, CGI-S and CGI-I. The genetic association analysis was performed with a total of 316 validated SNPs in 45 candidate genes involved in six different pathways directly or indirectly related to fluoxetine mechanism of action including the serotonergic and other neurotransmitter systems, the hypothalamic-pituitary-adrenal axis, the circadian rhythm, and inflammatory and neurodevelopmental pathways.
Results: Clinical improvement after treatment with fluoxetine in our pediatric population was significantly associated with two polymorphisms located in genes related to the serotonergic system: the 5-hydroxytryptamine receptor 1B (*HTR1B*) and the tryptophan 5-hydroxylase 2 (*TPH2*). Particularly, clinical improvement was lower in heterozygous (AT) patients for rs130058 (*HTR1B*) and higher in minor allele homozygous (TT) for rs4570625 (*TPH2*).
Conclusions: Although a wide range of candidate genes related to different pathways were assessed, it seems that the most directly related pathway with the action mechanism of fluoxetine is the one that had the most important genetic effect on clinical response.
Keywords: children, fluoxetine, HTR1B, pharmacogenetics, polymorphism, TPH2

P-101

PREVALENCE AND PATTERN OF POTENTIALLY INAPPROPRIATE MEDICATION IN OLDER PRIMARY CARE PATIENTS. PROMINENT ROLE OF BENZODIAZEPINES

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Potentially Inappropriate Medication (PIM) is one of the main risk factors for adverse drug events (ADEs) in older people. STOPP criteria (Screening Tool of Older Persons' Prescriptions) has proven greater correlations to ADEs with PIMs than with those defined by the Beers criteria. The aim of this work was to determine the prevalence and profile of PIMs and its predictive factors in elderly population.

Methods: This was a multicenter study with a cross-sectional design. The study population comprised 582 community-dwelling residents ≥65 years in Health District of Málaga. Data recorded included sociodemographic characteristics, clinical status, comorbidity, functional assessment and complete information about drugs intake (ATC classification, indication, dosage and length). The primary endpoint was the percentage of participants receiving at least one PIM according the recent update of STOPP criteria (v2, 2015).
Results: A total of 3626 prescriptions were analyzed. The mean age was 73.1 (±5.6) years (57.4% female). The median number of medications per patient was 6.8 drugs (range 0–23). 66.5% of the participants had prescribed at least one drug of N-group. The third part received benzodiazepines as usual treatment (long-acting: 15%). PIMs were detected in 66.8% of patients (51.7% if we exclude the most questionable criteria: lack of evidence-base clinical indication, drugs prescribed beyond the recommended duration and duplicate drugs). The most frequent PIMs were benzodiazepines for ≥4 weeks (31%) and drugs that predictably increase the risk of falls (benzodiazepines, neuroleptics, hypnotic Z-drugs and vasodilator drugs) (35%), followed by the use of opioids without concomitant laxative (8%), and long-term use of NSAID (>3 months) (2.7%).
Conclusions: The prevalence of PIM was high, and shows an increase over previous studies in Primary Care. Special attention should be paid on benzodiazepines, which keep being the most frequent PIM.
Funding: Consejería de Salud, Junta de Andalucía (Grant number: PI 0234/14)
Keywords: inappropriate prescribing; STOPP version 2; elderly adults; drug-related problems

P-102
EPIGENETIC AND GENETIC VARIANTS IN THE *HTR1B* GENE AND CLINICAL IMPROVEMENT IN CHILDREN AND ADOLESCENTS TREATED WITH FLUOXETINE

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The serotonin 1B receptor (5-HT_{1B}) is important to both the pathogenesis of major depressive disorder and the antidepressant effects of selective serotonin reuptake inhibitors. Although fluoxetine has been shown to be effective and safe in children and adolescents, not all patients experience a proper clinical response, which has led to further study into the main factors involved in this inter-individual variability. Our aim was to study the effect of epigenetic and genetic factors that could affect 5-hydroxytryptamine receptor 1B (*HTR1B*) gene expression, and thereby response to fluoxetine. A total of 83 children and adolescents were clinically assessed 12 weeks after of initiating an antidepressant treatment with fluoxetine for the first time. We evaluated the influence of single nucleotide polymorphisms (SNPs) specifically located in transcription factor binding sites (TFBSs) on their clinical improvement. A combined genetic analysis considering the significant SNPs together with the functional variant rs130058 previously associated in our population was also performed. Moreover, we assessed, for the first time in the literature, whether methylation levels of the *HTR1B* promoter region could be associated with the pharmacological response. Two, rs9361233 and rs9361235, were significantly associated with clinical improvement after treatment with fluoxetine. The heterozygous genotype combination analysis showed a negative correlation with clinical improvement. The lowest improvement was experienced by patients who were heterozygous for all three SNPs. Moreover, a negative correlation was found between clinical improvement and the average methylation level of the *HTR1B* promoter. These results give new evidence for the role of epigenetic and genetic factors which could modulate *HTR1B* expression in the pharmacological response to antidepressants.
Keywords: Fluoxetine, Pharmacogenetics, Methylation, Children, *HTR1B,* Polymorphism

P-103
HOST CHARACTERISTICS, PHARMACOLOGICAL PROPERTIES AND THEIR INTERACTIONS INFLUENCE TYPE OF LIVER INJURY IN HEPATOTOXICITY

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Background: The clinical course of drug-induced liver injury (DILI) is typically categorized as hepatocellular, cholestatic, or mixed. This categorization is important for the diagnosis of causative drugs and the assessment of possible clinical outcomes. Here we aimed to evaluate host and pharmacological factors that were associated with the development of liver injury pattern.
Methods: 564 DILI cases classified according to biochemical parameters as having hepatocellular and cholestatic type of liver injury were selected out of 701 cases enrolled in the Spanish DILI registry. Univariate analyses and logistic regressions (R software) were performed to compare the pharmacological properties of causative drugs and host factors between the two groups (hepatocellular vs. cholestatic injury).
Results: Of the study cohort, 437 (77%) cases had hepatocellular and 127 (23%) cholestatic type of liver damage. Seven parameters were identified as being best predictors for liver injury pattern by using the logistic regression analysis. Parameters increasing the risk of hepatocellular damage included: age <60 years (Odds ratio or OR=2.06; $P = 0.0029$), previously known allergic records (OR=2.61; $P = 0.0493$), ≥50% hepatic metabolism (OR=1.70; $P = 0.0417$), enterohepatic circulation (OR=4.94; $P < 0.0001$) and causative drugs with a chemical double/single bond ratio >0.5 (OR=2.04; $P = 0.0285$). In contrast, presence of comedications for cardiac disease and causative drugs with a higher number of heterorings (OR=0.68, $P = 0.0270$) were found to decrease the risk of a hepatocellular injury pattern (OR=0.47, $P = 0.0030$). Interaction between older age (≥ 60 y) and culprit drugs with low hepatic metabolism, but not high hepatic metabolism, increase the risk of cholestatic damage (OR=3.49, $P = 0.0001$).
Conclusions: Host factors and pharmacological properties seem to play a complementary role in the prediction of injury pattern in DILI. The pharmacological properties facilitating hepatocellular damage seem to be related with increased drug exposure or hepatocyte damage. A high drug metabolism could be associated with an increased possibility of generating reactive metabolites.
Funding: Fondos FEDER(PI15/01440);AEMPS;CIBERehd_by_ISCIII.

P-104
PREVALENCE OF UGT1A1 GENETIC VARIANTS IN ARGENTINEAN POPULATION, POTENTIAL IMPLICATIONS FOR PHARMACOGENOMIC TESTING

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Introduction: The uridine diphosphate glucuronosyltransferase (UGT) enzymes constitute a superfamily of enzymes responsible for the

glucuronidation of target substrates. The UGT family (UGT1A, UGT2, UGT3 and UGT8) is responsible for the glucuronidation of hundreds of compounds and drugs, which makes the enzymes in this group relevant to pharmacogenetic research. The UGT1A family is located on chromosome 2q37. There are genetics variants in the promoter region of *UGT1A1*, upstream to exon 1. The normal sequence of the TATAA element within the promoter is A[TA]6TAA. These variants generates longer version of the TATAA sequence, A[TA]7TAA (UGT1A1*28), A[TA]8TAA (UGT1A1*37), that cause reduced production of bilirubin-UGT. Since bilirubin-UGT is involved in the glucuronidation of several important drugs, individuals with UGT1A1*28 variant may be more susceptible to the toxic effect of substances that require bilirubin-UGT-mediated hepatic glucuronidation prior to excretion. Is known this variant increase the risk of toxicity with irinotecan and the hyperbilirubinemia with atazanavir. Also there is a shorter version A[TA]5TAA (UGT1A1*36) that causes increased production of bilirubin-UGT.

Objectives: Determine the frequency of *UGT1A1* variants in Argentinean population.

Methods: 100 DNA samples from healthy volunteers were analyzed. The presence of variant UGT1A1*28 was confirmed by direct sequencing of PCR products.

Results: Among the 100 subjects analyzed, 49% (49) were males and 51% (51) were females with a median age of 43 and a range of 32–80 years. The distribution of the UGT1A1 was: 70.5% (70.5) for the *1 allele. for the 21.5% *28 allele and *36 allele 1%, 48% (48) presented the *1/*1 genotype, while 43% (43) had *1/*28, 2% (2) had *1/*36 and 7% (7) showed the *28/*28. No preferential sex distribution was observed in between genotypes.

Comment: The high prevalence of low-of-function alleles in argentinean general population provide support for further evaluation of the practical consequences of pharmacogenomic test.

P-105
DRUG INDUCED LIVER INJURY ASSESSMENT: ACUTE HEPATITIS DUE TO HEPATITIS E VIRUS, A DIAGNOSIS TO CONSIDER

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Introduction: Drug-induced liver injury (DILI) remains one of the most challenging diseases due to the absence of diagnostic tests and biomarkers. DILI usually presents as an acute hepatitis-like picture requiring extensive differential diagnosis, is a diagnosis of exclusion requiring prudent causality assessment. Hepatitis E virus (HEV), which is considered a rare condition in Spain, is not usually ruled out during acute hepatitis assessment.

Material and Methods: Analysis of a cohort of 180 patients from the Spanish DILI Registry diagnosed with DILI was undertaken. We analyzed HEV Immunoglobulin (Ig)G and M from two groups of serum samples based on the time point of collection (27 samples during the episode of liver damage and 153 at different time points after resolution). In patients showing anti-HEV-IgM+, AgHEV and RNA-HEV were performed.

Results: Out of 180 patients included, 60 (33, 3%) were tested positive for anti-HEV-IgG and 6 for anti-HEV IgM (1 positive for HEV-RNA and 2 for Ag-HEV). In the group of samples collected during the episode, 3/27 (11%) were positive for IgM-HEV.

Table 1. Demographic and clinical data of patients with positive IgM anti-HEV.

Case n°	Age (years)	Sex	Drug	Latency (days)	Peak ALT(1) or AST(2)	HEV RNA	Ag HEV
1	49	Female	paracetamol	9	1840 (2)	Negative	Positive
2	74	Male	cefditoren	40	4191 (1)	Positive	Positive
3	26	Female	dexketoprofen	8	1561 (1)	Negative	Negative
4	56	Female	isoniazid	27	954 (1)	Negative	Negative
5	35	Male	erythromycin	27	2469 (1)	Negative	Negative
6	75	Male	amoxicillin	4	2967 (1)	Negative	Negative

Conclusions: Evidence of hepatitis E infection is present in a significant number of patients suspected to have DILI. Seroprevalence of IgG-HEV is also very high. HEV should be ruled out in all patients suspected to suffer from DILI in Spain.

Funding: ISCIII, FEDER, (PI15/01440, PI16/01748), Consejería de Salud (PI-0274-2016), AEMPS, CIBERehd, SCReN.

Keywords: hepatotoxicity; drugs; DILI; HEV

P-106
PROSPECTIVE DRUG-INDUCED LIVER INJURY REGISTRY: CLINICAL CHARACTERISTICS AND OUTCOMES OF 915 CASES

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Introduction: Idiosyncratic drug induced liver injury (DILI) is a rare adverse drug reaction that poses major challenges to healthcare practitioners and regulatory agencies. We aimed to update the clinical characteristics and outcomes of DILI patients, and the drugs frequently implicated in hepatotoxicity in Spain.

Methods: We analyzed 915 DILI cases (842 single-episodes, 55 re-challenges, 18 double-episodes) in 857 patients included in the Spanish Registry from 1994 to 2015. Cases were compared according to pattern of liver damage (hepatocellular, HC; cholestatic, Chol or mixed, Mix).

Results: Median age was 57 years with mean body mass index of 25.8 ± 3.8 kg/m2. HC, Chol and Mix patterns of liver damage were identified in 65%, 18% and 17% of cases, respectively. More than half of the cases were of moderate severity (58%). Patients with Chol and Mix pattern were older (median 64 y and 62 y, respectively) than HC patients (median 52 y), $P < 0.001$. Median time to DILI onset was 30 days in the HC group compared to 20 and 17 days for Chol and Mix, respectively, $P < 0.001$. The median time to resolution was

111 days, which was similar among the different patterns of liver injury. Jaundice and hospitalization were more common in Chol cases compared to HC (77% and 70% vs. 65% and 54%, $P < 0.05$, respectively). HC cases exhibited greater risk of developing fatal/severe outcomes, 15% vs. 7% and 3% in Chol and Mix cases, respectively, $P < 0.001$. Anti-infectives, central nervous system, cardiovascular and anti-inflammatory agents were the most commonly implicated therapeutic classes accounting for 37%, 14%, 11% and 9% of cases, respectively.

Conclusions: The pioneering prospective Spanish DILI Registry proved to be valuable for in-depth phenotyping of hepatotoxicity. It also constitutes an important tool for public health promotion in post-marketing drug surveillance.

Funding: Research grants from AEMPS and FEDER (PI15/01440, PI16/01748). CIBERehd by ISCIII.

Pharmacogenomic Alerts in an Electronic Medical History

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The human genome has different variants in each individual. This variability implies different responses to the same drug. The doctor who indicates a treatment may lack the specific knowledge about the effects that a drug can produce against the genetic variants of the patient. For this reason it is considered necessary the existence of a system of pharmacogenomic alerts as a tool to support the physician when making a prescription through an electronic medical record. The present work describes the development of the necessary components for the functioning of this system: a genetic database of patients, a pharmacogenomic knowledge base, an inference engine and a user interface. These components integrate a system that considers the genetic variants of the patient and verifies if there is any risk before the prescription of a drug, issuing an alert to the doctor if necessary. A system of pharmacogenomic alerts in an Electronic Clinical History has the potential to reduce adverse effects in people highly susceptible to suffering them.

P-107
OLEUROPEIN EXHIBITED ANTI-INFLAMMATORY AND ANTI-OXIDANT ACTIVITIES IN IL-1β-STIMULATED HUMAN SYNOVIAL FIBROBLASTS

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Rheumatoid arthritis (RA) is a chronic and systemic inflammatory autoimmune disease mainly characterized by aggressive hyperproliferation of synovial fibroblasts (SFs) and massive infiltration of inflammatory immune cells inducing a progressive matrix degradation, destruction of cartilage and bone erosion through the production of inflammatory mediators. Oleuropein, is the most prevalent phenolic component in olive leaves, seed, pulp and peel of unripe olives and is responsible for unprocessed olives characteristic bitter taste. This secoiridoid possesses a well-documented pharmacological properties, including antioxidant and anti-inflammatory, consequently is available as food supplement in Mediterranean countries. However, at the date, anti-arthritic effects of oleuropein on SFs have not been yet elucidated. Thus, the aim of the present study was to investigate the potential effects of oleuropein, on IL-1β-induced inhibitory production of inflammatory mediators and oxidative stress in human synovial sarcoma cell line (SW982). In order to gain a better insight into mechanisms of action, signaling pathways were also explored.

Cell viability was determined using sulforhodamine B (SRB) assay. The expression of inflammatory cytokines IL-6, TNF-α, MMP-1 and MMP-3 was evaluated by ELISA. Moreover, changes in the protein expression of cyclooxygenase (COX)-2, microsomal prostaglandin E synthase-1 (mPGES-1) as well as mitogen-activated protein kinase (MAPKs), nuclear factor kappa B (NF-κB), and nuclear factor e2-related factor 2 (Nrf2) and heme oxygenase-1 (HO-1) signalling pathways were analysed by western blot.

Oleuropein exerted anti-inflammatory and anti-oxidant effects via down-regulation of MAPKs and NF-κB signaling pathways and induction of Nrf2-linked HO-1 controlling the production of inflammatory mediators decreasing IL-6 and TNF-α cytokines, MMP-1 and MMP-3 levels as well as mPGES-1 and COX-2 overexpression. Thus, oleuropein might provide a basis for developing a new dietary strategy for the prevention and management of RA.

P-108
IKKα IS INVOLVED IN PRO-INFLAMMATORY ACTIVITY OF EXTRACELLULAR NICOTINAMIDE PHOSPHORIBOSYLTRANSFERASE (ENAMPT) IN BONE MARROW-DERIVED MACROPHAGES

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Introduction: The IKKα, a subunit of the NF-κB-activating IKK complex, has emerged as an important regulator of inflammatory gene expression. Although intracellular nicotinamide phosphoribosyltransferase (iNAMPT) is a key enzyme in controlling NAD^+ metabolism, circulating eNAMPT has been associated with several metabolic and inflammatory disorders, including cancer and cardiovascular diseases. Herein, the potential role of IKKα and the underlying mechanisms by which eNAMPT could exert the metabolic and inflammatory dysfunctions were investigated.

Material and Methods: Wild type (C57BL/6) and IKKα$^{-/-}$ (C57BL/6 IKKα$^{AA/AA}$) mice were used in this study. Bone marrow (BM) was isolated from wild type and IKKα$^{-/-}$ bones, then cells were pooled and cultured in RPMI supplemented with 15% L929-conditioned medium to generate BM-derived macrophages (BMDMs) and ex vivo studies were performed. These BMDMs were pretreated with FK866 (an iNAMPT inhibitor), Lentivirus iNAMPT (LV.iNAMPT), and eNAMPT treatment before the isolation of RNA and cell supernatants. mRNA levels of genes related to IKKα pathway, macrophage polarization, inflammation and secreted pro-inflammatory cytokines were determined by RT-qPCR and ELISA respectively.

Results: IKKα$^{-/-}$ BMDMs pretreated with eNAMPT, in contrast to those from wild type, revealed a significant decrease in pro-inflammatory (*Tnfa, Il1b,* and *Il6*) gene expression and cytokine (TNF-α, IL-1β, and IL-6) release. Moreover, wild type BMDMs pretreated with eNAMPT were polarised towards M1 phenotype, whereas IKKα$^{-/-}$ BMDMs were skewed to an unaltered macrophage phenotype compared to IKKα$^{-/-}$ BMDMs in absence of eNAMPT. Finally, pre-incubation of BMDMs with LV.iNAMPT enhanced the pharmacological benefits of IKKα inhibition; increasing the expression of PPARγ-related genes.

Conclusion: These findings provide evidence that NF-κB play a role in pro-inflammatory activity of eNAMPT and reveal that targeting IKKα kinase activity represents a pharmacological approach.

P-109
THE ANGIOTENSIN-(1-7)/ MAS RECEPTOR AXIS COUNTERACT PRO-INFLAMMATORY SIGNALING IN HUMAN VASCULAR SMOOTH MUSCLE CELLS

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Background and Aims: Vascular inflammation is the main characteristic of vascular diseases such as hypertension and atherosclerosis,

which are one leading causes of death worldwide. For this reason, targeting inflammation is nowadays regarded as a challenging pharmacological strategy to prevent or delay the development of vascular diseases. Angiotensin (Ang)-(1-7) is a heptapeptide of the renin-angiotensin system (RAS) that binds Mas receptors and participates in vascular homeostasis by antagonizing some of the actions of Ang II. Here, we explored the capacity of Ang-(1-7) to counteract human aortic smooth muscle cell (HASMC) inflammation triggered by both (Ang II) or RAS-independent stimuli, such as interleukin (IL)-1β.
Methods: HASMC cultures were isolated from aortic fragments obtained from organ donors at Hospital Universitario de Getafe. The expression of inducible nitric oxide (iNOS) and the release of nitric oxide were determined by Western blot and the Griess method, respectively. The activation of NADPH oxidase was determined by lucigenin-derived chemiluminescence, while NF-κB activation was measured by electromobility shift assay.
Results: Ang-(1-7) inhibited in a concentration-dependent manner the iNOS induction and NO release elicited by Ang II and IL-1β. The effect of Ang-(1-7) was equally blocked by two different Mas receptor antagonists, A77 and D-Pro-Ang-(1-7), suggesting the participation of a unique Mas receptor subtype. Both Ang II and IL-1β were able to activate NADPH oxidase and nuclear factor (NF)-κB upstream of iNOS induction, and such pro-inflammatory signaling was attenuated by Ang-(1-7).
Conclusion: Ang-(1-7) can act as a counter-regulator of the inflammation of vascular smooth muscle cells triggered by Ang II, but also by other stimuli beyond the RAS. Activating the Ang-(1-7)/Mas axis may represent a pharmacological opportunity to attenuate the pro-inflammatory environment that promotes and sustains the development of vascular diseases.

P-110
EVOO PHENOLS, HYDROXYTYROSOL AND HYDROXYTYROSOL ACETATE, SUPPRESSED IL-1β-INDUCED PROINFLAMMATORY MEDIATORS PRODUCTION IN HUMAN SYNOVIAL FIBROBLASTS

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The hallmark of rheumatoid arthritis (RA) pathology is characterized by both hyperproliferation of synovial fibroblasts (SFs) and massive infiltration of inflammatory immune cells, including CD4 + T-cells and innate immune cells. It is well known that various proinflammatory mediators, including IL-6, IL-1β, TNF-α, and MMPs, released from RA-SFs are involved in the destruction of both articular bone and cartilage. This study was conducted to evaluate the efficacy of hydroxytyrosol (HTy) and hydroxytyrosol acetate (HTy-Ac), phenolic compounds from EVOO in regulating IL-1β-induced production of metalloproteases (MMP-1 and MMP-3), IL-6 and TNF-α in human synovial cell line, SW982. Treatment with HTy and HTy-Ac significantly inhibited IL-1β -induced MMPs, IL-6 and TNF-α production when measured by ELISA. The nuclear transcription NF-κB represent an attractive target for RA since it induces the transcription of inflammatory cytokines and mediators (IL-6 and MMPs) being responsible in addition to phosphorylation of MAPKs and COX-2 up-regulation. The effects of HTy and HTy-Ac on COX-2 and m-PGEs1 protein expression and the activation of MAPKs and NF-κB were also examined in SW982 cells by western blotting IL-1β-up regulation COX-2 and m-PGEs1 were diminished by HTy and HTy-Ac. IL-1β-induced p38, JNK and ERK1/2 phosphorylation were inhibited by HTy and HTy-Ac treatment. Similarly, the phenolic compounds inhibited IL-1β-induced NF-κB activation. These results suggest that these EVOO phenols reduce the production of proinflammatory mediators in SW982 cells; the mechanism underlying these protective effects could be related to the inhibition of MAPKs and NF-κB signalling pathways.

P-111
VASCULAR DAMAGE INDUCED BY VISFATIN IN MICE INVOLVES NADPH OXIDASE ACTIVATION AND PROSTANOID RELEASE

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Introduction: Vascular aging, obesity, and type 2 diabetes mellitus are associated with high circulating levels of pro-inflammatory adipocytokines, namely IL (interleukin)-1β or visfatin. We investigated whether the *in vivo* infusion of these adipocytokines can produce vascular alterations mimicking those observed in aging or metabolic diseases.
Methodology: Osmotic mini-pumps were implanted in three-month-old male mice, and vehicle, visfatin (100 ng/kg/day) alone or with the specific inhibitor of visfatin enzymatic activity FK866 (2.4 mg/kg/day), or IL-1β (12 μg/kg/day) were infused for 7 days. Some mice also received i.p. the IL-1β-receptor antagonist anakinra (100 mg/kg/day) for 3 days. Reactivity of mesenteric arteries was studied *ex vivo* using a small vessel myograph. Microvessels from visfatin-treated mice were preincubated *ex vivo* with 10 μM apocynin (NADPH oxidase inhibitor), 10 μM SQ-29,548 (thromboxane A2 receptor antagonist) or 100 μg/ml anakinra. Moreover, microvessels from control mice were treated *ex vivo* with visfatin (50 ng/ml) alone or in the presence of the previously described inhibitors.
Results: The infusion of visfatin or IL-1β reduced the endothelium-dependent relaxations produced by acetylcholine (ACh, 10 nM-10 μM). The endothelial dysfunction induced by visfatin was reversed by the simultaneous *in vivo* infusion of FK866 or by the *ex vivo* preincubation of the segments with apocynin, SQ-29,548, or anakinra, indicating a role for superoxide anions, vasoconstrictor prostanoids, and endogenous pro-inflammatory IL-1β, respectively. Anakinra infusion also prevented IL-1β-induced endothelial dysfunction. Visfatin evoked endothelial dysfunction when acutely exposed *ex vivo* to mesenteric fragments from control mice, and this effect was blocked by apocynin and SQ-29,548, but not with anakinra. None of the tested drugs modified the contractility to 3 μM noradrenaline or the endothelium-independent vasodilations induced by sodium nitroprusside (10 nM-10 mM).
Conclusion: The infusion of the adipocytokines visfatin or IL-1β produces endothelial dysfunction in mice, supporting their possible role as mediators of vascular damage and premature aging associated with metabolic diseases.
Keywords: adipocytokines, vascular damage, visfatin

P-112
PROSTANOID-MEDIATED INHIBITION OF IL-6 TRANS-SIGNALLING IN PULMONARY ARTERIAL HYPERTENSION: A ROLE FOR EPAC-1 MEDIATED INDUCTION OF 'SUPPRESSOR OF CYTOKINE SIGNALLING 3' (SOCS3)

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Pulmonary arterial hypertension (PAH) is a devastating disease of the pulmonary vasculature associated with endothelial cell (EC) dysfunction, vascular inflammation and remodelling, which contribute to eventual heart failure and death. Recent research has highlighted inflammation as a key factor in PAH development, in particular the cytokine interleukin-6 (IL-6). IL-6 signals via a JAK/STAT signalling pathway to induce transcription of a number of pro-inflammatory and pro-angiogenic genes that enable PAH to progress, as well as the transcription of suppressor of cytokine signalling 3 (SOCS3) which is involved in a negative feedback loop that limits IL-6 signalling.

Current PAH therapies include prostanoid drugs which act by stimulating intracellular 3',5'-cyclic adenosine monophosphate (cAMP) levels. cAMP has been well documented as an inhibitor of endothelial dysfunction via exchange factor directly activated by cAMP (EPAC)1 mediated induction of SOCS3.
My studies are testing the hypothesis that an important mechanism by which cAMP-mobilising prostanoid drugs limit PAH is by inhibiting IL-6-mediated pulmonary inflammation and remodelling via Epac1-mediated SOCS3 inhibition of IL-6 induced JAK/STAT signalling. We have demonstrated that prostanoid drugs beraprost and treprostinil both induce SOCS3 mRNA and protein in pulmonary arterial ECs to inhibit IL-6 mediated Tyr705 phosphorylation of STAT3 by 30% ±8 ($P < 0.01$) and 25% ±9 ($P < 0.05$) respectively for $n = 4$ experiments. We will also present data assessing the functional significance of prostanoid mediated inhibition of IL-6 signalling in pulmonary arterial ECs, and determine the EPAC1 and SOCS3 dependence of their inhibitory effects on IL-6 signalling.
In summary, from these and future studies, it is anticipated that more effective strategies will emerge with which to target the IL-6/JAK/STAT signalling pathway in PAH.
Keywords: Pulmonary Arterial Hypertension; Cytokine Signalling; Jak-STAT; cAMP

P-113
HYDROXYTYROSOL DERIVATIVE MODULATES INFLAMMATORY RESPONSE IN LPS-INDUCED MURINE PERITONEAL MACROPHAGES

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Background: The beneficial effects of extra virgin olive oil have been corroborated by a large number of epidemiologic studies. Among the different components of olive oil, its phenolic compounds such as hydroxytyrosol (HTy), seem to have a special role in these beneficial effects. The present study was designed to investigate specially the antioxidant and anti-inflammatory role of HTy and its derivate in lipopolysaccharide (LPS)-stimulated murine peritoneal macrophages.
Methods: Isolated murine peritoneal macrophages were treated with HTy or its derivative in the presence or absence of LPS (5 μg/ml) for 18 h. Cell viability was determined using sulforhodamine B (SRB) assay and nitric oxide (NO) production was measured using the Griess reaction. Supernatants were collected, and then it were used to determined cytokines production, such as IL-1β, TNF- α, IFN-γ, IL-17 and IL-6, using Enzyme-linked immunosorbent assay (ELISA) kits. Pro-inflammatory enzymes and transcription factors expression were detected by Western blotting.
Results: HTy and its derivative reduced significantly nitrites levels and induced a significant decrease on inducible nitric oxide synthase (iNOS) expression. Moreover, HTy-derivative showed better results in the reduction of the most of the studied cytokines when compared with HTy. However, only HTy down-regulated significantly cyclooxygenase (COX)-2 protein expression. Our findings demonstrated no significant changes in p38 and JNK protein phosphorylation after treatments, suggesting that the MAPKs signalling pathway does not make a consistent contribution in the anti-inflammatory activities of HTy-derivative. However, the treatment with HTy and its derivative produced a significant up-regulation of Nrf-2 and HO-1 expression compared with LPS-DMSO cells. In the same way, both of them showed a significant reduction of STAT-3 expression.
Conclusions: This study establishes that HTy and its derivative could improve LPS-induced oxidative stress and inflammatory response reducing NO generation by down-regulation of iNOS and COX-2 protein expression via inhibition of Nrf-2 and HO-1 signaling pathway.

P-114
ANTI-INFLAMMATORY AND IMMUNOMODULATORY EFFECTS OF OSTEOSTATIN IN THE CIA MODEL

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Parathyroid hormone related protein (PTHrP) and its derived peptides have been recently studied for the treatment of bone diseases. One of them, osteostatin (PTHrP(107-111)), has shown *in vitro* anti-inflammatory and anti-senescence properties (1). The aim of the present study was to examine the effects of osteostatin in an animal model of rheumatoid arthritis.
We used the collagen-induced arthritis (CIA) model in DBA1/J mice. After the onset of arthritis, we administered 40 μg/kg/day and 80 μg/kg/day s.c. of osteostatin for 13 days. On day 14, serum was obtained to determine cytokines and bone metabolism mediators by Multiplex assay. After the sacrifice of mice, lymph nodes were extracted to characterize lymphocyte population by flow cytometry and cell proliferation by BrdU assay. Limbs were collected and homogenized to determine cytokines by ELISA.
Results demonstrated a dose-related improvement of the arthritic macroscopic score after 13 days of treatment with osteostatin. IL-1β (11731 ± 2535 pg/ml) and IL-17 (81.91 ± 22.34 pg/ml) levels in arthritic control paw homogenates were significantly decreased by osteostatin at 80 μg/kg/day (5319 ± 1016 pg/ml, $P < 0.05$ and 45.24 ± 5.88 pg/ml, $P < 0.05$, respectively). IL-6 levels in serum of control mice (2653 ± 601 pg/ml) were also reduced at the same dose (1482 ± 127 pg/ml, $P < 0.05$). In addition, osteostatin treatment significantly reduced lymphocyte proliferation (>50% inhibition) with respect to the cells obtained from control arthritic lymph nodes and the decrease of $CD4^+/CD8^+$ ratio observed in control mice was reverted in treated groups. These results confirm the ability of osteostatin to reduce inflammatory and immune responses in an *in vivo* model of chronic inflammation and suggest its potential interest for rheumatoid arthritis treatment.
1. Platas J, Guillén MI, Gomar F, Castejón MA, Esbrit P, Alcaraz MJ. Anti-senescence and Anti-inflammatory Effects of the C-terminal Moiety of PTHrP Peptides in OA Osteoblasts. J Gerontol A Biol Sci Med Sci. 2016;0(0):1–8.
KeyWords: arthritis, osteostatin, *in vivo*, CIA
Funded by SAF2013-48724R (MINECO, FEDER) and PROMETEOII/2014/071 (GENERALITAT VALENCIANA).

P-115
PSORIATIC FIBROBLASTS POLARIZE MACROPHAGES TO A PRO-INFLAMMATORY PHENOTYPE

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Fibroblasts are mesenchymal cells that regulate tissue homeostasis and are involved in the switch from acute resolving to chronic inflammation (1). We have recently described that psoriatic dermal fibroblasts showed a defective activation of the JNK pathway that resulted in diminished cytokine production and STAT3 signaling (2). In the present study we aimed to determine if fibroblast-released factors influence the polarization of macrophages.
Healthy and psoriatic fibroblasts were incubated during 24 h with IL1-β and supernatants were collected to obtain the correspondent healthy (HF) or psoriatic (PF) conditioned media, which were added to macrophage-derived THP-1 cells. After 24 h, levels of TNF-α and IL-10 were determined by ELISA. Macrophages incubated with PF conditioned media produced high levels of TNF-α (369.5 ± 27.5 pg/ml vs.

51.5 ± 17.5 pg/ml in untreated cells $P < 0.001$ $n = 5–8$) and low levels of IL-10 (63.4 ± 3.1 pg/ml $P < 0.0001$ $n = 5–10$). In contrast, macrophages incubated with HF conditioned media, produced lower levels of TNF-α (161.0 ± 21.8 pg/ml) and higher levels of IL-10 (213.0 ± 11.4 pg/ml). Psoriatic fibroblast showed a defect in COX-2 induction, which resulted in reduced PGE_2 production. Consistently, conditioned media of HF previously treated with indomethacin significantly increased TNF-α (574.9 ± 74.7 pg/ml $P < 0.0001$ $n = 5–8$) and reduced IL-10 (45.8 ± 5.3 pg/ml $P < 0.0001$ $n = 5–10$) production by THP-1. These results were corroborated directly stimulating macrophages with IL-1β in the presence or absence of exogenous PGE_2.

Our results suggest that healthy fibroblasts support the M2 anti-inflammatory phenotype in macrophages, which is lost in psoriatic fibroblasts. Therefore, dermal fibroblast could play a relevant role in the perpetuation of psoriasis by favouring the pro-inflammatory activation of macrophages while hindering resolution of inflammation. Additionally, our findings offer a possible explanation of the disease exacerbation triggered by NSAIDs.

(1) Buckley CD (2016). *Rheumatology (Oxford).* https://doi.org/10.1093/rheumatology/kew289.

(2) Arasa J et al. (2015). *Exp Dermatol.* 24:800–2.

KeyWords: psoriasis, macrophages, fibroblasts, COX-2

P-116
BENEFICIAL EFFECT OF ADENOSINE A_{2B} RECEPTOR ACTIVATION IN A MURINE MODEL OF SKIN HYPERPLASIA

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It is known that adenosine mediates, at least in part, the anti-psoriatic effect of methotrexate via interaction with one or more of four known cell-surface receptors (A_1, A_{2A}, A_{2B} and A_3). Recently, we have demonstrated that adenosine regulates inflammatory and proliferative response in human keratinocytes through A_{2B} receptor activation (1). In the present study we determine the effect of topical application of BAY60-6583 (BAY), a selective A_{2B} agonist, on the inflammatory murine model of epidermal hyperplasia induced by the protein kinase C activator 12-O-tetradecanoylphorbol-13-acetate (TPA).

BAY (1–10 μg/site), or vehicle (acetone) were applied on the shaved backs of female Swiss mice 30 minutes before TPA (2 nmol/site) for three consecutive days. The next day, animals were sacrificed and 1 cm^2 punch biopsies were collected, weighed and either homogenized or processed for histological analysis.

Topical application of BAY (10 μg/site) produced a significant reduction of score and edema determined by the weight of biopsies (115.7 ± 4.6 mg vs. 147.3 ± 9.3 mg in vehicle-treated mice, $n = 8–10$, $P < 0.01$). Treatment with the A_{2B} receptor agonist also reduced the levels of PGE_2 (4.6 ± 1.8 ng/ml vs. 10.6 ± 2.9 ng/ml in vehicle-treated, $n = 5–8$, $P < 0.05$) in skin homogenates. The possible effect on inflammatory skin infiltrate was observed in H&E stained paraffin-embedded tissue sections and correlated with the reduction of myeloperoxidase activity determined in homogenates ($A_{450\ nm}$ 0.568 ± 0.025 vs. 0.374 ± 0.025 in vehicle-treated $n = 5–8$ $P < 0.05$).

These results suggest that activation of the adenosine A_{2B} receptor, besides reducing keratinocyte proliferation, could regulate the inflammatory response in skin effect and therefore constitute a possible therapeutic strategy in psoriasis.

(1) Andrés RM *et al*, (2017). Adenosine A_{2A} and A_{2B} Receptors Differentially Modulate Keratinocyte Proliferation: Possible Deregulation in Psoriatic Epidermis. *J Invest Dermatol.137*,123-131.

Keywords: adenosine, A_{2B} receptor, mouse skin, BAY60-6583

P-117
SUPPRESSOR OF CYTOKINE SIGNALLING 3 (SOCS3) INTERACTION WITH CAVIN-1 LINKS SOCS3 FUNCTION AND CAVIN-1 STABILITY

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Caveolae are lipid raft microdomains essential for the compartmentalisation and regulation of several signalling pathways e.g. JAK/STAT signalling. Disruption of caveolae is a significant factor in multiple disorders including muscular dystrophy, cardiovascular disease, and cancer. Central to caveolae stability is cavin-1 which couples caveolae to the microtubule network to prevent degradation of a key structural element i.e. caveolin-1, and caveolae disassembly. Via an unbiased quantitative proteomics screen, we have identified SOCS3, a negative regulator of JAK/STAT signalling, as a novel cavin-1 interactor.

SOCS3-cavin-1 interactions were characterised by immunoprecipitation assays and probing overlapping peptide arrays. SOCS3 bound to multiple regions within cavin-1, while a PEST motif within the C-terminal region of the SOCS3 SH2 domain was required for interaction with cavin-1 independently of its capacity to bind phospho-tyrosine. Biochemical analysis and confocal imaging also demonstrated that SOCS3 localisation within lipid raft microdomains and at the plasma membrane required cavin-1. Interestingly, SOCS3 does not ubiquitinate cavin-1 but instead supports cavin-1/caveolae stability. Moreover, genetic deletion of cavin-1 results in the loss of SOCS3-mediated inhibition of cytokine signalling. Importantly, while the inhibitory function of SOCS3 relies on its induction, caveolae stabilisation occurs at basal SOCS3 expression levels. Thus, transmission electron microscopy demonstrated that SOCS3 knock-out endothelial cells show reduced levels of caveolae.

Our data suggest a novel role for SOCS3 in regulating caveolae assembly while cavin-1, acting as a scaffold-protein, might aid SOCS3-dependent regulation of JAK/STAT signalling. This is the first indication of a novel role for SOCS3 in caveola homeostasis and suggests that loss of caveolae represents a novel mechanism by which chronic activation of pro-inflammatory JAK/STAT signalling could be triggered in disease.

Keywords: JAK/STAT, caveolae, cavin-1, SOCS3, inflammation

P-118
CX_3CL1/CX_3CR1 AND CCL2/CCR2 AXES ARE POTENTIAL THERAPEUTIC TARGETS TO PREVENT ARTERIAL LEUKOCYTE ADHESION IN METABOLIC SYNDROME

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Introduction and Objectives: Metabolic syndrome (MS) is characterized by a cluster of physiological alterations that collectively convey in a low grade of systemic inflammation and endothelial dysfunction. Both are involved in atherosclerotic lesion formation yet the pathways involved in its development remain unknown. CX_3CL1/CX_3CR1 and CCL2/CCR2 axes have emerged as potential candidates for cardiovascular disease (CVD) prediction and targets for therapeutic intervention Therefore, the potential link of these chemokine/receptor axes and MS-induced endothelial dysfunction were investigated.

Material/Methods: Whole blood samples from 20 MS patients and 20 age-matched controls were analysed by parallel-plate flow chamber assay, to evaluate platelet-leukocyte and leukocyte adhesion to TNFα-stimulated arterial endothelium. Flow cytometry was employed to determine platelet (P-selectin expression and % of PAC-1^+ platelets), monocyte (CD11b expression) and lymphocyte (CD69 expression) activation. CX_3CR1 expression was also determined on platelets, monocyte subtypes (classical or M1, intermediate/pro-inflammatory or M2 and non-classical or M3) and lymphocyte subsets ($CD4^+$ and $CD8^+$) by flow cytometry.
Results: Enhanced platelet-leukocyte and leukocyte adhesiveness to TNFα-stimulated arterial endothelial cells was found in MS patients vs. age-matched controls. Neutralization of CX_3CL1 or CCL2 activity resulted in significant inhibition of adhesive interactions to the dysfunctional arterial endothelium in MS patients but not in control volunteers. In MS patients, greater platelet activation and CX_3CR1 expression than age-matched controls was detected. Additionally, MS patients presented augmented numbers of platelet-$CD8^+$ lymphocyte aggregates, enhanced lymphocyte activation and increased CX_3CR1 expression than control subjects. Monocyte activation and the percentage of CX_3CR1 expressing platelet/M1 monocyte aggregates were also increased in MS patients. These observations correlated with the augmented platelet-leukocyte- and leukocyte-arterial adhesiveness in MS.
Conclusions: Given that platelet/leukocyte- and leukocyte-arterial adhesion precedes atherogenic process development, blockade of either CX_3CL1/CX_3CR1 or CCL2/CCR2 axis may ameliorate the risk of suffering further cardiovascular events in patients with this metabolic disorder.
Keywords: Metabolic syndrome; Cardiovascular; Inflammation, chemokine/receptor axes

P-119
CHONDROITIN SULFATE INHIBITS MONOCYTE CHEMOATTRACTANT PROTEIN-1 RELEASE FROM 3T3-L1 ADIPOCYTES: A NEW TREATMENT OPPORTUNITY FOR OBESITY-RELATED METABOLIC SYNDROMES?

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Background: Monocyte chemoattractant protein-1 (MCP-1) overproduction from inflamed adipose tissue is a major contributor to obesity-related metabolic syndromes. We have recently published that chondroitin sulphate (CS) can attenuate the monosodium urate (MSU) crystal mediated THP-1 macrophage inflammatory response through an inhibitory effect on NF-κB activation.
Objective: We sought to determine whether CS had a similar inhibitory effect on MCP-1 release from lipopolysaccharide (LPS) stimulated adipocytes.
Methods: We treated 3T3-L1 adipocytes with LPS together with CS (Bioibérica, Spain) in a physiologically relevant range (10–200 μg/ml) and 24 h after we measured MCP-1 release (R&D Systems, Minneapolis, MN, USA). We also cultured THP-1 monocytes and tested whether CS (200 μg/ml) could inhibit cell migration induced by 24 h exposure to human recombinant MCP-1 (R&D Systems; 0, 3.125–100 ng/ml).
Results: We found that LPS (1 μg/ml) caused a significant rise in MCP-1 release ($P < 0.0001$) from 3T3-L1 adipocytes. CS in physiologically achievable concentrations (100–200 μg/ml) produced a dose dependent reduction ($P < 0.01$ at 100 μg/ml and $P < 0.001$ at 200 μg/ml) of MCP-1 release from 3T3-L1 adipocytes in response to LPS. Recombinant MCP-1(25–100 ng/ml) caused a dose dependent increase ($P < 0.001$ at 25 ng/ml and $P < 0.0001$ at 100 ng/ml) in cell migration of THP-1 monocytes. CS at the highest test concentration (200 μg/ml) had no effect on MCP-1 mediated THP-1 migration.
Conclusions: Our data demonstrate that CS inhibits the release of MCP-1 from 3T3-L1 adipocytes stimulated with LPS, but has no effect on the chemotactic action of MCP-1 on THP-1 monocytes. Furthermore, our work data strongly suggests that it is the inhibition of MCP-1 release by CS that underlies this effect and not a direct inhibition of the chemotactic action of MCP-1 by CS. Given the importance of MCP-1 over-production in obesity-related metabolic syndromes, the recruitment blocking of macrophages to adipose tissue exerted by CS, could provide a new treatment opportunity for these syndromes.
Keywords: metabolic syndromes; obesity; adipocytes; chondroitin sulfate; monocytes, macrophages, inflammation

P-120
A FLUOROMETHYLCHALCONE DERIVATIVE AS INHIBITOR OF CASPASE-1 ACTIVATION IN CPPD-INDUCED MOUSE AIR POUCH MODEL

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The inflammatory process includes the participation of many factors, such as the pro-inflammatory cytokines IL-1β and IL-18. Both require post-translational processing by active caspase-1 to generate their active forms. Caspase-1 activity is controlled by inflammasomes, a group of cytosolic protein complexes whose activity has been linked to many diseases including arthritis. This complex is activated by microbial pathogens and damage-associated molecular patterns such as calcium pyrophosphate dihydrate crystals (CPPD)[1].
Previous studies demonstrated the potential anti-inflammatory profile of 3,4,6-trimethoxy-6′-trifluoromethylchalcone (CH) by inhibition of NO release and iNOS expression in macrophages[2]. In the present study, we have characterized the involvement of caspase-1 activation in the CPPD-induced mouse air pouch model (MAP) in order to determine the anti-inflammatory effect of CH.
Six days after the initial air injection, CH (100 μg/pouch) was injected in the treated group. After 20 minutes, CPPD (100 mg/pouch) was injected in CH and control group. Mice were sacrificed 6 h later and pouch exudates were collected. Cells were measured using a Coulter Counter. IL-1β, IL-18 and TNF-α were determined by ELISA in supernatants. Cell pellet was used to measure the active p20 subunit of caspase-1 by Western Blotting.
Results demonstrated that CH treatment down-regulated cell migration into exudates (218.00 ± 29.90 cells/ml $P < 0.05$) with respect to control group (502.10 ± 75.32 cells/ml). IL-1β (517.10 ± 59.20 pg/ml) and IL-18 (94.94 ± 12.75 pg/ml) levels in control supernatants were also significantly decreased after CH treatment (IL-1β: 279.40 ± 32.68 pg/ml $P < 0.01$); IL-18: 51.39 ± 1.64 pg/ml $P < 0.05$). Besides, the increase in the expression of activated p20 subunit from control exudates demonstrated the involvement of caspase-1 in MAP, suggesting that inhibition of inflammasome activation is as a potential mechanism of CH anti-inflammatory effect.
1. Martinon *et al.* (2006), *Nature, 440*:237–41.
2. Rojas *et al.* (2002), *Bioorg Med Chem Lett, 12*:1951–4.
Keywords: Inflammation; Caspase-1; CPPD; Air Pouch Model
Funded by SAF2013-48724R (MINECO, FEDER) and PROMETEOII/2014/071 (GENERALITAT VALENCIANA).

P-121
VLDL AND APOLIPOPROTEIN CIII INDUCE ER STRESS AND INFLAMMATION AND ATTENUATE INSULIN SIGNALING VIA TOLL-LIKE RECEPTOR 2 AND ERK1/2 IN SKELETAL MUSCLE CELLS

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Aim/hypothesis: Here, we examined whether very low-density lipoproteins (VLDL) and apolipoprotein (apo)CIII induce endoplasmic reticulum (ER) stress, inflammation, and insulin resistance in skeletal muscle.
Methods: Studies were conducted on mouse C2C12 myotubes, isolated skeletal muscle, and in skeletal muscle from transgenic mice overexpressing apoCIII.
Results: C2C12 myotubes exposed to VLDL showed increased levels of ER stress and inflammatory markers, whereas peroxisome proliferator-activated receptor γ co-activator 1α (PGC-1α) and AMP-activated protein kinase (AMPK) levels were reduced and the insulin signaling pathway was attenuated. VLDL effects were also observed in isolated skeletal muscle incubated with VLDL. The changes caused by VLDL were dependent on extracellular signal-regulated kinase (ERK1/2) because they were prevented by the ERK1/2 inhibitor U0126 or by knockdown of this kinase by siRNA transfection. ApoCIII mimicked the effects of VLDL and its effects were also blocked by ERK1/2 inhibition, suggesting that this apolipoprotein was responsible for the effects of VLDL. Skeletal muscle from transgenic mice overexpressing apoCIII showed increased levels of some ER stress and inflammatory markers and of phosphorylated ERK1/2 levels, whereas PGC-1α levels were reduced, confirming apoCIII effects *in vivo*. Finally, incubation of myotubes with a neutralizing antibody against toll-like receptor 2 abolished the effects of apoCIII on ER stress, inflammation, and insulin resistance, indicating that the effects of apoCIII were mediated by this receptor.
Conclusions/interpretation: These findings indicate that VLDL and apoCIII induce ER stress, inflammation, and insulin resistance by activating ERK1/2 through TLR2, suggesting that targeting VLDL overproduction can ameliorate insulin resistance and it might prevent type 2 diabetes mellitus.

P-122
ORM2 AND APOA2 SERUM LEVELS CAN PREDICT OA PATIENT RESPONSE TO CHONDROITIN SULFATE/ GLUCOSAMINE HYDROCHLORIDE: RESULTS FROM THE MOVES STUDY

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Background: A shotgun proteomic analysis performed on sera from patients enrolled in the Multicentre Osteoarthritis interVEntion trial with Sysadoa(MOVES) led to the discovery of a panel of predictive protein biomarkers useful to stratify osteoarthritis(OA) patients into responders and non-responders, either to Chondroitin sulfate/ glucosamine hydrochloride(Droglican®, Bioiberica S.A., Barcelona, Spain) or Celecoxib.
Objectives: To validate the sensitivity and specificity of a panel of six serum proteins useful to predict the patient response to Droglican treatment.
Methods: We analyzed the serum levels of:APOA2, APOA4, APOH, C4BPa, ITIH1 and ORM2 by enzyme-linked immunosorbent assays (ELISAs).All the subjects studied belonged to the MOVES cohort at baseline(Droglican sub-cohort, $n = 260$).Non-parametric and multivariate analysis were performed to test the effects of the clinical variables gender, age, BMI, radiologic Kellgren/Lawrence(K/L) grade and WOMAC score at baseline, as well as the biomarker serum levels, on the response to Droglican treatment according to the OMERACT-OARSI criteria and the WOMAC pain score(20%,30%,50% and 70% reduction) recorded at the end of the trial (after 6 months of treatment).
Results: We found decreased serum levels of ORM2 at baseline in responders to Droglican according to the OMERACT-OARSI criteria vs. non-responders (76,11 ± 53,25vs104,25 ± 84,93; $n = 171$ vs 46; $P = 0.047$).APOA2 appeared statistically increased in responders with a 50% reduction in WOMAC pain score compared to non-responders (79,95 ± 58,53vs66,05 ± 46,49; $n = 129$vs112; $P = 0.028$).Patients with lower levels of ORM2 (median concentration= 69.8 μg/ml) and higher level of APOA2 (median concentration= 63.8 μg/ml) responded better to pharmacotherapy. Statistical interactions between ORM2 and APOA2 levels and radiologic K/L grade were also detected ($P = 0.048$ and $P = 0.002$, respectively).No statistically significant differences were found for the other four proteins.
Conclusion: Our results show that ORM2 and APOA2 levels significantly correlates with patients response to Droglican suggesting the possibility of their use in predictive assays in order to optimize therapeutic outcomes in OA. Validation studies in different cohorts are needed to identify and validate a cut-off point for these biomarkers.

P-123
EVALUATION OF THE ANTI-INFLAMMATORY ACTIVITY OF LXR AGONISTS IN *IN VITRO* AND *IN VIVO* MODELS

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Evidence in the literature supports a role for topical liver X receptor (LXR) agonists in reducing skin inflammation and enhancing skin barrier function in different mouse models, making LXR an interesting target for treating skin inflammatory diseases such as atopic dermatitis. However, most evidence has been generated using non-selective LXR agonists. In this work we have assessed the effect of selective LXR agonists in several *in vitro* and *in vivo* mouse models of skin inflammation. Inhibition of cytokine secretion in human keratinocytes (HaCaT cell line and primary keratinocytes) and immune cells (PBMCs and THP-1 cell line) in response to a pro-inflammatory stimulus (TNF-α and Poly(I:C) + IL-4 for keratinocytes and LPS for immune cells) was analyzed. Gene expression analysis of different markers of skin inflammation and barrier function was performed in a commercial *in vitro* model of atopic dermatitis using skin equivalents stimulated with a mixture of cytokines (IL-4, IL-13, IL-22, TNF-α). Efficacy was evaluated through the topical route in mouse models of TPA-induced contact dermatitis and oxazolone-induced delayed type hypersensitivity (DTH), the latter considered a more relevant model of atopic dermatitis. We observed a poor anti-inflammatory activity *in vitro* and a lack of therapeutic effect in the DTH model. Only a dose-dependent effect was observed in the TPA-induced acute inflammation model. In addition, LXR agonists did not modify biomarkers of skin barrier function in human skin equivalents. These results call into question the potential of LXR agonists as a therapeutic option for treating skin inflammatory diseases such as atopic dermatitis.
Keywords: LXR; atopic dermatitis; inflammation; skin barrier function

P-124
CHARACTERIZATION OF THE *IN VITRO* AND *IN VIVO* PROFILE OF EPIGALLOCATECHIN-3-GALLATE

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Epigallocatechin-3-gallate (EGCG) is the main polyphenol in green tea reported to have anti-inflammatory, anti-carcinogenic, antioxidant and antimicrobial activities. A potential use for EGCG in different skin conditions has been put forward. The aim of this study was to characterize the pharmacological activity of EGCG in *in vitro* and *in vivo* models of skin inflammation. The anti-inflammatory effect of EGCG was assessed in human primary keratinocytes by the ability to inhibit the production of TSLP induced by Poly:IC/IL4, and in mice by the ability to reduce oxazolone-induced ear edema in a DTH-type reaction. In addition, cutaneous local tolerance was assessed in mice upon topical repeated administration for 4 days. In both *in vivo* models, EGCG formulated as an ointment (3%) or solubilized in ethanol (1, 3 and 10%) was compared to the commercial green tea extract approved for warts treatment (Veregen® containing around 5% EGCG). EGCG modestly inhibited TSLP production in human primary keratinocytes with an IC_{50} of 1.7 μM. However, assessment of the stability of EGCG demonstrated that it was completely degraded in the assay medium, suggesting that EGCG may not be responsible for this activity. In addition, no anti-inflammatory effect of two stable formulations (ointment and ethanol-based) was observed in the oxazolone-induced DTH model, whereas Veregen significantly increased ear edema. Local tolerance studies did not reveal any relevant clinical, macroscopic or histological changes compared to vehicle treated animals in the 4-day scheme. Taken together, these results question the therapeutic use of EGCG for inflammatory dermatoses and warrant further discussion.

Keywords: Epigallocatechin-3-gallate (EGCG); Veregen®; keratinocyte; skin inflammation models

P-125
DISCOVERY OF SMALL MOLECULES FOR IL-17 MEDIATED INFLAMMATORY DISEASES

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Inflammation is an adaptive response of the body to harmful stimuli. Chronic inflammation is the classic sign of autoimmune diseases and is associated with the malfunction of tissue. Such diseases have a great impact in the quality of life and the outcomes of patients, since they occur at early age. Although treatments have improved significantly with the introduction of biologics, novel small molecule approaches may enable further development of therapeutics.

Lately, interest has been sparked in interleukin-17 (IL-17), which binds to surface receptors with dimer structure to activate inflammation, as a novel pharmacological target for inflammatory autoimmune diseases. Therapeutic antibodies selectively targeting IL-17A have been approved for clinical use in psoriasis, a condition characterized by abnormal accumulation of skin cells (psoriatic plaques) due to overactivation of the immune system. Recently, a class of macrocyclic compounds was discovered, indicating that the IL-17 druggability with molecules might be feasible.

We aim to identify chemical tools to study the IL-17 mediated inflammatory mechanisms in psoriasis. For this purpose, we developed a protocol for the production of recombinant IL-17A in mammalian cells that can be used in label-free binding assays. IL-17A was cloned into pcDNA3.1.+ vector containing a C-terminal His_6 tag. The plasmid was transfected in HEK 293 cells and supernatants, where IL-17A is released, were collected after 48 hours. Subsequently, protein was purified by affinity chromatography on Ni-Sepharose Fast Flow column. We confirmed IL-17A expression by means of western blotting and we achieved a protein production of > 85% purity.

In order to identify novel ligands for IL-17A, we used DMR (Dynamic Mass Redistribution) technology. We tested the protein immobilization at different concentrations and pH, obtaining suboptimal binding levels. Further assay development and screening may provide a discovery platform for novel IL-17A ligands.

Keywords: inflammation; psoriasis; IL-17; label-free

P-126
EFFECT OF THE INTAKE OF THE STRAIN BIFIDOBACTERIUM ANIMALIS SUBSP. LACTIS CECT 8145 IN ZÜCKER FATTY RATS

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We evaluate the effect of oral administration of the strain *Bifidobacterium animalis* subsp. *lactis* CECT 8145 in Zücker fatty rats. The Zücker fatty rats were randomly divided into two groups of 10 animals that were respectively fed with either *B. animalis* subsp. *lactis* CECT 8145 (10^{10} CFU/day) suspended in skim milk, or skim milk (control group), from week 5 until week 17 of life. A lean Zücker rat group (standard group) was included to provide normal values for the Zücker strain. To this group was administered skim milk for the same experimental period as Zücker fatty rats. Body weight gain was more accentuated in the fatty control group than in the fatty rats that received daily *B. animalis* subsp. *lactis* CECT 8145. In line, dry and liquid food intakes significantly decreased in the Zücker fatty group fed with this bacterial strain. These rats also showed decreased plasma ghrelin levels as compared with the Zücker fatty control group. *Bifidobacterium animalis* subsp. *lactis* CECT 8145 intake also decreased the pro-inflammatory cytokine tumoral necrosis factor-alpha and the oxidative stress biomarker malondialdehyde. Moreover, the ratio plasma total cholesterol/plasma cholesterol transported by high-density lipoproteins, considered as an indicative index for cardiovascular disease, also significantly decreased in the Zücker fatty rats that were fed with *B. animalis* subsp. *lactis* CECT 8145. On the contrary, this bacterial strain significantly increased plasma adiponectin (an insulin-sensitizing adipokine) and slightly improved plasma triglycerides and glucose metabolism biomarkers. Further research would be needed to support the effects of *B. animalis* subsp. *lactis* CECT 8145 in humans as a probiotic for metabolic syndrome prevention. Nevertheless, our results are promising and set the health and anti-obesity properties of this bacterial strain.

Supported by Biópolis S.L. (78/2012 U.C.M Project, art. 83 L.O.U.).

P-127
EFFECTS OF PEPSIN EGG WHITE HYDROLYSATE ON METABOLIC SYNDROME

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Metabolic syndrome (MetS) is a complex disorder which refers to the clustering of central obesity, insulin resistance, impaired glucose tolerance, hypertension and dyslipidemia. The prevalence of this disorder is increasing fast, and it is becoming an important and worldwide public health problem. The purpose of this study was to evaluate the effect of the administration of a pepsin egg white hydrolysate (HEW) on some metabolic complications developed in MetS. Thirty-four 8-week old Wistar rats where used and divided in four groups: Control group (C); Control + HEW group (CH); high-fat diet with a 25% dextrose solution (MS) and high-fat diet with a 25% dextrose and HEW 1 g/kg/day (MSH). Body weight, food and liquid intake were estimated weekly. The occurrence of neuropathic sign (tactile allodynia) was assessed once every 6 weeks using the von Frey hair test. At the end of the study, the abdominal circumference was registered. Blood samples for biochemical determinations (plasma glucose, lipid metabolism and oxidative stress biomarkers), and epididymal adipose tissue and liver for histopathological analyses were collected. Abdominal circumference, body, adipose tissue and liver weights were decreased after HEW consumption in MSH group. Plasma glucose values were slightly reduced in MS animals after HEW intake, and the tactile allodynia was significantly improved in this experimental group. The oxidative stress biomarkers, such as plasma malondialdehyde and liver glutathione were also decreased in MSH group, and both groups fed with HEW (CH and MSH) showed lower adiponectin levels. No significant differences were observed in the antioxidant capacity and leptin levels in plasma. Our results suggest that HEW may improve simultaneously several metabolic complications developed in MetS, and could be used as an alternative therapy to control the MetS progress in obese patients.
Supported by MINECO (AGL212-32387).

P-128
ANTIOXIDANT AND BIOACTIVE PROPERTIES OF *JASONIA GLUTINOSA* (COMPOSITAE) IN *CAENORHABDITIS ELEGANS*

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Jasonia glutinosa (Compositae) is an endemism from the Iberian Peninsula traditionally used as a medicinal herbal tea to improve digestion and gastrointestinal disorders. The plant is known as té de roca (rock tea) or té de Aragón and has been highly cited in ethnopharmacological studies as a medicinal plant. Previous studies from our group have demonstrated the spasmolytic activity. We have now measured the antioxidant and protective effects of *Jasonia glutinosa* extract using *in vitro* and *in vivo* procedures as the living organism *Caenorhabditis elegans*. The antioxidant activity in living organisms was evaluated using *C. elegans* pretreated with the plant extract and exposed to juglone as oxidizing agent. Life span of *C. elegans* treated with increasing concentrations of the plant extract was also tested. Antioxidant activity against free radicals was performed using enzymatic systems such as xanthine oxidase or monoamine oxidase as well as the free radical DPPH. *Jasonia glutinosa* exerted antioxidant and protective properties both in cell free systems (enzymatic and chemical assays) and in *C. elegans* stressed with juglone. *Jasonia glutinosa* was able to scavenge free radicals in a dose dependent manner and to significantly increase *C. elegans* survival up to 10% compared to control groups, where all individuals died after 24 h of juglone treatment. Life span of *C. elegans* significantly augmented when worms were treated with 100 μg/ml of extract. We can conclude that the increase in life span and in the survival of *C. elegans* exposed to juglone could be mediated through antioxidant mechanisms of compounds present in *Jasonia glutinosa* extract.
Acknowledgements: Universidad San Jorge is thanked for financial support.

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DUAL EFFECTS OF RESVERATROL ON CELL DEATH AND PROLIFERATION OF COLON CANCER CELLS

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Colorectal cancer remains a main cause of death worldwide, and novel agents have been searched as polyphenols which have emerged as promising therapeutic tools in cancer. Resveratrol (3,5,4′-trihydroxy-trans-stilbene) induces cell death in different tumor cell lines, but it also stimulates the proliferation of specific breast and prostate cancer cell lines. Here, we studied the impact of resveratrol over a 100-fold concentration range on cell death and proliferation of HT-29 colorectal adenocarcinoma cells. A biphasic pattern was observed after 96 h of exposure to resveratrol. At lower concentrations (1 and 10 μmol/L), resveratrol promoted an increase in cell number. However, enhancing the resveratrol concentration up to 50 or 100 μmol/L resulted in reduced cell number, together with increased percentage of apoptotic or necrotic cells, thus indicating cytotoxicity. These proliferative properties at lower concentrations were shared by the flavonoid polyphenol quercetin. However, on a second colon cancer cell line, such as HCT116, the growth promoting properties of resveratrol could not be observed. The cytotoxic effect of resveratrol on HT-29 cells was associated with the over-activation of the superoxide-generating enzyme NADPH oxidase and increased levels of histone γH2AX, a marker of DNA damage. A parallel enhancement in the levels of sirtuin 6 was also observed, probably as a repair mechanism to counterbalance the damaged exerted by resveratrol to the cells. In conclusion, resveratrol seems to be an effective tool to be used in anti-tumor chemotherapy. However, the biphasic behavior of resveratrol indicates that this polyphenol may in some conditions favor tumor cell growth. Thus, appropriate local concentrations must be achieved to minimize unwanted harmful effects of resveratrol as an anti-cancer drug.

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SAFETY STUDY ON THE USE OF MEDICINAL PLANTS

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Summary: Since ancient times, man has used medicinal plants for the prevention and treatment of various diseases. Despite their beneficial and traditional role in different communities, it must be taken into consideration that they can also cause adverse reactions and toxicity. The aim of this study was to analyze the reports on Communication of Adverse Events for the use of Herbal Medicines, Plant Products and/or Preparations of Vegetable Drugs, received as Chaco's Peripheral Reporter for the National Pharmacovigilance System, part of the National Administration of Drugs, Food and Medical Technology (ANMAT) of Argentina during the 2010–2016 period. A retrospective

analysis of the reports received from pharmacies and health centers was made using the Anatomical, Therapeutic, Chemical (ATC) classification guide for herbs. The causality was assessed by the Naranjo et al. algorithm and the severity was classified into three categories: mild, moderate, and severe. From the total of notifications received (n = 135), 80% involved the use of a single plant product, 15% the mixture of two or more and 5% the use of herbal medicines. Thirty-four percent of reports about adverse reactions involved children, while female sex prevailed over male. According to the ATC classification of herbs, 71% corresponded to the HA group (alimentary tract and metabolism) as the most reported, followed by 14% to the HN group (nervous system) and the rest to other groups. The most common adverse reactions proved to be probables and milds. The most frequently reported medicinal plants involving children were *Chenopodium ambrosioides* L. and *Illicium verum* Hook. F., with severe adverse effects such as seizures being described, whereas in the adult population it was *Cassia angustifolia* Vahl., with moderate adverse effects. It is essential to monitor and identify the risks posed by plants, especially when used by vulnerable populations, thereby promoting their safe use.

P-131
DIOSMETINE AND DIOSMINE INHIBIT THE PROINFLAMMATORY CYTOKINES RELEASE

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Nowadays cardiovascular diseases are major causes of death in the developed world. Platelets play a key role in thrombus formation. Experiments carried out in our laboratory showed diosmetine and diosmine, polyphenols of flavonoids family, had a potential antiplatelet activity. Pro-inflammatory cytokines promote the activity of specific adhesion molecules on the vascular endothelium contributing to the leukocyte rolling and their extravasation from capillaries, what leads to exacerbate the thrombosis clinical course. Therefore, we tested the impact of these products on the inhibition of these cytokines release.
Material/Methods: Whole blood aliquots extracted from 10 healthy volunteers were incubated with diosmetine/diosmine, both to 0.5 mM, 1 mM, and 2 mM concentration. Lipopolysaccharide (LPS) was added for inducing pro-inflammatory cytokines release. Samples were centrifuged and supernatant was collected in order to measure the IL-1β and TNF-α secretion from monocytes using specific immunoassay techniques.
Results: LPS (10 mg/ml) induced a significant increase on the IL-1β and the TNF-α release compared to control values. This production was inhibited by diosmetine and diosmine at 0.5 mM, 1, and 2 mM concentrations. IL-1β inhibition mean with diosmetine was 21.94%± 3.38, 43.04%± 4.91, 71.05%± 3.44, and with diosmine was 0.00%, 0.00%, 1.51%±1.99 respectively. TNF-α inhibition mean with diosmetine was 43.36%± 1.89, 77.00%± 2.06, 84.11%± 2.12, and with diosmine was 0.00%, 7.91%± 2.61, 15.60%± 2.37, respectively.
Conclusions: Diosmetine was able to inhibit the pro-inflammatory cytokines released induced by LPS in a dose-dependent way, while diosmine provokes only certain effect over TNF-α at its higher dose. The diosmine antiplatelet activity was higher than diosmetine. The activity differences shown could be related with the different chemical structures of the heteroside and the aglycon. However these studies suggest both flavonoids could have a potential therapeutic use in cardiovascular disease.
Keywords: diosmetine, diosmine, IL-1β, TNF-α

P-132
ANTHELMINTIC ACTIVITY OF ESSENTIAL OILS AGAINST *ANISAKIS SIMPLEX* L3 LARVAE

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Anisakis simplex is a foodborne pathogen with increasing interest due to the high consumption of raw fish products and the prevalence of anisakiasis worldwide. Nineteen essential oils obtained from medicinal and culinary plants were screened against *Anisakis simplex* L3 larvae isolated from blue whiting fish (*Micromesistius poutassou*). The experiments were performed in small Petri dishes containing 10 larvae. The percentage of anisakis mortality after 48 h of treatment at 1 µl/ml was superior to 50% only for C*innamomum zeylanicum*, *Cinamomum cassia*, *Tanacetum annum* and *Origanum compactum*. Inhibition of the enzyme acetylcholinesterase through a colorimetric assay in 96-well plates was used to elucidate the pharmacological mechanism. The four essential oils were able to inhibit acetylcholinesterase, which could explain in part the mechanism of action against the parasites. The median lethal doses (DL_{50}) after 24 and 48 h were lower than 0.5 µl/ml for *C. zeylanicum*, *C. cassia* and *O. compactum*, revealing a good potential as anthelmintic natural products.
Acknowledgements: Pranarom International is thanked for financial support and supplying essential oils.

Table 1.

	Baseline				6 months			
	No bDNA		bDNA		No bDNA		bDNA	
	CS/GH	CE	CS/GH	CE	CS/GH	CE	CS/GH	CE
Adrenaline (pg/ml)	98 (88–127)	100 (77–118)	92 (78–114)	98 (75–115)	78* (65–92)	81* (62–92)	76* (58–97)	77* (66–105)
Noradrenaline (pg/ml)	1357 (790–1693)	1228 (738–1967)	1723# (1385–2381)	2765# (1232–3250)	1050 (811–1600)	1588 (882–2133)	1563 (1209–2180)	1528 (1174–2229)
IL-8 (pg/ml)	5.5 (1.3–9.4)	5.6 (1.7–14)	4.8 (3.9–5.7)	11 (1.9–15)	3.3* (0.0–11)	0.0* (0.0–5.9)	6.1 (3.7–14)	5.6 (1.5–16)
VAS score (0–100)	72 (66–82)	75 (65–81)	79 (67–90)	76 (70–80)	32* (22–45)	20* (10–60)	37* (27–50)	52* (24–60)
WOMAC score	364 (341–389)	354 (321–389)	382 (372–407)	360 (340–400)	150* (87–217)	135* (64–238)	180* (84–218)	282* (139–318)

*$P < 0.05$ vs. baseline values (Mann-Whitney-Wilcoxon Test, # $P < 0.05$ vs. No bDNA values.

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THE REDUCTION IN ADRENERGIC AND INFLAMMATORY SERUM MARKERS IN OSTEOARTHRITIS PATIENTS AFTER TREATMENT WITH CHONDROITIN SULFATE/ GLUCOSAMINE HYDROCHLORIDE AND CELECOXIB IS DIFFERENT ACCORDING TO THE PRESENCE OF BACTERIAL DNA IN BLOOD

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Background: Inflammation in osteoarthritis(OA) has been characterized by infiltration of immune cells and secretion of cytokines into synovial tissues. Adrenergic activity has been associated with subchondral bone loss and increased osteoclast activity in OA. The relationship between activation of adrenergic and immune system with OA progression and treatment response is unknown.
Objectives: To assess the adrenergic and immune activation in OA patients treated with Chondroitin Sulfate/Glucosamine Hydrochloride (CS/GH)(Droglican®, Bioibérica, Barcelona) or Celecoxib(CE).
Methods: serum samples from patients participants in a 6-month controlled, double blind, randomized clinical trial comparing the analgesic efficacy of CS/GH and CE were analyzed to determine cytokines(IL-2, IL-4, IL-6, IL-10, IL-8, IL-1beta using BD™ CBA immunoassay),catecholamines(noradrenaline, adrenaline and dopamine using ELISA LDN®) and the presence of endotoxin or lipopolysaccharide(LPS; LAL test Lonza®) and bDNA(16S ribosomal DNA-based PCR).
Results: Samples from 100 OA patients(age:62 ± 8 yr.; BMI:31 ± 6 kg/m^2; 86 females; VAS:73 ± 15; WOMAC:369 ± 43) treated with CS/GH(50) or CE(50) were analyzed. There were no baseline significant differences between the two treatment groups regarding demographics, clinical and experimental variables. Thirty-four patients(*n* (CS/GH)=17; *n*(CE)=17) showing bDNA in blood had significantly higher levels of noradrenaline compared with patients without bDNA (1993[1354–3183]vs.1324[738–1899];$P = 0.0002$).After 6 months, both groups showed a similar reduction in VAS and WOMAC score and serum adrenaline levels independent of presence/absence of bDNA (Table 1).Thirty-three patients showed bDNA presence at the end of the study. Patients with bDNA at 6 months showed reduced serum noradrenaline levels compared with those observed in patients with bDNA at baseline(1993[1354–3183] vs.1561[1174–2193]; $P = 0.0325$).IL-8 was significantly reduced after 6 months only in patients without bDNA. There were no significant differences between baseline and 6 months samples in the other experimental variables.
Conclusions: OA patients show bDNA fragments in blood associated to higher serum noradrenaline levels. 6-month treatment with CS/GH or CE reduces significantly pain and adrenaline levels.IL-8 levels were also reduced except in patients with bDNA fragments in blood. Systemic adrenergic and inflammatory activity in OA patients is influenced by the presence of bDNA in blood and this may be taken into consideration to evaluate severity, evolution and treatments' response.

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TOPICAL PRE-TREATMENT WITH A CAROTENOID FROM MICROALGAE REDUCES CUTANEOUS UVB-INDUCED INFLAMMATION IN HAIRLESS MICE

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Introduction: Acute exposition to UVB (360 mJ/cm^2) induces increase in skin thickness, loss of elasticity and moisture, increase the melanin production, and promote inflammation. These characteristics are well known as photoaging parameters and play a crucial role in skin cancer induction. Photochemoprevention with natural products is a potent strategy for reduce the development of skin cancer. F-111 is a carotenoid isolated from *Isochrysis galbana* microalgae. The aim of this study was to investigate whether pre-treatment with the carotenoid emulsion is capable to reduce the UVB-induced inflammation in SKH-1 hairless mice.
Materials: O/W emulsions were prepared by gradually adding solutions of F-111 (10 mg/ml) or β-carotene (10 mg/ml), used as reference control, and prepared from ethanol absolute to a cold mix excipients. Female SKH-1 hairless mice were topically pre-treated one time per day for three days. Then the animals were exposed to an UVB-exposition (360 mJ/cm^2) to induce erythemal response. Animals were sacrificed 48 h later, skin parameters were measured and tissue samples were taken out determination of skin inflammation markers.
Results: Our results showed that F-111 emulsion reduced the loss of skin moisture and animals significantly recovered the elasticity comparing with sham; furthermore, melanin index and skin edema were decreased. F-111 also significantly reduced myeloperoxidase (MPO) activity *($P < 0.001$)* and inflammatory mediators such as cyclooxygenase-2 (COX-2*) ($P < 0.01$)* and inducible nitric oxide synthase (iNOS) levels following our experimental model. In addition, we have demonstrated that this compound is involved in the modulation of the nuclear factor-erythroid-derived 2 (Nrf2) *($P < 0.05$)* and heme oxygenase 1 (HO-1) *($P < 0.05$).*
Conclusions: We found that topical treatment with F111-emulsion reduces photoaging parameters and pro-inflammatory skin markers in UVB-irradiated hairless mice. These results indicate that this microalgal carotenoid plays an important role in prevention of inflammatory skin process consequence of sunburn, avoiding important complications including skin cancer.
Keywords: Photoaging, Photochemoprevention, UV-irradiation, Microalgae, Skin Inflammation
Acknowledgements: Proyecto-ALGALIMENTO, INNPACTO-IPT-2012-1370-060000. Ayudas a la gestión de la investigación V-Plan-Propio-US-PPI2015-1.5. Polfanat-II-(P12-AGR-430).

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ANTI-INFLAMMATORY AND SEDATIVE ACTIVITIES OF *PEPEROMIA GALIOIDES*: *IN VIVO* STUDIES IN MICE

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The aerial parts of *Peperomia galioides* popularly known in Ecuador as 'congona blanca' or 'tigresillo' is commonly used in folk medicine in Ecuador for wound healing, auricular inflammation, hysteria, gastrointestinal disturbances, and as sedative in 'susto' illness. Despite its widespread use, few scientific studies support the above claims. Thus, we aimed to confirm the anti-inflammatory and sedative uses of *P. galioides* in folk medicine. The anti-inflammatory activity was evaluated *in vivo* mice models, including croton oil-induced ear edema and myeloperoxidase (acute inflammation); cotton pellet induced granuloma (chronic inflammation) and by *Escherichia coli* Lipopolysaccharide (LPS) induced inflammation (cellular mediators). The sedative activity was evaluated by the pentobarbital-induced sleeping time. Single intraperitoneal administered doses (300 and 600 mg/kg) of *P. galioides* extract significantly inhibited the croton oil-induced ear edema ($P < 0.001$) and myeloperoxidase ($P < 0.05$) activity. Repeated (6 days) administration of the lowest dose of the extract (300 mg/kg) to mice previously implanted with cotton pellets reduced ($P < 0.05$) granuloma formation by 37.8%. Intraperitoneal injection of LPS (20 mg/kg) increased ($P < 0.001$) both plasma nitrites and TNF-α levels that were significantly ($P < 0.05$–0.01) inhibited by the extract. The duration but not the onset of sleeping time, by sodium pentobarbital (40 mg/kg; i.p.), was dose-dependently enhanced by 300 and

600 mg/kg of the extract. Our results demonstrate that *P. galioides* has anti-inflammatory as well as sedative activities in mice, which validates some of its traditional uses.

Keywords: anti-inflammatory activity, sedative activity, *Peperomia galioides*

This work was funded by: DIUC (Cuenca University); VLIR-UOS project 'Pharmacological Characterization of Medicinal Plants from the South of Ecuador'. E.V. by *Proyecto Prometeo, Secretaria de Educación Superior, Ciencia, Tecnología e Innovación de la República del Ecuador.*

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HEPATOPROTECTIVE EFFECT OF *CROTON HYPOLEUCUS* FOLLOWING THIOACETAMIDE-INDUCED NECROSIS IN RATS

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Introduction: *Croton hypoleucus* (Ch) is used by traditional medicine in Central Mexico for inflammatory processes or to treat respiratory illnesses like cough. However, scientific evidence does not exist in any literature to corroborate the claim of therapeutic success of Ch in these targets or any other. In the present report the effect of Ch extract were studied in reference to postnecrotic liver damage induced in rats by thioacetamide (TA).

Material & Methods: Rats, pretreated with a single dose of *Croton hypoleucus* ethanol extract (300 mg/kg) every 24 h for four days, were intraperitoneally injected with TA (6.6 mmol/Kg) at fourth day of treatment. Samples of blood and liver were obtained at 24 h following TA intoxication and parameters related to liver damage like AST and ALT were carried out in blood using well established protocols and methods. Results

The results showed that the pre-treatment with crude ethanol extract significantly reduced liver damage. *Croton hypoleucus* decreased liver injury by 40% and 30% for ALT and AST (biochemical markers of liver damage) respectively at 24 h the peak of maximum regeneration. Also the LD (50) of the plant species was obtained and this was greater than 5000 mg/kg (p.o.).

Conclusion: The data obtained indicate that the crude extract of *Croton hypoleucus* has hepatoprotective activity. However, further investigation on the mechanism of the hepatoprotective effect of the plant species needs to be carried out.

Keywords: croton Hypoleucus, hepatoprotective effect, thioacetamide

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EFFECTS OF OLIVE LEAF EXTRACT IN HIGH-FAT DIET-FED MICE: IMPACT ON VASCULAR DYSFUNCTION

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Introduction: Different studies have reported the beneficial effects of the olive (*Olea europaea*) leaf extract (OLE)in experimental models of metabolic syndrome, being this activity ascribed to the ability of its phenolic compounds, like oleuropeoside, to exert immunomodulatory, hypoglycemic and hypocholesterolemic activities. However, little information is available about its impact on obesity-associated vascular dysfunction.

Aim: To evaluate the effects of a well defined olive leaf extract in high fat diet (HFD)-induced obesity in mice, and to investigate the underlying mechanisms involved in the beneficial effect, with special attention to vascular dysfunction.

Methods: Male C57BL/6J mice were divided into several groups: control, control-treated, obese and obese-treated with OLE (1, 10 and 25 mg/kg/day) or Metformin(250 mg/kg/day).Control mice received a normal chow diet(NCD), whereas obese mice were fed(HFD).The treatment was followed for 6 weeks, and animal body weight periodically assessed. At the end of the experiment, metabolic plasma analysis was performed, including lipid profile, as well as glucose and insulin levels. Additionally, the HFD-induced inflammatory status was studied biochemically in liver and fat, by determining the RNA expression of different inflammatory mediators and markers of intestinal epithelial barrier function by qPCR. Also, endothelial dysfunction in aortic rings was also evaluated.

Results: OLE administration reduced body weight gain, basal glycaemia and insulin resistance, and showed improvement in plasma lipid profile when compared with HFD-fed mice. The extract significantly ameliorated the HFD-induced altered expression of key adipogenic genes, like PPARs, adiponectin and leptin, in adipose tissue. Furthermore, the extract reduced the RNA expression of TNFα, IL-1β, IL-6 in liver and adipose tissue, thus improving the inflammatory status associated to obesity. Additionally, the extract reversed the endothelial dysfunction observed in the aortic rings of obese mice.

Conclusion: OLE exerts beneficial effects in HFD-induced obesity in mice, which was associated to an improvement in plasma and tissue metabolic profile, inflammatory status and vascular dysfunction.

Keywords: experimental obesity, olive leaf extract, polyphenols, endothelial dysfunction

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HEMP (*CANNABIS SATIVA* L.) SEED OIL AS A SOURCE OF BIOACTIVE COMPOUNDS FOR THE TREATMENT OF FIBROMYALGIA-RELATED SYMPTOMS

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Introduction: This study was designed to evaluate the effect of hemp (*Cannabis sativa* L.) seed oil (HSO) on an experimental mouse model of fibromyalgia syndrome (FMS) induced by intermittent cold stress (ICS) and to evaluate the anti-inflammatory effect of the bioactive components in HSO.

Material and Methods: HSO phytochemical characterization was carried out by GC-MS and HPLC techniques. Sixty mice (C57BL/6J) were used for the experiments and randomized into six groups (n = 10): healthy control group and ICS group, were fed a standard diet, and the remaining groups received a standard diet supplemented with the respective dietary fats (10%, w/w): ICS+sunflower oil, ICS+refined olive oil, ICS+hemp seed oil, and ICS+fish oil. Freshly human monocytes were used to analyse the effects of phytol and cycloartenol (10–100 mM) on inflammatory response using FACS analysis, RT-qPCR, and ELISA procedures.

Results: The lipid profile showed that linoleic, α-linolenic, and oleic were the most abundance fatty acids. A yield (1.84–1.92%) of unsaponifiable matter was obtained and the most interesting compounds were: β-sitosterol, campesterol, phytol, cycloartenol, and γ-tocopherol. In ICS-induced FMS, HSO consumption improved mechanical and thermal allodynia and mechanical hyperalgesia (Paw

pressure, hot plate, and tail immersion tests) and improved behavioral changes related to cognitive disturbances, anxiety, and depression (hole board, traction, and evasion tests). Phytol and cycloartenol skewed the monocyte plasticity towards the anti-inflammatory non-classical $CD14^{+}CD16^{++}$ monocytes macrophage phenotype and reduced the inflammatory IL-1β, IL-6, and TNF-α production.
Conclusions: This study is an important contribution for *Cannabis sativa* L. valorization as a source of bioactive compounds contributing to research novel applications (e.g., fibromyalgia syndrome) for hemp seed oil in the food, pharmaceutical, cosmetic, and other non-food industries.
Keywords: Hemp seed oil; Cannabis sativa; Fibromyalgia; Inflammation

P-139
HOW DID THE PRESS APPROACH THE DISCOVERY AND USE OF SULPHONAMIDES DURING THE 20TH CENTURY?

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Introduction: The advent of the sulphonamides changed the treatment of infectious diseases during the 20th century. The scientific literature clearly records the development and use of these drugs; however, how the mass media transmitted these advances has not been extensively studied. We aimed to quantitatively and qualitatively evaluate reports concerning the discovery and use of sulphonamides in the press during the 20th century.
Methods: We searched for news reports on sulphonamides published between 1908 and 1979 in *The New York Times* (NYT), *The Times of London* (TTL), and *La Vanguardia* (LVG). The selected reports were quantitatively analyzed and categorized in function of their year of publication and other general features. Reports considered scientifically informative were analysed qualitatively using the Oxman Index of Scientific Quality (ISQ).
Results: We retrieved 2101 reports; 788 (37.5%) were excluded (duplicate reports, ads and newspaper indexes). Thus, 1313 (62.5%) reports were selected for analysis: 986 (75.1%) from NYT, 193 (14.7%) from TTL, and 134 (10.2%) from LVG. Of these, 812 (61.8%) reports were published between 1938 and 1949. The number of reports published peaked in 1943 (n = 142, 10.8%). Most reports (n = 982, 50.1%) dealt with general aspects about sulphonamides. Reports on pharmacology aspects (n = 523, 26.7%) dealt predominantly about clinical studies (n = 197, 26.4%) and route of administration (n = 147, 18.3%) issues. The qualitative analysis of the 20 (1.5%) reports considered informative (9 from NYT, 7 from TTL, and 4 from LVG) yielded a mean ISQ score of 24.1 (SD=3.8; range 17.0–30.0), equivalent to 6.1 on a 10-point scale.
Conclusions: The number of news reports published in the press adequately reflects the development of sulphonamides. A low percentage of reports were considered truly disseminative, and the scientific quality of these was acceptable.
Keywords: health communication; history; mass media; sulphonamides

P-140
ATTRACTIVE KEY HISTORICAL FACTS AS A TOOL TO TEACH PHARMACOLOGY

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Introduction: Pharmacology is an important subject in many university undergraduate programmes. Different pedagogical strategies, from lectures to problem-based learning sessions, can be used for teaching pharmacology. This study aimed to select key historical information related to the first antimicrobials to enhance the teaching of pharmacology.
Methods: We examined review articles and material in pharmacology textbooks related with the first antimicrobials: arsphenamine, sulphonamides, and streptomycin. Subsequently, we searched *The New York Times*, *The Times of London*, and *La Vanguardia* for news published on these drugs between 1908 and 1979 to identify key historical information that could be attractive for teaching. Finally, we wrote a brief summary of each selected issue.
Results: A review of 50 scientific articles, 82 books, and 3,078 news articles yielded 28 key historical issues: 13 on arsphenamine, 9 on sulphonamides, and 6 on streptomycin. Examples include the mistaken attribution of the discovery of arsphenamine to Paul Ehrlich's Nobel Prize; U.S. President Franklin Roosevelt's son's miraculous recovery thanks to sulphonamides; and sexist discrimination against Elizabeth Bugie in assigning credit for the discovery of the streptomycin. Other selected facts addressed issues such as new pharmaceutical dosage forms, drug toxicity, new indications for drugs, patents, commercial interests, and the use of drugs during war.
Conclusions: Reviewing historical information related to drugs makes it possible to identify material that can enhance the teaching of pharmacology. Interesting anecdotes about arsphenamine, sulphonamides, and streptomycin can serve not only for teaching antimicrobials but also for pharmacology in a much broader sense.
Keywords: antimicrobials; history; pharmacology; teaching

P-141
USING CINEMA FILMS AS AN INNOVATIVE EDUCATIONAL STRATEGY TO CREATE MEANINGFUL LEARNING IN PHARMACOLOGY TEACHING

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The teaching of Pharmacology for fourth year students of Biochemistry represents an interesting challenge for the teachers in the field because it is taught simultaneously with vocational training subjects that demand a lot of time and energy from students. The application of the *Honey-Alonso Learning Styles Questionnaire (CHAEA)* evidenced that these students′ predominant learning styles were the reflexive-theoretical modes. Since one of the activities that strengthens the learning process in this group of students is the analysis of films or videos on specific subjects, five popular films related to different aspects of Pharmacology were chosen and exhibited as part of the course syllabus in a films series called 'Movie Mornings in Pharmacology'. Each film session included a complete technical file and a list of expected learning outcomes to guide the students in the analysis of the film. At the end of the course, the students had the opportunity to write an analytical essay about these films or to give an oral presentation including their own conclusions regarding each movie. This innovative teaching tool implemented in the subject of Pharmacology has allowed the students to integrate the basic knowledge of the discipline with ethical and social issues involved in the use of medicines, develop a critical attitude towards possible advantages and risks of pharmaceutical drugs commonly used by the community and increase their motivation regarding the subject and the program. It has also provided teachers with the opportunity to contextualize the contents of the subject, to implement alternative assessment methods, to enhance teacher-student interactions and to contribute effectively to create and promote meaningful learning experiences regarding pharmacology.
Keywords: pharmacology, cinema, meaningful learning

P-142
ACTIVE LEARNING: ALIGNMENT OF ASSESSMENT AND OBJECTIVES

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BHSc (Honours) Program/Medicine

Effective learning in any format needs close alignment with objectives and assessments. This is particularly so in courses that foster active learning since these make more demands on students. Here we report on our 11-year experience in a first-year introductory undergraduate course in cellular biology in a large class setting (160–255 students). In term 1, interactive didactic sessions gave students a broad overview of cellular signalling (gene expression, synthesis, storage, release, effects and termination of responses to specific signalling molecules: histamine, acetylcholine, catecholamines, prostaglandins, nitric oxide and steroids). Students learnt not just the 'facts' but the 'way' to those facts, with an emphasis on approaches used to gather them. In term 2, students were expected to go beyond the mere facts to explore their construction, demonstrate creativity in transferring learning to novel situations, as well as flexibility in responding to challenges. A range of assessment exercises that fostered active engagement were used: (i) process-oriented problem-solving exercises; (ii) abstract writing exercises to get students to recognise key elements of peer-reviewed publications; (iii) a project to explore key discoveries in cell signaling through the Nobel archives; (iv) a project to explore the molecular basis of specific sins (greed, lust, anger, sloth); and, (v) a project to design problem-solving exercises and frame acceptable solutions and leave these as legacies for future classes. The course received high ratings from 813 students who felt that it provided them a valuable learning experience (9.0 $\pm$ 1.6 on a 10-point scale).

P-143
PREPARING PHARMACY STUDENTS TO PRACTICE: ESPECIALICED REPORTS AND ORAL COMMUNICATION ACTIVITIES

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Introduction: Students in the 5th year of the Pharmacy degree must complete the compulsory subject 'Clinical Pharmacy and Pharmaceutical Care'. Students must acquire the skills and ability to identify, integrate and apply the knowledge in the medical and pharmaceutical sciences to resolve through communication, information and education to the patient and other health professionals. Seminar sessions are one of the best settings to interact with the students in small groups reinforcing the transmission of these types of skills.
Method: Teacher presents the types of seminar they can perform: 1) Medication reconciliation at transitions in care and active dispensing of emergency contraception pills prescription; 2) Drug selection by means the System of Objectified Judgement Analysis method, and 3) Health education through a service-learning (S-L) project. Students, in teams of four, choose the activity they prefer to work with and prepare the necessary material. Following, each working team, with the help of the teacher and the valuable sources uploaded to the virtual platform, prepare a documented analysis of their cases or the activities and materials to perform the S-L project. The latter is done outside the classroom, in a social institution. Finally, all the groups give the dossier to the teacher and share and discuss their works with their colleagues.
Results and Discussion: Evaluation was carried out with a survey about the quality of the presentation and debate, and the usefulness of the activity towards their future role as pharmacists. The students become aware of the role of the pharmacist to reduce the number of medication errors and improve the quality of care. Moreover, these activities promote students' communication skills and their ability to search information and produce well-reasoned written reports concerning therapeutic issues, addressed to either patients or health professionals.

P-144
MOTIVATIONAL TOOLS TO ENCOURAGE STUDENTS TO BECOME ENGAGED IN A PHARMACOLOGY SUBJECT

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Teachers play a crucial role in students' motivation, which is seen as one of the most important and determinant factors for academic success as well as promoting long life learning. The aim of this survey was to implement strategies for active learning in an elective subject of the Degree in Physiotherapy (Drugs and Sport), in order to improve students' motivation for the subject and encourage their autonomous learning.
Two enforcement activities (reading of news related to the subject and questionnaires) were used. A satisfaction survey was also provided to students to value their opinion. 32 students took part voluntarily in the innovative strategy (94.1% of those enrolled in the subject): 75% were female and 25% male. Four articles on doping and its adverse consequences were read and commented at the end of the corresponding topic. Four on line questionnaires were provided via Moodle in order to self-assess the knowledge of the contents. They had to be answered in a limited period of time, and three attempts were allowed.
Scores achieved in quizzes were high, and response times were progressively reduced, as well as the number of attempts needed by the participants. Final scores in the subject were slightly higher than those enrolled in the previous academic year (no significant differences, Mann-Whitney U test, $p < 0.05$). In satisfaction survey most students considered that the number of articles and questionnaires was suitable, and also their degree of difficulty. Moreover, both strategies have improved their interest and motivation for the subject, and they were highly satisfied with them.

P-145
RUBRIC-BASED SCORING IN A HANDS-ON SESSION OF VETERINARY PHARMACOLOGY

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At present, the assessment at University is undergoing profound shift from traditional evaluation of factual knowledge towards assessment for learning and competences. The use of rubrics could improve the quality of the evaluation made. The objective of this study was to develop a skill assessment method in a hands-on session conducted with mice in the subject Veterinary Pharmacology.
A specific analytic rubric was designed to evaluate, by means of performance criteria and indicators, the acquisition of skills related to the pharmacological action of the drug used in the practical session, their ability to work in team, a respectful treatment of animals, or their capacity of analysis and synthesis. 132 students (74.2% female) were evaluated with the rubric. Skills defined for this practical session were reached by all students. They acquired 76.0 $\pm$ 5.6% of those competencies previously set. Female students were capable of acquiring 78.7 $\pm$ 5.8% of skills, whereas male students showed a significantly lower level (76.2 $\pm$ 4.9%, Mann-Whitney U test, $P < 0.05$). Competences addressed to work in team and handling of animals achieved the highest scores, and those related to capacity of analysis and summary of results obtained the lowest values.
Rubrics are a suitable and useful instrument to assess complex performances and provide evidence of student achievements, although it is necessary the commitment of the entire teaching staff who collaborates in a certain subject.

P-146
STUDENTS OF THE PRIMARY EDUCATION DEGREE, AS THE NEW PLAYER IN HEALTH AND NUTRITION PROMOTION

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Introduction: Primary Education Degree (PED) students are the future teachers for children aged 6 to 11 years old. As professors, it is our responsibility to encourage these students to achieve both scientific and social skills. This is remarkable if we consider that they must develop teaching strategies concerning health and nutrition. The main goal of this proposal is to improve scientific training concerning lipid metabolism and diet of the future PE teachers.
Material and Methods: The students of the first year of PED are distributed into groups. They choose several 'key words' related with the metabolism of lipids present in our diet to prepare a scientific project during classroom sessions, to finally explain their conclusions in class. We also give them a test including questions about this topic, before and after the activity. A control group of students do not participate in any project but work both pre and post tests. Professors will be available for additional tutorial sessions to support student′s activity out of the classroom.
Results: After evaluating the scientific projects and the answers of the tests, we can conclude that the participants reveal a significant increase of the level of scientific knowledge concerning lipid effects in our diet, in contrast to the students control group. Moreover, one of the questions of the tests let them propose teaching activities in their future classes, and it is remarkable that they have many and very interesting ideas. We must highlight the level of implication of the students in this proposal.
Conclusions: We provide evidence that new teaching strategies should allow the students become the main actors of the learning process. Our students have improved both their knowledge concerning nutrition and their skills in scientific education research. It is also important to develop teaching proposals which are quite appropriate with the needs of our future citizens.

P-147
THE LAST FRONTIER: INTER PROFESSIONAL LEARNING EVENTS BETWEEN DENTISTRY AND PHARMACY

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Undergraduate students from across a range of healthcare professional courses often struggle to apply fundamental pharmacological principles to their clinical practice. Problem-based or case-study centred curriculums provide some resolution to this problem but often lack the depth of detail and complexity needed to showcase how basic pharmacokinetic and pharmacodynamic (PK/PD) understanding should be used to address drug safety issues. Interprofessional (IPL) learning, as a complimentary teaching method, not only supports students understanding of pharmacological decision making processes but also prevents the development of a 'silo mentality' by encouraging greater professional teamwork and communication (Harden, 2000). Both Wilson and Soni (2016) and Pemberton (2014) argue that greater focus should be placed on the benefits of encouraging IPL links between dentistry and pharmacy to support patient safety.
This pilot project developed two unique IPL workshops which allowed students to explore, collaboratively, how PK/PD knowledge can be used to address drug interaction issues in patient's prescribed anticoagulant or antibiotic therapy. The study aimed to determine if students would further their knowledge of drug mechanisms of action through discussion with other professional groups. 23 students attended the 3 hour session (18 pharmacy, 5 dentistry) and completed a perception survey before and after the workshop to rate their therapeutic knowledge and understanding of healthcare professional roles. Students reported a 52% and 30% increase in their antibiotic and anti-coagulant therapeutic knowledge as a result of the workshop. Students also reported that their appreciation of the practice decisions of other healthcare professionals increased by 18% post-workshop. 82% of students agreed that the workshop helped them to understand how to work with other professionals to resolve clinical practice issues.
The results from this study show how IPL events can provide an enriched pharmacology curriculum, enhance therapeutic knowledge and provide a unique learning opportunity for undergraduate students.
Keywords: Interprofessional learning

P-148
RATIONAL PHARMACOTHERAPY IN THE ASSESSMENT OF CLINICAL COMPETENCE USING AN OBJECTIVE STRUCTURED CLINICAL EXAMINATION 'OSCE'

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Drugs are the mainstay of medical treatment. Pharmacology training for most medical students concentrates more on theory than on practice, but in clinical practice it must be known how to select the best option for the particular patient. On the other hand it is necessary to encourage innovation in medical education. The examination should meet three main criteria: validity, reliability and close to real practice. The Objective Structured Clinical Examination 'OSCE' was designed to assess student's clinical competence. The aim of this work has been to assess the results of the implementation of a pharmacotherapy station in the OSCE for sixth-year Medicine students.
Methods: A multidisciplinary team of clinical specialists organized an OSCE including 20 stations. There were stations with actors, patient simulators, radiological images and others. In the pharmacotherapy station students were presented a clinical case, laboratory data and medication used. They had to optimize the therapeutic plan and fill in on a model of common electronic prescription. The clinical case was a patient with hypertension, diabetes and dyslipidemia. The patient presented lower back pain, persistent dry cough, microalbuminuria and high blood pressure levels. We established 10 items that assessed clinical judgment and therapeutic management plan.
Results: 150 students were examined in the hospital setting (Málaga). The average rating of the whole stations ranged between 4.2 and 8.9. Pharmacotherapy station reached 7.48 (range 1.5–10), with Gaussian distribution. The easiest items were pharmacodynamic interaction and side effects. However, the correct choice of analgesia was the most difficult skill. There was a very good level of discrimination.
Conclusions: We have found that the Therapeutic OSCE is feasible and useful to assessing prescribing competences. Given that prescribing requires the acquisition of knowledge, skills and attitudes, we think that the incorporation of clinical pharmacology in this final examination is essential.
Keywords: OSCE; medical education; rational prescribing skills; clinical pharmacology

P-149
DOES THE DESIGN AND DEVELOPMENT OF OBJECTIVE STRUCTURED CLINICAL EXAMINATIONS BY UNDERGRADUATE STUDENTS OF PODIATRY DEGREE IMPROVE THEIR LEARNING AND ASSESSMENT SKILLS ABOUT MEDICINES?

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We aim to evaluate the impact of designing, developing and presenting Objective Structured Clinical Examinations (OSCEs) by undergraduate students on the Pharmacology course of Podiatry Degrees in their learning of medicines uses.
We carried out a three-year prospective study in which students on the Pharmacology course of Podiatry Degree were invited to voluntarily design and perform an OSCE. Each group (4–5 students) reports a clinical situation/problem involving medicines for 10 min max. Clinical histories, cameras, a mobile-phone's video editor, photos, actors, dolls, simulators or whatever they may use was allowed. After each OSCE performance the other students of the class were encouraged to ask questions and they should criticize the work of each OSCE-group indicating the possible committed errors in both the approach and development of each OSCE. The results in the final exam of the students of the three previous courses without OSCEs were used as historical controls for comparison purposes.
This study improved the learning of pharmacology and medicines uses of undergraduate students on the Pharmacology course of Podiatry degree (N = 189, 59.8% female, 19 $\pm$ 2.7 years old) and allowed us to make 43 OSCEs showing a clinical situation or a clinical problem. The students did not spend a lot of time making the OSCEs (31.7 $\pm$ 14.7 h). Spoken participation and communicative skills of the students in class increased as they were developing the OSCEs. The percentage of success in the final subject assessment questions was significantly higher in the OSCEs related question (92.5%) compared with the OSCEs un-related question (68.2%) and with the historical controls 76.5 ($P < 0.05$). The percentage of students satisfied with this way of studying pharmacology was 92.7%.
Objective Structured Clinical Examinations designed and performed by undergraduate students on the Pharmacology course of Podiatry Degree improved their knowledge about medicines use and their final assessment results.

P-150
IMPLEMENTATION AND EVALUATION OF AN ONLINE SELF-ASSESSMENT SYSTEM TO IMPROVE STUDENT PERFORMANCE IN PHARMACOLOGY

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Active learning tools have been shown to enhance student learning and promote knowledge retention in several disciplines. The aims of the present study were to incorporate a self-assessment tool in the virtual platform Moodle and to evaluate its potential to improve student's performance in a Pharmacology course.
Students enrolled in Pharmacology (3rd course of Medical Degree, UCM) were the potential participants of this study (n = 360). A test bank of true/false questions covering 9 topics addressed during each semester was made available to students through Moodle. Using a crossover design, participants had unlimited access to self-assessment quizzes (10 questions/quiz) for 12 days after finishing the theory lessons. The week before the formal assessment, the participants were offered two full test attempts (85 questions each). Results on student's performance on both optional and formal assessments were collected from Moodle and exported to perform statistical analysis (2-way ANOVA; $P < 0.05$). Adherence to protocol ('active participants') was defined as completing 75% of self-assessments and the formal assessment in each semester.
During the first semester, 41.8% of the students completing the midterm formal assessment (n = 153) showed adherence to the protocol. Active participants obtained higher marks (6.04 $\pm$ 1.99) than students in the control group (4.88 $\pm$ 2.33) in the topics included in the self-assessment quizzes ($P < 0.001$). These differences remained significant despite including the non-adherent participants in the analysis (5.43 $\pm$ 2.27; $P = 0.019$). However, during the second semester, the participation dropped to 22.9% of the students completing the final formal assessment (n = 109) and only active participants obtained significantly higher marks in the relevant topics as compared to those in the control group (7.38 $\pm$ 1.68 vs. 6.49 $\pm$ 1.92; $P = 0.017$).
Although the low participating rate suggest that this system has a limited value to engage students in learning activities on a daily basis, offering this tool might improve the performance of medical students in pharmacology courses.

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NEW ACADEMIC PERSPECTIVES IN TEACHING PHARMACOLOGY AND CLINICAL THERAPEUTICS: AN INTERNATIONAL SERVICE LEARNING EXPERIENCE

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Introduction: Service-learning (SL) is an educational method that is expanding the involvement of universities into their neighbouring communities. It also tends to promote civic and moral development of students. Service-learning can be defined as 'service performed by students, aimed at attending to a real need of the community, and oriented in an explicit and planned way to enhance the quality of academic learning.' (1)
Furthermore International Service Learning (SL) has enabled health professional students the opportunity to provide healthcare, under the direction of a trained faculty, to underserved populations in developing countries.
Objective: To verify the usefulness of International SL experience in learning pharmacology, to support or not the design of a new pharmacology subject based on SL.
Methods: We designed a cooperation project with ACOES-Honduras to develop a training experience for students of medicine. This study is based on the qualitative analysis of the final reports of 8 participants.
Results: The analysis was focused on 27 transversal and 19 specific competences that cover areas of practice and others related to Pharmacology. In the case of transversal competences, around 70% were covered in the activities carried out. Specific competences were covered up to 75%. Volunteer narratives provided an image of the experience that showed the effectiveness of this type of program, both in increasing sensitivity and attention to diversity and in the implementation of 'theoretical' training, and the original response to unforeseen situations.
Conclusions: SL was found to be an effective method to acquire personal and professional competences and skills in the area of pharmacology demand by our present society. Hence we will suggest this methodology in the design of a new subject: 'Pharmacotherapeutics in disadvantaged environments'.
(1) Tapia, MN, et al. (2006). 'Service-Learning in Argentina Schools.' Casey, Karen McKnight, et al. (eds.).

P-152
PEER INSTRUCTION IN VETERINARY PHARMACOLOGY: A PRELIMINARY EXPERIENCE

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Peer Instruction (PI) is a student-centered approach in which the class time is used for learners to construct concepts rather than for instructors to lecture. Differing from those in traditional lectures, PI students requires to read and complete assignments before attending class (1). There are different research data that supports the effectiveness of PI over more traditional teaching methods, such as pure lecture (2).
We used PI for first time in our Pharmacology cours, specificaly we applied this teaching methodology in the Pharmacology of Hemostasis. The class time was focused on two different ConcepTests: Antiplatelet agents and Anticoagulants/ Fibrinolytics drugs with a previous 5 min minilecture. The number of questions were 6 and 8, respectively, according to the main key points of each section. Each question had four options and one or two of them were correct.
For this purpose the free platform SOCRATIVE was used, and students, through their laptops or cell phones, answered the test based on what they have learned during the preinstructional reading assignments. In the first assessment of ConcepTest the global incorrect answers were between 64–56%. Consequently students discussed answers with others classmates and some general questions were discussed by the instructor with the class as a whole. After, students revoted the first ConcepTest and the number of incorrect answers decreased up to 10–7%. Then we moved to the next ConcepTest. Similar results were observed with the ConcepTest of anticoagulant/fibrinolitic drugs.
Developing good ConcepTests certainly takes a great deal of effort, however during the development of this inverted class students showed a high degree of involvement. We obtained a positive feedback from them. The only criticism was regarding the time spent in preparing the subject.
(1) E. Mazur, Peer Instruction: A User's Manual (Prentice-Hall, Upper Saddle River, NJ, 1997).
(2) P Zhang, L Ding and E Mazur. PRPER 113, 010104 (2017).

P-153
EPIGENETIC MECHANISMS UNDERLYING NEUROPATHIC PAIN IN RATS

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Neuropathic pain (NP) subsequent to lesions or diseases affecting the somatosensory nervous system is a prevalent and debilitating chronic syndrome highly refractory to current analgesics. The development and maintenance of NP encompass long term pathological plasticity in the nervous system.
Objective: To investigate the involvement of epigenetic mechanisms in NP pain establishment.
Methods: Neuropathic pain was induced to rats by sciatic spared nerve injury (SNI). Mechanical allodynia was assessed with von Frey monofilaments. At the moment of maximal allodynia (10 days post-SNI) lumbar (L3, L4, L5) spinal dorsal horns (SDH) and dorsal root ganglions (DRG) were obtained and processed for quantitative PCR or immunofluorescence.
Results: The transcript levels of DNA methyltransferase-3b (DNMT3b) and histone deacetylases (HDACs) 4 and 9 were significantly up-regulated in the SDH and DRG from neuropathic rats. In parallel, both structures exhibited increased methylation at cytosine in CpG islands, assessed by immunofluorescence with Ab to 5-methylcytosine, as well as methylation of histone H3K9, assessed by Ab to H3K9me3.
Conclusions: Epigenetic modifications including DNA and histone methylation suggest transcriptional repression in pain-relevant structures associated to neuropathic pain development. (Supported by SAF2016-77732-R).

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SENSITITIZATION OF TRPV1 NEURONS BY PGE2: LOST OF SENSORY SELECTIVITY FOR NOXIOUS HEAT AND PARTICIPATION ON MECHANICAL HYPERALGESIA

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Introduction: Peptidergic (TRPV1-expressing) C-fibers code for noxious heat under normal conditions, but not for mechanical stimulus. Prostaglandin E2 (PGE2) sensitizes peptidergic C-neurons by TRPV1 phosphorilation through protein kinase A (PKA) activity, leading to heat hyperalgesia, whereas glial cell derived neurotrophic factor (GDNF) sensitizes other neuronal types (which do not express TRPV1), leading to mechanical hyperalgesia. PGE2 also triggers the development of mechanical hyperalgesia although its mechanisms are poorly understood. We hypothesized that PGE2 might also induce mechanical hyperalgesia through TRPV1 sensitization.
Material & Methods: All experiments were performed on CD-1 mice. Nociceptive pain to mechanical stimulus was evaluated by the paw pressure (450 g) test and nociceptive pain to heat stimulus was evaluated using the unilateral hot plate (55°C) test. PGE2- and GDNF-induced mechanical and thermal hyperalgesia were assessed by the same procedures as above, although at lower stimulation intensities (100 g and 42°C).
Results: The *in vivo* ablation of TRPV1-expressing neurons (by resiniferatoxin treatment) decreased nociceptive pain to heat stimulus without altering mechanical nociceptive pain. PGE2 administration induced both heat and mechanical hyperalgesia, and both types of sensory hypersensitivity were sensitive to resiniferatoxin treatment. The intraplantar administration of ruthenium red (a nonselective TRP antagonist), SB366791 (a selective TRPV1 antagonist), or H-89 (a PKA inhibitor) reversed PGE2-induced mechanical hyperalgesia. Any of these treatments modified GDNF-induced mechanical hyperalgesia, indicating that they do not induce nonselective actions affecting the sensitization of other neuronal populations different from TRPV1 neurons.
Conclusions: TRPV1 neurons are needed for nociceptive pain to heat stimulus but not for mechanical nociceptive pain. However, these neurons are involved in PGE2-induced hyperalgesia to both heat and mechanical stimuli, involving TRPV1 sensitization through the action of PKA.
Acknowledgements: *MINECO (SAF2013-47481P and SAF2016-80540R),* Junta de Andalucía (grant CTS 109), Laboratorios Esteve, FEDER funds and the Research Program of the University of Granada.
Keywords: TRPV1, prostaglandin E2, hyperalgesia

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ACTIVATION OF EXTRACELLULAR SIGNAL-REGULATED KINASES (ERK 1/2) IN THE LOCUS COERULEUS CONTRIBUTES TO PAIN-RELATED ANXIETY IN ARTHRITIC RATS

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Chronic pain is now considered a disease with repercussions that reach far beyond hypersensitivity. Many studies have emphasized that chronic pain exponentially increases the risk of someone to suffer emotional disturbances characterized by persistent anxiety and low mood, creating a self-perpetuating cycle that magnifies and exacerbates the painful experience. However, the mechanisms underlying this co-morbidity are unknown. Prolonged arthritis can produce anxiety-like behavior in rats, along with enhanced phosphorylation of the extracellular signal-regulated kinase 1/2 (pERK1/2, a marker of activation and plasticity) in the Locus Coeruleus (LC). Therefore, we propose that ERK1/2 activation in the LC plays a crucial role in pain-related anxiety. To test this hypothesis, the monoarthritis model of painful arthritis was induced in rats by injection of complete Freund′s adjuvant. The behavioral attributes of pain and anxiety, as well as LC function, were evaluated at an early (1 week, MA1W) and late-phase (4 weeks, MA4W) of the disease. Next, these procedures were repeated in MA4W rats in order to explore the effect of pERK1/2 blockade by intra-LC administration of the mitogen-activating extracellular kinase (MEK) inhibitor, SL327.

Results showed that pain was evident in monoarthritis rats after 1 week, although anxiety did not appear until 4 weeks later. This late-phase of the disease was accompanied by diminished tonic LC activity, which was coupled to exacerbated evoked LC responses to noxious stimulation of the inflamed and the healthy paw. When ERK1/2 activation was dampened by intra-LC administration of SL327, the exaggerated evoked response of MA4W rats was blocked. Importantly, SL327 did not change pain hypersensitivity but rather, it reversed both the anxiogenic-like behavior and the increase in pERK1/2 in the anterior cingulate cortex (ACC) of MA4W rats. Therefore, this study provides a direct evidence of how a subcortical structure interferes with ACC activity, leading to pain-related anxiety relief.

Keywords: anterior cingulate cortex; anxiety; Locus Coeruleus; pain

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POSTOPERATIVE PAIN AND MORPHINE CONSUMPTION AFTER ULTRASOUND-GUIDED FEMORAL AND SCIATIC COMBINED NERVE BLOCK VS. NEUROSTIMULATION FOR FEMORAL AND SCIATIC COMBINED NERVE BLOCK OR NEUROSTIMULATION FOR FEMORAL NERVE BLOCK IN PRIMARY ELECTIVE TOTAL KNEE ARTHROPLASTY

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Total knee arthroplasty injuries are extremely painful and merit prompt attention to adequate postoperative analgesia. We aim to compare femoral and sciatic ultrasound-guided combined nerve block vs. neurostimulation for femoral and sciatic combined nerve block or for femoral nerve block in postoperative pain in primary elective total knee prosthesis.

A three arms, prospective longitudinal study of patients having primary elective unilateral knee prosthesis and randomly assigned to catheter insertion guided by ultrasound or neurostimulation was done: (1) Ultrasound-guided femoral and sciatic combined nerve block (USFSCN) ($N = 15$); (2) Neurostimulation for femoral and sciatic combined nerve block (NSFSCN) (N = 17); (3) Neurostimulation for femoral nerve block (NSFN) ($N = 11$). Total analgesia (morphine) consumption after 48 hours was the primary endpoint. The postoperative pain intensity (visual analogue pain scale (VAS)) at post-anaesthetic recovery unit (PARU), 6, 24, 48 h, and during movement and postoperative complications were secondary outcomes.

43 patients (68.3 ± 8 years old, 77% female) subjected to elective unilateral knee prosthesis were enrolled. There were no differences in the demographic, anaesthetic and surgical variables between groups. Pain intensity was lower in the USFSCN group compared with NSFSCN and NSFN during the first 48 h post-surgery (% of intense pain at PARU/6 h/24 h/48 h): USFSCN 0.8/1.4/3.2/1.6; NSFSCN 5.6/8.3/7.5/3; NSFN 7.2/5.3/6.4/5.4. The average consumption of morphine within 48 h after surgery was similar in the groups USFSCN and NSFSCN (3 mg vs. 3.11 mg), and significantly lower than NSFN (4.19 mg) ($P < 0.05$). And the number of complications was significantly lower in the USFSCN group compared with NSFSCN and NSFN during the first 48 h of postoperative.

Ultrasound-guided femoral and sciatic combined nerve block presented better analgesia and was more safety than neurostimulation for femoral and sciatic combined nerve block or for femoral nerve block in primary elective total knee arthroplasty.

P-157
THE CHEMOKINE CCL4 INDUCES OPIOID ANALGESIA THROUGH LYMPHOCYTE STIMULATION AND INHIBITS INFLAMMATORY HYPERALGESIA IN MICE

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Introduction: Previous studies from our laboratory described that the s.c. administration of small doses of chemokine CCL4 (10–100 pg/Kg) to healthy mice produces analgesia through the release of endogenous opioids. The inhibition of this analgesia in mice treated with cyclophosphamide suggested the involvement of immune cells.

Aims: To elucidate the type of immune cells involved in the analgesic responses evoked by systemic CCL4 and to explore the ability of this chemokine to inhibit inflammatory hyperalgesia.

Material and methods: Thermal hyperalgesia was measured in Swiss CD1 mice by the unilateral hot plate test. Inflammation was induced by i.pl CFA one week before. The degree of immune cell depletion was checked with a differential hematology analyzer (Abacus). The expression of CCR5 in lymphocytes was assessed by immunofluorescence.

Results: The s.c. administration of CCL4 induced analgesia in mice treated with an antibody against granulocytes (antibody anti-Ly6G, 100 μg), but disappeared after selective lymphocytic depletion (antibody anti-CD4 30 μg + anti-CD8 20 μg). Supporting the involvement of these cells, the expression of CCR5, the main receptor for CCL4, was demonstrated in lymphocytes by immunofluorescence assays. The hyperalgesic response measured in CFA-inflamed mice was dose-dependently inhibited either after the systemic (0.1–1 pg/kg) or intrathecal (0.3–1 pg) administration of CCL4. However, whereas the

antihyperalgesic effect evoked by s.c. CCL4 was reversed by the CCR5 antagonist, DAPTA (3–10 mg/kg), or naloxone (0.1–1 mg/kg), only DAPTA (1–5 μg) but not naloxone (10 μg) inhibited its spinal antihyperalgesic effect.
Conclusions: Our results indicate that the systemic administration of CCL4 evokes opioid analgesia through the stimulation of CCR5 and the activation of lymphocytes. Furthermore, this chemokine counteracts inflammatory hyperalgesia evoked when administered either systemically or spinally, being the antihyperalgesic effect evoked after spinal administration unrelated to opioid mechanisms.
Fundings came from FICYT (FC-15-GRUPIN14-125).
Keywords: CCL4, CCR5, DAPTA, Inflammation, Naloxone

P-158
TREATMENT OF NEUROPATHIC PAIN ASSOCIATED TO THE SPARED NERVE INJURY MODEL IN MICE: ROLE OF SIGMA-1 RECEPTORS

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Background and aims: Sigma-1 receptors (σ_1Rs) are an emerging target for the treatment of neuropathic pain. The role of σ_1Rs in neuropathic pain induced by nerve compression is known, but their participation on peripheral neuropathy induced by nerve transection is unexplored. Therefore, we investigated the effect of pharmacological and genetic blockade of σ1Rs in the neuropathic pain associated to the sciatic nerve spared nerve injury (SNI) model. This model involves a lesion of two of the three terminal branches of the sciatic nerve, leaving the sural nerve intact.
Methods: SNI was performed on female CD1 wild-type (WT) and σ_1Rs knockout (σ_1Rs-KO) mice. Mechanical allodynia (von Frey test), cold-allodynia (acetone test) and heat hyperalgesia (Hargreave's test) were tested previously to SNI and during three weeks following injury. The effect of acute σ_1Rs antagonist S1RA (16–64 mg/kg, s.c.) and agonist PRE-084 (32 mg/kg, s.c.) were tested seven days after SNI. Animals were also treated with S1RA (25 mg/kg i.p.) twice daily for 10 days.
Results: WT mice developed prominent cold and mechanical allodynia after SNI, whereas σ_1Rs-KO did not develop cold allodynia and showed reduced mechanical allodynia. These effects were mimicked by the administration of S1RA to neuropathic WT mice, and the effects of the σ_1 antagonist were reversed by PRE-084. σ_1Rs-KO and WT mice showed a similar thermal hyperalgesia after SNI. However, S1RA abolished SNI-induced thermal hyperalgesia in WT mice, an effect which was reversed by PRE-084. Mechanical allodynia and cold allodynia were reduced by the repeated administration of S1RA.
Conclusion: σ_1 receptors have a relevant role in SNI-induced neuropathic pain. The pharmacological antagonism of this receptor can be useful to prevent and/or treat neuropathic pain produced by nerve section.
Keywords: Neuropathic pain; Allodynia; hyperalgesia; Sigma-1 receptors; S1RA
Acknowledgments: Spanish Government (SAF2016-80540-R, FPU, Juan-de-la-Cierva-Incorporación-grants), Junta de Andalucía (P11-CTS-7649) and the Research Program of University-of-Granada.

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DEVELOPMENT OF PHENOTYPIC *IN VITRO* MODELS OF PAIN IN EARLY DRUG DISCOVERY

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Background: Despite considerable efforts for treating neuropathic pain, new analgesics identified in preclinical studies failed in clinical trials. One explanation could be that the sole modulation of a given target may not be enough to have a therapeutic effect. Another explanation is the scarce translationality of *in vitro* assays in early drug discovery, where immortalized cells unrelated to the disease and overexpressing the target of interest are used.
Aim of the Study: Our aim is to develop High-Throughput Screening (HTS) phenotypic and translational assays to discover new analgesics for neuropathic pain using immortalized Dorsal Root Ganglion (DRG) neuronal cells better resembling the physiology of nociceptors than conventional cell cultures, while keeping throughput, assay sensitivity and reproducibility for HTS.
Methods: We evaluated several protocols to differentiate immortalized DRG cell lines (F11 and ND7/23) to a neuronal phenotype considering both their morphology and expression of neuronal markers. Also, we functionally evaluated their response to activation by ATP and to the depolarization induced by KCl using dynamic mass redistribution label-free measurements (Epic system, Perkin-Elmer).
Results: We found an effective protocol to differentiate both cell lines to DRG-like neurons using 30 μM forskolin, 0.5 mM dibutyryl cAMP and 0,5% FBS or 60 μM forskolin, 1 mM dibutyryl cAMP, 1,3 ng/ml NGF and 0,5% FBS for F11 and ND7/23 respectively, as shown by the advent of neuron-like processes and the increased expression of TrkA receptor. Both cell lines responded to ATP and KCl in a concentration-dependent manner in the label-free assay, obtaining with ATP an EC_{50} of $4{\cdot}10^{-3}$M and $7{\cdot}10^{-3}$M in F11 and ND7/23 cells, respectively. In the case of KCl, F11 cells responded with an EC_{50} of 0.2M.
Conclusions: These differentiated cell lines could represent new biological tools for establishing novel phenotypic *in vitro* models for HTS of drugs to treat neuropathic pain.
Keywords: High Throughput Screening (HTS); *in vitro* assay; neuropathic pain; Dynamic Mass Redistribution (DMR)

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TREATMENT WITH SULFORAPHANE ENHANCES THE ANTINOCICEPTIVE EFFECTS OF Δ OPIOID RECEPTORS IN DIABETIC MICE: MECHANISMS IMPLICATED

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Background: The involvement of heme oxygenase 1 (HO-1) in the modulation of the antinociceptive effects of opioids in type 1 diabetes has been demonstrated but the role played by the transcription factor Nrf2 in the regulation of painful neuropathy and in the effects and expression of δ-opioid receptors (DOR) in type 2 diabetic mice have not studied.
Methods: In male BKS.Cg-m+/+Leprdb/J (db/db) mice, the anti-allodynic effects produced by the administration of a Nrf2 transcription factor activator sulforaphane (SFN) alone and combined with two DOR agonists, [d-Pen(2),d-Pen(5)]-Enkephalin (DPDPE) and (+)-4-[(αR)-α-((2S,5R)-4-Allyl-2,5-dimethyl-1-piperazinyl)-3-methoxybenzyl]-N,N diethylbenzamide (SNC-80) were evaluated. The effects of SFN on glucose levels and body weight as well as on the proteins levels of Nrf2, HO-1, NAD(P)H: quinone oxidoreductase 1 (NQO1),

MAPKs (JNK) and DOR in sciatic nerve from db/db mice were also assessed.
Results: This study showed that the administration of SFN dose dependently reversed mechanical allodynia, reduced hyperglycemia and body weight gain associated to type 2 diabetes and significantly increased the anti-allodynic effects of DPDPE and SNC-80 in db/db mice. This treatment also enhanced the protein levels of HO-1, normalized the down regulation of Nrf2, NQO1 and DOR, and inhibited JNK phosphorylation in the sciatic nerve of diabetic mice. Our data indicated that SFN treatment is effective in reversing mechanical allodynia and enhancing DOR antinociceptive effects by activating the Nrf2/HO-1/NQO1 signaling pathway, decreasing the phosphorylation of JNK and normalizing DOR expression in db/db mice.
Conclusions: These results propose SFN, alone and combined with DOR agonists, as interesting approaches for the treatment of painful diabetic neuropathy associated to type 2 diabetes in mice.
This work was supported by Ministerio de Economía y Competitividad, Instituto de Salud Carlos III and Fondo Europeo de Desarrollo Regional (FEDER), Unión Europea [Grant PI1400927].

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EFFECTS OF VASCULAR ENDOTHELIAL GROWTH FACTOR-A_{165}B AND SRPK1 INHIBITION IN THE MONOSODIUM IODOACETATE MODEL OF OSTEOARTHRITIS IN RAT

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Osteoarthritis (OA) is a major cause of musculoskeletal pain worldwide, and it is still inadequately managed. Serine-arginine protein kinase 1 (SRPK1) controls the alternative splicing of vascular endothelial growth factor-A into the pro-nociceptive VEGF-A_{165}a variant and the anti-nociceptive VEGF-A_{165}b. This study evaluated the effects of VEGF-A_{165}b and SRPK1 inhibition on pain behaviour and chondropathy severity in the monosodium iodoacetate (MIA) model of OA in rats.
Male Wistar rats (n = 56, 200–250 g) were used. In study 1, arthritis was induced with intra-knee-joint injection of MIA (1 mg/50 µl saline, n = 23). Controls were untreated (n = 9). Rats were treated with: VEGF-A_{165}b (20 ng/g body weight, days 0–14) followed by PBS (days 15–28, twice weekly, I.P.), or PBS (d0–14) followed by VEGF-A_{165}b (d15–28). In study 2, MIA rats (n = 16) were treated with SRPK1 inhibitor EXN109 (0.8 µg/g body weight in 1% DMSO). Weight bearing and mechanical withdrawal thresholds were measured twice weekly. Rats were euthanized (100 mg pentobarbital i.p.) on day 28, and knee joint macro- and microscopic chondropathy assessed.
Weight bearing asymmetry was significantly reduced in MIA/VEGF (d0–14) rats (weight borne ipsilaterally 44.7 ± 2.7% body weight) compared to controls (38.6 ± 2.7%) on days 25 and 28. EXN109 had no significant effect on pain behaviour (weight borne ipsilaterally 44.4 ± 1.3% body weight) compared to controls (42.7 ± 1.2%) (two-way ANOVA, post-hoc Tukey's tests).
Both gross and microscopic chondropathy scores were significantly higher in MIA animals (gross score: 15.2 ± 2.0; microscopic score 12.2 ± 0.9) and in all treated animals (VEGF$_{165}$b(d0–14) gross: 11.2 ± 1.3; microscopic 10.7 ± 1.0, EXN109 gross: 14.3 ± 0.9; microscopic: 8.2 ± 1.3) than in controls (gross: 0.2 ± 0.1; microscopic: 1.7 ± 0.4, one-way ANOVA).
Systemic VEGF-A_{165}b has an anti-nociceptive action, but no effect on joint damage in the MIA model of OA in rat only when given early in disease development. The lack of effect of EXN109 on OA pain and chondropathy is probably due to the early stage of drug development.
Keywords: osteoarthritis, pain, VEGF, SRPK

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PLASMA LEVELS OF THERAPEUTIC DRUGS SIGNIFICANTLY INHIBIT BLOOD-BRAIN BARRIER ENDOTHELIAL CELL ABC TRANSPORTERS

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Introduction: Clinically relevant interactions between therapeutic drugs and transporters, including ATP-binding cassette (ABC) efflux transporters, are of growing interest, as highlighted by the FDA [1]. Inhibition or induction of ABC transporters can cause pharmacokinetic interactions leading to unpredicted toxicities or sub-optimal treatment. ABCB1 and ABCG2 are highly expressed in blood-brain barrier endothelial cells, and modulation of their activities may influence CNS drug penetration of ABCB1 and ABCG2 substrates [2].
Methods: Primary porcine cerebromicrovascular endothelial cells were isolated and maintained as described previously [3]. The effect of indomethacin, olanzapine, chlorpromazine and glibenclamide, at concentrations reported in plasma (5 µM indomethacin, 650 nM olanzapine, 1 µM chlorpromazine, 1 µM glibenclamide), on cell viability was measured using the Neutral Red assay. The impact of drugs on ABCB1 and ABCG2 transporter activity was determined by measuring intracellular calcein and Hoechst 33342 accumulation, respectively, in cells pre-treated with these drugs.
Results: The drugs demonstrated no significant effect on the viability of porcine cerebromicrovascular endothelial cells at the concentrations used.
Indomethacin, olanzapine, chlorpromazine and glibenclamide all significantly increased intracellular accumulation of calcein, indicating inhibition of ABCB1. Whereas indomethacin, olanzapine, chlorpromazine significantly increased intracellular accumulation of Hoechst 33342, indicating inhibition of ABCG2.
Conclusion: This is the first study to demonstrate plasma levels of therapeutic drugs significantly inhibit ABCB1 and ABCG2 in primary porcine cerebromicrovascular endothelial cells. The findings suggest that these drugs, which are widely used in polypharmacy, have the potential to influence the CNS permeability of co-administered ABCB1 and ABCG2 drug substrates.
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IMPACT OF CEREBRAL ISCHEMIA/REPERFUSION AND URIC ACID TREATMENT ON MIDDLE CEREBRAL ARTERY ANGIOGENESIS IN NORMOTENSIVE AND SPONTANEOUSLY HYPERTENSIVE RATS

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High blood pressure is the single most important risk factor for stroke and hypertensive patients have poor post-stroke functional outcomes. Angiogenesis plays a role in stroke recovery, though excessive neovascularization may be detrimental. It is unclear whether hypertension may alter angiogenesis after stroke and the effect of uric acid (UA) antioxidant therapy, which is showing encouraging neuroprotective

effects in clinical trials. We analysed the influence of cerebral ischemia (90 min)/reperfusion (24 h) on the time course of *in vitro* middle cerebral artery (MCA) angiogenic growth in normotensive Wistar-Kyoto (WKY) and spontaneously hypertensive rats (SHR) treated (*i.v.*; 120 min after MCA occlusion) with UA (16 mg/kg) or vehicle (Locke's buffer). Ipsilateral and contralateral to ischemia MCA explants were cultured in Matrigel and vessel sprouting was measured from day 4 through day 14. Systolic blood pressure ($P < 0.001$) and infarct volumes($P = 0.05$) were larger in SHR than in WKY, and UA attenuated ($P < 0.05$) infarct volume in both strains. Ischemia/reperfusion induced an early (from day 4 to 7) increase in angiogenesis in ipsilateral MCA from WKY ($P < 0.05$) and SHR ($P < 0.01$) compared to sham-operated rats that was prevented by UA only in SHR. Vessel sprouting was higher ($P < 0.05$) at day 4 (230.3 ± 16.4 μm, $n = 6$) and lower ($P < 0.05$) at day 14 (482.4 ± 34.8 μm, $n = 6$) in SHR than in WKY (day 4: 139.2 ± 34.9 μm, $n = 5$; day 14: 591.0 ± 46.8 μm, $n = 5$) after ischemia/reperfusion, an effect that was prevented by UA. In conclusion, transient cerebral ischemia potentiates early stage angiogenesis in the ipsilateral MCA independently of high blood pressure. However, hypertension is linked to early exacerbated and late impaired angiogenic growth after ischemia/reperfusion. UA treatment does not modify early increased angiogenesis in WKY, while it prevents both early and long-term alterations in SHR.

Supported by Ministerio de Ciencia e Innovación (SAF2014-56111-R):

Keywords: cerebral ischemia/reperfusion; hypertension; angiogenesis; antioxidant treatment

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SIMVASTATIN BLOCKS SOLUBLE SSAO/VAP-1 RELEASE IN EXPERIMENTAL MODELS OF CEREBRAL ISCHEMIA: POSSIBLE BENEFITS FOR STROKE-INDUCED INFLAMMATION CONTROL

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Beyond cholesterol reduction, statins mediate their beneficial effects on stroke through pleiotropic actions. They have shown anti-inflammatory properties by different mechanisms, including the inhibition of the NF-κB transcriptional activity and the consequent increase and release of adhesion molecules. We have studied simvastatin effects on the vascular enzyme semicarbazide-sensitive amine oxidase/vascular adhesion protein 1 (SSAO/VAP-1), which is involved in the stroke-mediated brain injury. SSAO/VAP-1 has leukocyte-binding capacity and mediates the expression of other adhesion proteins through signaling molecules generated from its catalytic activity, which when overproduced induce vascular damage. Our results indicate that soluble SSAO/VAP-1 is released to the bloodstream after an ischemic stimulus, in parallel with an increase in E-selectin and VCAM-1 in the contralateral brain side. Simvastatin blocks the soluble SSAO/VAP-1 release and prevents E-selectin and VCAM-1 overexpression as well. Thus, simvastatin efficiently blocks SSAO/VAP-1-mediated leukocyte adhesion, although it is not an enzymatic inhibitor of SSAO. In addition, simvastatin-induced changes in adhesion molecules are greater in human brain endothelial cell cultures expressing SSAO/VAP-1, compared to those non-expressing it, indicating some synergic effect with SSAO/VAP-1. We think that combined therapy with SSAO/VAP-1 inhibitors and statins could enhance the beneficial effects of both individual treatments in stroke patients.

Keywords: stroke; statins; SSAO/VAP-1; inflammation

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OLIVE LEAF EXTRACT EXHIBITS IMMUNOMODULATORY ACTIVITY IN HUMAN PBMCS AND ANTI-INFLAMMATORY EFFECT IN THE DSS MODEL OF MOUSE COLITIS

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Introduction: Olive (*Oleaeuropea*) leaf extract (OLE) is used in Mediterranean traditional medicine as an anti-inflammatory remedy, most probably due to its presence of antioxidant phenolic compounds, like oleuropeoside. It would be interesting to validate it use for the treatment of inflammatory conditions associated with oxidative stress in humans, like inflammatory bowel disease.

Aim: To evaluate the intestinal anti-inflammatory properties of an olive leaf extract in the dextran sodium sulfate (DSS) model of mouse colitis, which resembles human IBD.

Methods: Male C57BL/6J mice were assigned into five groups: non-colitic, colitic control and colitic treated groups with olive leaf extract (0.5-1-10 mg/kg).Colitis was induced by incorporating DSS(3%) in the drinking water for 5 days. Treatment started the same day of colitis induction, and maintained until one week after the establishment of the colitic process. The inflammatory status was evaluated macroscopically, assigning a disease activity index (DAI), and biochemically by determining the colonic expression of mediators involved in the inflammatory response or in the intestinal epithelial barrier function. In addition, *in vitro* immunomodulatory properties of the extract were determined when incubated (0.1–100 μg/ml) with peripheral blood mononuclear cells(PBMCs)obtained from healthy and Crohn's disease patients.

Results: The treatment with the extract improved the recovery of the colitic mice, since a significant reduction in DAI values was observed. This effect was associated with a reduced expression of colonic pro-inflammatory mediators(IL-1β, IL-6, TNFα, ICAM-1 and MIP-2),while increasing that of key players of the intestinal epithelial integrity(occludin, ZO-1 and MUC-3). Besides, it displayed immunomodulatory properties *in vitro* since it decreased pro-inflammatory cytokines production in LPS-stimulated PBMCs.

Conclusion: OLE showed intestinal anti-inflammatory activity in the DSS model of mouse colitis, maybe related to its antioxidant properties, which may result in the downregulation of the immune response and the improvement of the intestinal epithelial barrier function. In addition, this extract has a direct effect on immune cells, as demonstrated in the *in vitro* studies.

Keywords: Inflammatory bowel disease, mouse experimental colitis, olive leaf extract, polyphenols

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NEW CURCUMIN-COUMARIN DERIVATIVES AND RESVERATROL REDUCE PATHOPHYSIOLOGICAL PROCESSES ASSOCIATED WITH PANCREATITIS AND PANCREATIC CANCER

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Background: Pancreatic diseases have not a curative treatment nowadays. Progressive inflammatory unbalance and physiopathology

associated events, as intracellular trypsin activity and abnormal increase of the cytoplasmic concentration of Ca^{2+} ($[Ca^{2+}]_c$), are common pathways for both pancreatitis and pancreatic cancer.

Methods: In the present study, we have used an *in vitro* model, based on the stimulation of the pancreatic acinar cells (PAC) with a supramaximal concentration (100 nM) of the cholecystokinin hormone (CCK) to evaluate the activity of two new hybrid curcumin-coumarin derivatives (H1K3 and H1K5) and resveratrol against several physiological processes associated with both pancreatic diseases, such as premature trypsin intracellular activation, abnormal $[Ca^{2+}]_c$ or PAC necrosis. All the measures were carried out with fluorescence quantitative techniques with different specific fluorochromes (rhodamine, fluo4 and propidium iodide).

Results: Our experiments show that H1K3 and H1K5 (10, 100 μM) and resveratrol (200 nM, but not 20 nM) significantly inhibit both CCK-induced intracellular trypsin activity (at min 20 and 40) and PAC necrosis. Also, H1K3 and H1K5 (10, 100 μM) significantly reduce CCK-induced $[Ca^{2+}]_c$ increases in PAC.

Conclusions: Our results with H1K3 and H1K5 confirm the potential therapeutic interest of the combination of curcumin-coumarin structures on the cellular basis of pancreatitis and pancreatic cancer. In addition, a potential beneficial effect of resveratrol against both pancreatic pathologies is confirmed. Further studies relating those findings with inflammatory process as main target of these new molecules will be performed.

Keywords: pancreatitis, pancreatic cancer, curcumin-coumarin, resveratrol

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