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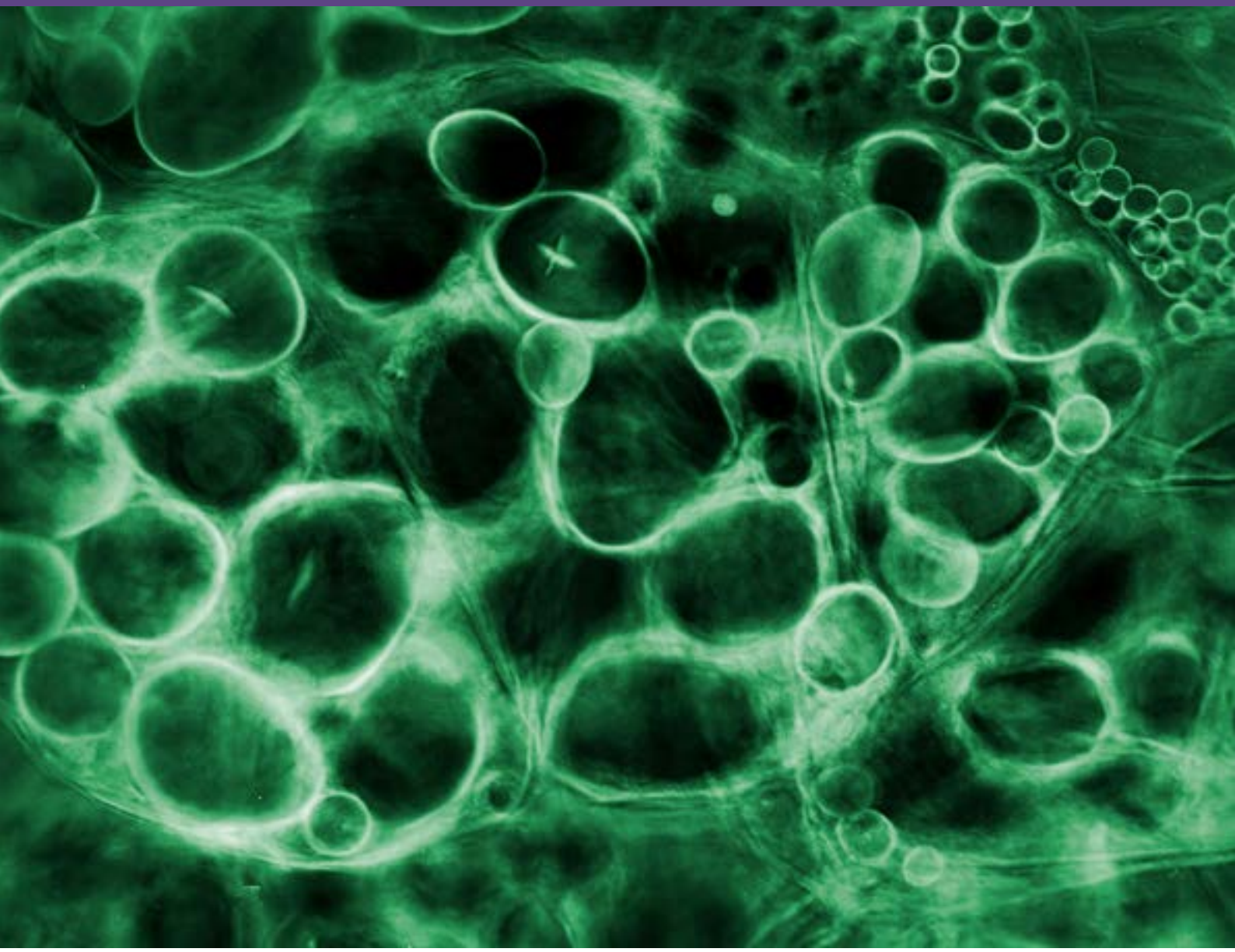

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Physiological Mini Reviews

Physiological Mini-Reviews

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Physiological Mini-Reviews is a scientific journal, publishing brief reviews on "hot" topics in Physiology. The scope is quite broad, going from "Molecular Physiology" to "Integrated Physiological Systems". As indicated by our title it is not our intention to publish exhaustive and complete reviews. We ask to the authors concise and updated descriptions of the "state of the art" in a specific topic. Innovative and thought-provoking ideas are welcome.

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PROGRAM & ABSTRACTS

SAFIS
2023

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08:00 - 09:00	**REGISTRATION**	08:00 -10:00	**POSTER PRESENTATION**	09:00 - 10:00	**CAMILION DE HURTADO AWARD PRESENTATIONS**
09:00 - 10:30	**VII MEETING OF PHYSIOLOGY AND BIOLOGICAL PHYSICS TEACHER CONFERENCE** Artificial intelligence and immersive techniques as teaching	10:00 -12:00	**SYMPOSIUM III** Neuroendocrine regulation of immunity	10:00 - 12:00	**SYMPOSIUM V COMMISSION OF YOUNG RESEARCHERS** Endocannabinoid System and its modulation: from laboratory to practice
10:30 - 11:00	**BREAK**				
11:00 -12:00	Simulation models in physiology teaching				
12:00 -13:30	**TIME FOR LUNCH**	12:00 -13:30	**TIME FOR LUNCH**	12:00 - 13:30	**TIME FOR LUNCH**
13:30 -15:30	**SYMPOSIUM I** Aging, repair and regeneration / Gene therapy	13:30 -15:30	**SAFIS YOUNG INVESTIGATOR AWARD PRESENTATIONS**	13:30 - 15:30	**SYMPOSIUM VI** Oncology and inflammation
15:30 -17:30	**POSTER** VII TEACHERS 2023 MEETING I MEETING OF UNDERGRADUATE STUDENT INVESTIGATORS	15:30 -17:30	**POSTER PRESENTATION**	15:30 - 17:30	**POSTER PRESENTATION**
17:30 -19:30	**SYMPOSIUM II** Metabolism in situations of insulin resistance	17:30 -18:30	**SCIENCE ROUNDTABLE DEBATE** How to communicate science in the era of fake news	17:30 - 19:30	**JOINT SYMPOSIUM HYPERTENSION COUNCIL OF THE ARGENTINEAN SOCIETY OF CARDIOLOGY AND SAFIS** Renal Physiology and Hypertension
19:30 - 20:30	**SAFIS OPENNING CEREMONY INAUGURAL CONFERENCE** Physiological Role of Autophagy in the Heart	18:30 - 20:30	**SYMPOSIUM IV** Neuroimmunomodulatory regulation of ventricular function and remodeling	19:30 - 20:30	**CLOSING CONFERENCE** Physiology of immune system and SARS-CoV-2
		20:30 - 21:30	**SAFIS ASSEMBLY**	20:30 - 21:30	**SAFIS CLOSING AND AWARD CEREMONY**

Welcome from the President of SAFIS
Dr. Graciela Cremaschi

Dear Colleagues and friends,
I want to give you the warmest welcome to the 2023 Meeting of the Argentine Society of Physiology, which we have the pleasure of celebrating in person at the Pontifical Catholic University of Argentina in the city of Buenos Aires.

Last year we celebrated the first in person meeting after the COVID-19 pandemic from which we are still recovering. The 2022 meeting was a true celebration after 2 years of virtual events. This year we are going to celebrate resilience and our ability to overcome adversity, taking advantage of the virtual tools brought to us by almost 2 years of "confinement". Thus, we will have not only in person, but also synchronous virtual lectures of excellent scientists who are in other regions of the country and the world.

I especially want to welcome and express my deepest gratitude to Dr. Junichi Sadoshima (from Rutgers University, USA) and Dr. Merry Lindsey (Meharry Medical College, USA, and editor-in-chief of the American Journal of Physiology: Heart and Circulatory Physiology) for their generous participation. We feel very honored by their presence which gives prestige to our congress. Having them on site is a wonderful and not frequent opportunity for our local physiologists and fellows to hear first-hand results and feedback from them. Also, I want to extend my gratitude to all the excellent national and international speakers that will give lectures in different areas of physiology who are the main architects of the success of this meeting.

This year we will have a very interesting meeting of physiology and biological physics teachers dedicated to the use of artificial intelligence in the teaching of physiology, organized by the SAFIS Education Committee. Many professors of physiology point out the problem of studying organs and systems of the human body in the form of sealed compartments without relating them and integrating knowledge. For this reason, we organized the 1st meeting of undergraduate students, who are beginning their scientific work in different branches of physiology. In this meeting, these students will present their work in a multidisciplinary poster session.

We also have several Symposiums on different thematic areas in which prestigious national and international scientists will participate and which include a joint SAFIS symposium with the Hypertension Council of the Argentine Society of Cardiology. We will also have the symposium organized by the Young Investigators Committee of SAFIS on the modulation of the endocannabinoid system and its impact at a therapeutic level. I especially want to congratulate the entire commission for their dedication and work that guarantees the future of our Society. The program will include a panel discussion on fake news in science, the classic poster sections, and the SAFIS and María Cristina Camilión de Hurtado awards, the latter for the best work in the cardiovascular area.

A few days ago, the first Argentine vaccine against COVID-19 was approved. In this regard, we will have Dr. Jorge Geffner, the 2023 Konex Platinum Award winner, at the closing conference of the event to talk about the immunogenicity of the SARS-CoV-2 virus.

Finally, I want to express my gratitude to all the authorities of the congress, and especially to our Secretary, Dr. Germán González and to our Treasurer, Dr. Alicia Klecha for their great help in the organization, and to Valeria Cassaza, Secretary of SAFIS, for her permanent support. Likewise, my gratitude to the Pontifical Catholic University and its School of Medical Sciences, CONICET, and FONCYT for financial support. I also thank our sponsors, and the Camilion de Hurtado Family, which finances the homonymous award. Last but not least, I want to extend my gratitude to the chairs, the awards' jury, and all the participants who make this event possible. In fact, this year we have doubled the number of registrations. We hope to have a successful meeting that can be an excellent framework for learning, improving knowledge, and networking. You are welcome to enjoy the congress.

CONFERENCES

SAFIS INAUGURAL CONFERENCE
Junichi Sadoshima (Department of Cell Biology & Molecular Medicine. Rutgers New Jersey Medical School, Newark, New Jersey, USA.)
Physiological Role of Autophagy in the Heart

SAFIS CLOSING CONFERENCE
Jorge Geffner (Instituto de Investigaciones Biomédicas en Retrovirus y SIDA (INBIRS). Facultad de Medicina. Universidad de Buenos Aires-CONICET, Buenos Aires, Argentina)
Immune response against SARS-CoV-2 infection

SYMPOSIA

SYMPOSIUM I • Aging, repair and regeneration / Gene therapy

Microbiome, brain, and cognition in early stages of Alzheimer's disease
Laura Morelli (*Laboratorio de envejecimiento cerebral y neurodegeneración. Fundación Instituto Leloir. IIBBA (CONICET), Buenos Aires. Argentina).*

Neurogenesis and aging: how to rewire a stubborn hippocampus.
Alejandro Schinder (*Laboratorio de Plasticidad Neuronal, Fundación Instituto Leloir. CONICET. Buenos Aires. Argentina).*

Anti-inflammatory mesenchymal stem cell-derived extracellular vesicles for donor heart preservation.
Gustavo Yanarelli (*Instituto de Medicina Traslacional, transplante y bioingeniería Fundación Favaloro-CONICET. Buenos Aires. Argentina).*

Mesenchymal Stem Cells and Regenerative Medicine in Chronic Liver Diseases
Guillermo Mazzolini (*Instituto de Investigaciones en Medicina Traslacional, Universidad Austral. Buenos Aires. Argentina).*

SYMPOSIUM II • Metabolism in situations of insulin resistance

Adipose tissue in obesity and GLP-1 agonist treatment
Verónica Miksztowicz (*Laboratorio de Patología Cardiovascular Experimental e Hipertensión. Instituto de Investigaciones Biomédicas (BIOMED), Facultad de Ciencias Médicas, Pontificia Universidad Católica Argentina (UCA) y Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos Aires, Argentina).*

Effect of chronic stress on obesity and metabolic syndrome
Bibiana Fabre (*Departamento de Bioquímica Clínica, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires; Buenos Aires. Argentina).*

Intrauterine proinflammatory environment and fetal programming in maternal diabetes
Alicia Jawerbaum (*Laboratorio de Reproducción y Metabolismo. CEFYBO-CONICET. Facultad de Medicina. Universidad de Buenos Aires, Buenos Aires, Argentina).*

Dietary bioactives in the mitigation of insulin resistance: role of the gastrointestinal tract.
Patricia Oteiza (*Department of Nutrition, and Department of Environmental Toxicology, University of California, Davis, Davis, CA, USA).*

SIMPOSIUM III • Neuroendocrine regulation of immunity

Neuroendocrine-immune interactions: Interleukin-1β as an example of immune mediators linking the periphery and the brain.
Adriana del Rey (*Research Group Immunophysiology, Division Neurophysiology, Inst. f. Physiology and Pathophysiology, Medical Faculty, Philipps University, Marburg, Germany).*

Pregnancy and Autoimmunity: Influence of Female Sex Hormones on B Lymphocyte Activation.
Federico Jensen (*Centro de estudios farmacológicos y botánicos (CEFYBO-CONICET), Facultad de Medicina, UBA. Buenos Aires, Argentina).*

Gut microbiota, probiotics and health.
Carolina Maldonado (*Laboratorio de Inmunología, Centro de Referencia para Lactobacilos (CERELA-CONICET), Tucumán, Argentina. Cátedra de Inmunología, Facultad de Bioquímica, Química y Farmacia, Universidad Nacional de Tucumán. Tucumán, Argentina).*

Immunomodulation mechanisms in joint inflammation.
María Silvia Di Genaro (*Facultad de Química, Bioquímica y Farmacia, Universidad Nacional de San Luis, Instituto Multidisciplinario de Investigaciones Biológicas-San Luis (IMIBIO-SL, UNSL, CONICET), San Luis, Argentina).*

SIMPOSIUM IV • Neuroimmunomodulatory regulation of ventricular function and remodeling

Repurposing sildenafil for the treatment of hypertensive cardiac hypertrophy.
Néstor Gustavo Pérez (*Centro de Investigaciones Cardiovasculares, Facultad de Medicina, Universidad Nacional de La Plata, La Plata, Buenos Aires. Argentina).*

Cardiac inflammation and repair following myocardial infarction.
Merry Lindsey (*Meharry Medical College, School of Graduate Studies, Meharry Medical College and Nashville VA Medical Center, Nashville, TN, USA. Editor-in-Chief American Journal of Physiology: Heart and Circulatory Physiology USA; American Physiological Society)*

Vagal neuromodulation for myocardial infarction: new insights into mechanisms and future perspective.
Bruno Buchholz (*Instituto de Bioquímica y Medicina Molecular (IBIMOL), Departamento de Anatomía, CONICET. Facultad de Medicina. Universidad de Buenos Aires, Buenos Aires, Argentina).*

Judging Arterial Hypertension: unveiling cardiac mitochondria as vital co-conspirators in pathological cardiac hypertrophy.
Irene Ennis (*Centro de Investigaciones Cardiovasculares, Facultad de Medicina, Universidad Nacional de La Plata, La Plata, Buenos Aires. Argentina).*

SYMPOSIUM V COMMISSION OF YOUNG RESEARCHERS • The Endocannabinoid System and its modulation: from the laboratory to practice.

Neuronal sets that co-express the growth hormone secretagoge receptor and the cannabinoid receptor type-1 in the mouse brain.
Camila Saenz (*Laboratorio de Neurofisiología del Instituto multidisciplinario de Biología Celular (IMBICE), La Plata, Buenos Aires, Argentina).*

Maternal cannabis consumption disinhibits male VTA dopamine neurons but does not affect cocaine conditioned place preference in mice.
Colleen S. Peterson, Nada Sallam, Sarah Mina, Stephanie L. Borgland (*Faculty of Medicine, University of Calgary. Department of Neuroscience, Faculty of Medicine, University of Calgary, Canada).*

The "B-side" of cannabis on the heart: Cardioprotective effects.
Erica Pereyra (*Centro de Investigaciones Cardiovasculares, Facultad de Medicina, Universidad Nacional de La Plata, La Plata, Buenos Aires. Argentina).*

Use of cannabis sativa for the development of dermal scaffolds and Bioinks for 3D Bioprinting.
Pablo Antezana (*Instituto de Química y Metabolismo del Fármaco (IQUIMEFA, Facultad de Farmacia y Bioquímica, UBA, Buenos Aires, Argentina)).*

SIMPOSIUM VI • Oncology and Inflammation

Histaminergic System as a Promising Target for Breast Cancer Treatment.
Vanina Medina (*Laboratorio de Biología Tumoral e Inflamación, Instituto de Investigaciones Biomédicas (BIOMED), Facultad de Ciencias Médicas, Pontificia Universidad Católica Argentina (UCA) y Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos Aires, Argentina).*

Ugly pro-inflammatory molecular actors helping the liver?
Daniel Francés (*Instituto de Fisiología Experimental (IFISE-CONICET), Universidad Nacional de Rosario, Rosario. Argentina).*

Suppression of salivary gland autoimmunity by galectin-1.
Marta Toscano (*Hospital Arturo Oñativia Salta).*

The mRNA-binding protein TTP acts as a tumor suppressor gene and regulator of the inflammatory response in head and neck carcinogénesis.
Ana R. Raimondi (*Instituto de Fisiología, Biología Molecular y Neurociencias (IFIBYNE) CONICET-Universidad de Buenos Aires, Buenos Aires, Argentina).*

JOINT SYMPOSIUM HYPERTENSION COUNCIL OF THE ARGENTINEAN SOCIETY OF CARDIOLOGY AND THE ARGENTINEAN SOCIETY OF PHYSIOLOGY • Renal Physiology and Hypertension.

Impact of chloride anion in the development of arterial hypertension.
Nicolás Kouyoumdzian (*Instituto de Investigaciones cardiológicas Dr. Alberto Taquini, Universidad de Buenos Aires, Buenos Aires. Argentina).*

Renal Autoregulation and Hypertension.
Cesar Romero (*Renal Division, Department of Medicine, Emory University, Atlanta, GA, USA).*

Role of Natriuretic Peptide in SRH and normotensive rats.
Carolina Canifi (*Instituto de la Química y Metabolismo del Fármaco (UBA-CONICET). Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Buenos Aires. Argentina. Consejo Argentino de HTA de la Sociedad Argentina de Cardiología).*

MAS receptor regulation in hypertension.
Mariela Gironacci (*Cátedra de Química biológica de la Facultad de Farmacia y Bioquimica, Universidad de Buenos Aires. Buenos Aires. Argentina).*

SCIENCE ROUNDTABLE DEBATE ABSTRACTS

SCIENCE ROUNDTABLE DEBATE • How to communicate science in the era of fake news.
Moderator: Leonardo Lacoa (*Scientific Journalist, National University of La Matanza, Buenos Aires, Argentina).*

Communicating science to non-expert audiences
Ma. Soledad Casasola (*Directora de Comunicación de la Ciencia. Universidad Nacional de Rosario, Argentina)*

Fighting fake news with a "multi-dose" of science.
Soledad Gori *(Instituto de Química Biológica. Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Buenos Aires. Argentina*).

Understanding and debunking misinformation about science and health.
Florencia Ballarino *(Chequeado ciencia, Buenos Aires. Argentina).*

Preprints' and misinterpretation of scientific studies as a source of misinformation.
María González Dionis *(Newport, España).*

CONFERENCE ABSTRACTS

INAUGURAL CONFERENCE

Junichi Sadoshima (Department of Cell Biology & Molecular Medicine. Rutgers New Jersey Medical School, Newark, New Jersey, USA.)

Physiological Role of Autophagy in the Heart

Autophagy is an essential mechanism for the degradation of cellular materials through lysosomes and is characterized by the presence of autophagosomes. Autophagy also selectively degrades damaged mitochondria, termed mitophagy, thereby maintaining the quality of mitochondria. Autophagy and mitophagy are activated by stress and generally protect the heart against stress. However, stress-induced activation of autophagy and mitophagy is often transient, and they are inactivated in chronically stressed hearts, including heart failure induced by myocardial infarction, high blood pressure and metabolic syndrome. Downregulation of autophagy and mitophagy is also observed in aging hearts. On the other hand, autophagy is dysregulated in some conditions and causes harmful effects, including cell death. One form of cell death induced by dysregulated autophagy is termed autosis, which is characterized by unique morphological and biochemical features. In this seminar, I will discuss the regulation of autophagy in chronically stressed hearts and the role of autophagy and mitophagy in heart failure and aging. In particular, I will discuss 1) the molecular mechanism by which autophagy is inhibited in the chronically stressed heart, including phosphorylation of Beclin 1 by mammalian sterile 20-like kinase 1 (Mst1), 2) activation of autosis during ischemia/reperfusion injury, which may contribute to slow myocardial death after reperfusion, together with the therapeutic potential of autosis inhibition during ischemia/reperfusion, 3) the role of conventional and unconventional form mitophagy in obesity cardiomyopathy heart, and 4) the role of autophagy and mitophagy in myocardial aging and underlying signaling mechanisms, including the role of autophagy suppression in induction of cardiomyocyte senescence, 5) therapeutic interventions to modulate the activity of autophagy and mitophagy in the failing hearts.

CLOSING CONFERENCE

Jorge Geffner (Instituto de Investigaciones Biomédicas en Retrovirus y SIDA (INBIRS). Facultad de Medicina. Universidad de Buenos Aires-CONICET, Buenos Aires, Argentina)

Immune response against SARS-CoV-2 infection

The SARS-CoV-2 pandemic has so far caused more than 7 million officially recognized deaths worldwide and possibly the real number of deaths is close to 15 million. Although the majority of infected people usually suffer an asymptomatic or mild disease, a fraction of them will suffer severe symptoms associated with the development of acute respiratory distress. Moreover, beyond the acute condition, a significant fraction of infected individuals will suffer long-COVID-19. Both, severe COVID-19 and long-COVID-19 are strongly associated with the development of a systemic inflammatory response driven by monocytes/macrophages, neutrophils, and endothelial cells. The development of this deleterious immune response appears to be related with the inability of the innate immune response to control viral replication at the early stages of the infection. Production of type I interferons, plasmacytoid dendritic cells, and natural killer cells appear to play an important role in anti-SARS-CoV-2 immune response. By contrast, an inflammatory response mediated by macrophages, neutrophils (production of oxygen reactive intermediates and neutrophil extracellular traps (NETs), platelets and endothelial cells together with the activation of the coagulation cascade, were shown to be involved in tissue injury during or after acute SARS-CoV-2 infection. Of note, severe COVID-19 is also associated to the production of a diversity of autoantibodies directed to type I interferons, pulmonary surfactant proteins, cardiolipin, pancreas β cells, G protein-coupled receptors (GPCR) and RAS-related molecules, among others, suggesting that autoimmunity contributes to tissue injure. The concept of stratified medicine correctly assumes that the same clinical picture in a given disease can be induced by different mechanisms. This concept seems applicable not only to the acute condition associated with severe COVID-19, but also with long-COVID-19. The stratification of patients affected by COVID-19 based on the underlying mechanisms responsible for tissue injury will surely open new therapeutic horizons.

SYMPOSIUM ABSTRACTS

SYMPOSIUM I • Aging, repair and regeneration / Gene therapy

Microbiome, brain, and cognition in early stages of Alzheimer's disease

Laura Morelli. *Laboratorio de envejecimiento cerebral y neurodegeneración. Fundación Instituto Leloir. IIBBA (CONICET), Buenos Aires. Argentina.*

Emerging literature indicates that gut microbiota could impact on the development of Alzheimer's disease (AD), the leading cause of dementia in older adults. Bacterial associations have been reported in transgenic (Tg) animal models of AD and in human case-control studies. However, the metabolic pathways linking intestinal microbiota and brain functionality were not deeply established. We exposed McGill-R-Thy1-APP rats (a Tg model of early AD-like amyloid pathology) and wild-type (WT) animals to control (CTL) (7 % fat) and high fat (HF) (21% fat) diets during 6 months. At the end of the experimental paradigm, animals were evaluated by the Morris water maze test to assess learning and memory. Neuroinflammation was estimated on hippocampal sections by quantification of branched (inactive) and non-brunched (reactive) microglia Iba-1 positive cells. Bacterial abundance in feces was determined by 16s genotyping (Illumina-StartSeq-Germany), and metabolomics profile of plasma and feces was assessed by UPLC-MS/MS (Metabolon-USA). Bioinformatics was performed with R packages (mixOmics, Metaboanalyst, dplyr,Fella) and post-hoc analysis (KEGG, PMBDID) was done to define candidate pathways. Our results suggest that HF diet impairs cognition in WT but it was unable to worsen learning or reference memory in Tg rats. However, HF diet promotes neuroinflammation regardless the genotype. 189 operational taxonomic units (OTUs) were detected in feces and 14 OTUs at genus levels (abundance > 1%) showed significant differences ($p<0.05$) between genotype. Four clusters were obtained after an integrative analysis of metabolites (plasma, n=702; feces, n= 686) and bacteria from animals exposed to CTL and HF diets. We found that transgenesis promotes L-histidine metabolism while inhibits bile acids, and fatty acids β-oxidation, highlighting the role of brain amyloidosis on ROS generation, bioenergetics dysfunction and neurodegeneration. The main impact of HF diet seems to be restricted to impairments of β-oxidation and enhancement of ceramides synthesis, contributing to oxidative stress and inflammation. This work represents a first step towards a comprehensive research on the “gut-brain” axis in early stages of amyloid pathology. Future experiments modulating the highlighted pathways will clarify the interaction “brain amyloidosis-microbiota“ and its consequence on cognition.Dieta en intestino cerebro y la cognición en etapas tempranas Alzheimer-

Neurogenesis and aging: how to rewire a stubborn hippocampus.

Alejandro Schinder. *Laboratorio de Plasticidad Neuronal, Fundación Instituto Leloir. CONICET. Buenos Aires. Argentina.*

The hippocampus plays a fundamental role in memory acquisition, storage and retrieval, and it is also involved in processing spatial representations. The dentate gyrus, the main gateway to the hippocampus for information arriving from the entorhinal cortex, continuously generates new granule cells that require several weeks to develop and integrate in preexisting networks. My lab is interested in understanding the regulation and the functional consequences of adult neurogenesis. New granule cells developing in an adult context recapitulate immature features that occur during early neural development, displaying enhanced excitability, poor inhibition, active synaptogenesis, and exacerbated activity-dependent synaptic modification. With time, connections become stabilized, synaptic inhibition becomes efficient, excitability decreases, and neurons reach a mature phenotype similar to that of perinatally-born granule cells. Thus, the host hippocampal circuit is continuously perturbed and remodeled by the integration of new granule cells. The crosstalk between new granule cells and host circuits is bidirectional; as developing cells remodel preexisting connections, local activity of preexisting cells influences their integration. These processes are different in the aging brain, where neurogenesis is reduced and development is slow. I will discuss recent data from my lab on different forms of behavioral stimulation that elicit rapid integration of new neurons in the aging brain. Our findings reveal a remarkable potential for circuit plasticity in the aging brain that remains dormant and can be awaken by activity.

Anti-inflammatory mesenchymal stem cell-derived extracellular vesicles for donor heart preservation.
Gustavo Yanarelli. *Instituto de Medicina Traslacional, transplante y bioingeniería Fundación Favaloro-CONICET. Buenos Aires. Argentina.*
Cardiac transplantation is an established therapy for patients with end-stage heart failure (HF). Heart transplants have not increased over the past 2 decades due to donor shortage, despite the increase in number of HF patients. At present, several strategies are being used to increase the number of organs suitable for transplantation, including improvement in donor organ preservation, use of organs from donors with extended/marginal characteristics such as cardiac death donors (DCD), and ex-vivo perfusion to assess and repair injured organs. Mesenchymal stem cell-derived extracellular vesicles (MSC-EVs) have shown significant anti-inflammatory, antioxidant, and pro-regenerative effects so, in this context, they could represent a novel therapeutic alternative to improve donor heart preservation. We have developed an ion exchange chromatography (IEX) procedure for the isolation of biologically active MSC-EVs from conditioned medium, compatible with large-scale production, necessary for clinical translation. We performed the isolation using an anion exchange resin (Q sepharose) and eluted the EVs in a single peak fraction, with a mean particle size of <150nm and expression of CD9, CD63, CD81, and TSG101 markers. In addition, we compared this procedure against ultrafiltration, and found that IEX produces a better product in terms of composition and functional properties of the EVs. Moreover, we established and standardized an in-vitro functional macrophage assay to test the anti-inflammatory activity of MSC-EVs, essential to evaluate the biological activity of different EV preparations. Finally, we showed that normothermic preservation is superior to hypothermic preservation for DCD hearts using a pig model of cardiac transplantation. Moreover, the administration of MSC-EVs during the ex- vivo perfusion may have a protective role on donor hearts. In conclusion, our data demonstrate the anti-inflammatory effect of MSC-EVs and postulates EV therapy as a novel tool for donor heart preservation.

Mesenchymal Stem Cells and Regenerative Medicine in Chronic Liver Diseases
Guillermo Mazzolini. *Instituto de Investigaciones en Medicina Traslacional, Universidad Austral. Buenos Aires. Argentina.*
Liver cirrhosis is caused by different etiological agents such as alcohol, hepatitis B or C viruses, autoimmunity, metabolic disorders, among others. It is characterized by repeated cycles of hepatocellular necrosis, inflammation, and scarring, where hepatic stellate cells (HSCs) become activated and extracellular matrix components (ECM). Hepatic macrophages (hMØ) play a critical role in disease progression and fibrosis resolution. It is estimated that 2 million people die annually due to liver diseases. Liver transplant, the only curative option for decompensated liver cirrhosis, faces the difficulty of donor scarcity. Therefore, there is an urgent need for new treatments. Cell therapy emerges as a therapeutic alternative for tissues regeneration. Mesenchymal stem/stromal cells (MSCs) are multipotent cells capable of selectively migrating to damaged tissue and contributing to its repair/regeneration. The pro-regenerative effect of MSCs is due to their ability to produce and secrete factors that promote cell protection and survival, as well as regulate the immune response. The fibrogenic microenvironment, characterized by high levels of cytokines (VEGF, PDGF, TGF-β1, (MCP-1), IL-8, TNF-β, IL-1β, IL-6, and SDF-1), plays a key role in guiding MSCs migration. When incorporated into damaged tissues, MSCs can collaborate with vasculogenesis and healing through the secretion of cytokines and growth factors. Particularly in liver diseases, it has been shown that MSCs are promising candidates for treating both cirrhosis and acute liver failure, as well as other types of liver diseases, as demonstrated in dozens of clinical studies in patients with chronic liver disease. Although the initial therapeutic interest was due to their differentiation capacity into hepatocytes, evidence indicates that the therapeutic mechanisms of MSCs in liver fibrosis depend on a wide range of effects exerted on different cell populations involved in liver damage and fibrogenesis, creating a hepatoprotective environment through cell-cell interactions or paracrine effects by secreting biologically active molecules in soluble form or through extracellular vesicles (EVs). This presentation delves into these intricate mechanisms and summarizes the key clinical findings from phase I and II trials conducted in patients with hepatic cirrhosis.

SYMPOSIUM II • Metabolism in situations of insulin resistance

Adipose tissue in obesity and GLP-1 agonist treatment

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Abdominal obesity is a multifactorial chronic inflammatory disease characterized by visceral adipose tissue (VAT) expansion. Obesity is an epidemic worldwide, and it has become one of the main public health problems. Therefore, in recent years, new drugs have been studied for obesity treatment, being glucagon like peptide (GLP)-1 agonists one of the most promising options. It has been demonstrated that Liraglutide (LGT), a GLP-1 agonist, decreases body weight in obese individuals; however, their mechanisms of action on AT remodeling are not complete dilucidated. Physiological expansion of AT involves adipogenesis and angiogenesis processes, and both require a highly regulated extracellular matrix (ECM) remodeling. Metalloproteinases (MMPs) are a family of endopeptidases responsible for ECM components degradation, which have several regulatory mechanisms essential for tissue homeostasis. In obesity, excessive visceral fat is characterized by hypertrophy and hyperplasia of adipocytes and a deficient vascularization, leading to AT dysfunctionality. Regarding adipogenesis, galectin-3 (Gal-3) has recently emerged as a new adipogenic factor. Gal-3, a β-galactosidase-binding lectin, modulates different biological functions and it is involved in the pathogenesis of cancer, inflammatory, cardiovascular, and metabolic diseases. Numerous studies have suggested the key function of Gal-3 in AT maturation and glucose homeostasis. Finally, different evidence suggest a relation between ECM and mitochondria. Since mitochondria are dynamic organelles, changes in their structure, function, and cellular distribution produce changes in metabolism. It has been suggested that shifts in ECM organization during AT expansion may also alter mitochondrial behaviour. In this talk I will develop the new insights in VAT expansion associated with obesity and the effects of LGT on factors associated with tissue remodeling and mitochondrial dynamics.

Effect of chronic stress on obesity and metabolic syndrome

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The association between stress, obesity, metabolic syndrome and diabetes has been widely studied. However, it remains unclear whether increased cortisol levels contribute to the development of obesity or if obesity itself prompts an elevation in cortisol levels. Chronic stress leads to chronic hyperactivation of the hypothalamus-pituitary-adrenal axis. On the other hand, visceral obesity leads to an increase in local cortisol production. In situations of insulin resistance, the greatest effects of glucocorticoids are through adipose tissue, where it has been proved there is a higher density of glucocorticoid receptors. Moreover, it has been noted that cortisol plays a role in the transformation of pre-adipocytes into adipocytes. In the adipocyte, the enzyme 11βeta hydroxysteroid dehydrogenase is involved in the conversion of inactive cortisone to active cortisol. The synthesis of this enzyme is stimulated by several cytokines and is found to be increased in inflammatory states and in expanded adipose tissue (Cooper M and Stewart P, 2009). The increased production of these cytokines, especially IL-6 and leptin, as well as free fatty acids, have a direct effect by increasing the synthesis and secretion of CRH. Taking this into account, we studied the association between chronic stress, obesity and metabolic syndrome including the evaluation of the hypothalamus-pituitary-adrenal axis. The assessment of chronic stress involved the assessment of hair cortisol as biomarker, along with the application of psychometric tools to evaluate psychological factors.

Intrauterine proinflammatory environment and fetal programming in maternal diabetes

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Diabetes in pregnancy impairs fetal and placental development and leads to the programming of metabolic and cardiovascular diseases in the offspring. A prooxidant and proinflammatory intrauterine environment is involved in these developmental alterations. Peroxisome proliferator activated receptors (PPARs) are ligand activated transcription factors with key regulatory functions in developmental, metabolic and anti-inflammatory processes. PPARs can be activated by nutrients, being unsaturated fatty acids their natural ligands. Studies in our laboratory have addressed the effect of diets supplemented with extra virgin olive oil, enriched in PPAR activators and with potent antioxidant effects, in experimental models of diabetes and pregnancy and in patients with GDM. Using experimental models of pregestational diabetes, we provided evidence of the capacity of diets enriched in extra virgin olive oil to reduce prooxidant and proinflammatory markers during fetal and placental development. This maternal dietary treatment provided during pregnancy also benefits the offspring, in which lipid metabolism impairments, cardiac alterations and alterations in the uteri of females are prevented. Translational studies performed in GDM patients provide evidence of the capacity of extra virgin olive oil supplementation to prevent maternal hypertriglyceridemia, to reduce maternal weight gain, to reduce insulin resistance markers and to regulate metabolic and proinflammatory pathways in the placenta. Our results suggest that extra virgin olive oil supplementation may be beneficial for the mothers, the placentas and the offspring in pregnancies complicated by maternal diabetes.

Dietary bioactives in the mitigation of insulin resistance: role of the gastrointestinal tract.

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The gastrointestinal (GI) tract is highly relevant for the absorption, distribution, metabolism, and excretion of bioactives. Additionally, bioactives and/or their metabolites can modulate events at the GI tract that can have both local and systemic effects. At the GI tract, bioactives can modulate nutrient absorption, GI barrier permeability, the activity of luminal enzymes, neutralize luminal toxins and oxidant species, and mitigate dysbiosis, tumorigenesis and intestinal inflammation. The capacity of bioactives to regulate glucose homeostasis and/or mitigate insulin resistance through their actions at the GI tract can occur at different levels, e.g. by regulating the synthesis/secretion of gut hormones that have GI trophic actions and modulate glucose/lipid metabolism, by modulating the activity of enzymes involved in carbohydrate digestion, by protecting the intestinal barrier from permeabilization and the associated systemic inflammation, by modulating the composition of the microbiota. Among bioactives, dietary fiber has a recognized beneficial effect on the modulation of glycemia, as supported by numerous epidemiological studies. For other bioactives, research mostly relies on preclinical studies. As examples, experimental evidence supports the capacity of select flavonoids, i.e. (-)-epicatechin (EC) and anthocyanins (AC), to have beneficial effects in the modulation of glucose homeostasis and insulin resistance. Thus, dietary supplementation with EC or AC mitigates high fat diet-induced intestinal permeabilization, endotoxemia, hyperglycemia, hyperinsulinemia and liver insulin resistance in mice. EC and AC act preventing intestinal barrier permeabilization which decreases the passage to the circulation of luminal lipopolysaccharides (LPS) that initiate systemic inflammation. Through this mechanism, EC and AC mitigate liver and adipose tissue inflammation and prevent the associated activation of signaling cascades (NF-κB, JNK1/2) that inhibit the insulin receptor pathway leading to insulin resistance. Additionally, EC and AC increase the synthesis/secretion of GI tract hormones (GLP-1 and GLP-2) that have GI trophic actions and modulate glucose metabolism by promoting satiety, pancreas insulin release and glucose uptake by peripheral tissues. In summary, dietary approaches including select bioactives that, in part through their actions at the GI tract, have beneficial effects on glucose homeostasis can be of value for the management of insulin resistance/T2D.

SIMPOSIUM III • Neuroendocrine regulation of immunity

Neuroendocrine-immune interactions: Interleukin-1β as an example of immune mediators linking the periphery and the brain.

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Humoral and neural routes contribute to neuro-endocrine-immune interactions with immunoregulatory consequences. Interleukin-1b (IL-1b), originally described as an immunederived product, is well known for its capacity to promote inflammation and its role in shaping adaptive immune responses. However, other properties and cell sources of the cytokine have been later reported. IL-1b was the first cytokine shown to be able to stimulate the hypothalamus-pituitary-adrenal axis by increasing CRH release. The resulting elevation of glucocorticoid blood levels, in turn, contributes to control an excessive production of potentially harmful cytokines and unwanted cellular proliferation. Under non-pathological conditions, and despite its glucocorticoid-increasing activity, IL-1b also induces hypoglycemia in an insulin-independent manner by mechanisms acting at central and peripheral levels. IL-1b is not only produced by immune cells, but also by other cell types, including neurons, and its expression is markedly increased in the brain during long-term potentiation of hippocampal neurons, and during hippocampal-dependent learning paradigms. At the same time, IL-1b can increase brain energetic metabolism and induce glucose incorporation in neurons. Among other targets, the cytokine can also increase glucose incorporation, oxigen consumption rate, and extracellular acidification rate in stimulated immune cells. These effects of the cytokineon neural and immune cells contribute to balance glucose distribution between the periphery and the CNS, depending on the actual physiological needs. A most interesting property of IL-1b is its capacity to induce its own production. For example, peripheral administration of very small amounts of recombinant human (rh) IL-1b to rodents results in upregulation of mouse IL-1b gene expression in several brain regions. More recently, it has been shown that intracerebroventricular injection of nanogram amounts of rhIL-1b induces increased mouse IL-1b expression in the spleen. Thus, the pleiotropic effects of IL-1b indicate that this cytokine is an important mediator that can link the periphery and the brain.

Pregnancy and Autoimmunity: Influence of Female Sex Hormones on B Lymphocyte Activation.

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During pregnancy, the maternal immune system undergoes a series of finely regulated and temporal adaptations that enable tolerance of paternal alloantigens present in the fetus. While these adaptations were evolutionarily selected to prevent immune rejection of the fetus, they can also alter the course of pre-existing autoimmune diseases or induce their development. In this regard, it is known that women with conditions such as multiple sclerosis or rheumatoid arthritis experience significant improvement in their symptoms during pregnancy. This has been attributed to changes in the immune profile during gestation. On the other hand, certain pregnancy-associated pathologies, such as recurrent miscarriages or preeclampsia, have a strong autoimmune component. While the mechanisms that regulate immune changes during pregnancy are not fully understood, it has been postulated that female sex hormones, estradiol (E2) and progesterone (P4), as well as the pregnancy hormone, the human chorionic gonadotropin (HCG), may play a significant role. Our laboratory has demonstrated that B lymphocytes, essential components of the adaptive immune response, not only express high levels of hormone receptors (E2, P4, and HCG) but that these hormones also have a profound impact on the functionality of this cell population. During my presentation, I will attempt to demonstrate, based on results obtained in our laboratory over the past few years, how these hormones regulate some of the fundamental functions of B lymphocytes and how this affects the proper development of pregnancy, as well as potentially the course of autoimmune diseases.

Gut microbiota, probiotics and health.

Carolina Maldonado. *Laboratorio de Inmunología, Centro de Referencia para Lactobacilos (CERELA-CONICET), Tucumán, Argentina. Cátedra de Inmunología, Facultad de Bioquímica, Química y Farmacia, Universidad Nacional de Tucumán. Tucumán, Argentina.*

There are currently a significant number of studies that relate the intestinal microbiota to the health and functioning of the immune system. In fact, dysbiosis or intestinal imbalance is associated with different diseases. Several environmental factors, such as diet and poly-antibiotic therapies, produce changes in the microbiota composition, making these individuals more susceptible to gastrointestinal disorders. Among the different factors, we can mention senescence. Aging is associated with the progressive deterioration of physiological functions, leading to different pathologies. The immune system is especially affected in this process, which is known as "immunosenescence." In recent years, the intestinal bacteria have become one of the most important focuses of biological and medical research. The possibility of achieving a positive balance of the intestinal microbiota through dietary manipulation constitutes an interesting perspective to preserve a diverse and healthy gastrointestinal microbial community and improve the regulation of the immune system.

Immunomodulation mechanisms in joint inflammation.

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Spondyloarthritis (SpA) are chronic inflammatory joint diseases that includes axial and peripheral SpA. Reactive arthritis (ReA) is a form of peripheral SpA that develops 1-3 weeks after a gastrointestinal or urogenital infection. Tumor necrosis factor alpha (TNF-α) and interleukin-17 (IL-17) are two pro-inflammatory cytokines involved in the pathophysiology of SpA. TNF mediates its function through two receptors: TNFRp55 (TNFR1) and TNFRp75 (TNFR2). We have demonstrated that TNFRp55 is essential for the protection against Yersinia enterocolitica (Ye)-induced ReA through mechanisms that involve IL-12/23p40 production. Dendritic cells (DCs) participate in IL-12/23p40 overproduction, which leads to increased Th1 and Th17 polarization. Migration of intestinal DCs to the regional lymph nodes of the joints was detected, providing evidence on gut-joint axis in ReA. In addition, we investigated the role of the synovial microenvironment on fibroblast-like synoviocytes (FLS). Stimulation with IL-6 co-triggered MMP-9 and IL-10 secretion. MMP-9 secretion depended on JNK and p38 MAPKs, whereas IL-10 secretion was dependent on the JAK pathway as a potential feedback mechanism controlling IL-6-induced MMP-9 expression. Using a proteomic approach, we identified S100A8 in the synovial fluids of patients with peripheral SpA. Our data revealed a relationship between S100A8 alarmin with IL-6 and metalloproteinase (MMP)-9 secretion by FLS in the real synovial microenvironment of peripheral SpA. These data shed new insights into the immunopathogenesis of SpA and suggest new suitable targets for SpA treatment.

SIMPOSIUM IV • Neuroimmunomodulatory regulation of ventricular function and remodeling

Repurposing sildenafil for the treatment of hypertensive cardiac hypertrophy.

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Originally developed as antianginal drug, Sildenafil (SIL) was later on revealed as an effective medication for the treatment of erectile dysfunction and pulmonary hypertension, therefore signifying a clear example of drug repurposing. In a previous study, we showed that an increased protein Kinase G (PKG) activity after phosphodiesterase 5 (PDE5) inhibition by SIL leads to myocardial Na+/H+ exchanger (NHE1) inhibition. Since it was shown that NHE1 hyperactivity is responsible for the development of pathologic cardiac hypertrophy in different animal models of cardiovascular disease we thought to evaluate the potential antihypertrophic effect of SIL in the myocardium of spontaneous hypertensive rats (SHR). We initially confirmed that SIL also inhibits

NHE1 in this pathological myocardium, and that this effect correlated with a reduction in the exchanger phosphorylation level primary assigned to activation of a PKG-p38MAP kinase-PP2A phosphatase signaling pathway. Furthermore, animals chronically treated with SIL showed a decreased oxidative stress that appeared to be consequence of decreased mitochondrial NHE1 phosphorylation. In addition, treated SHR showed a significant decrease in the pro-hypertrophic phosphatase calcineurin, which correlated with a significant decrease in cardiac hypertrophy and interstitial fibrosis that resulted in a reduced myocardial stiffness. The study provided novel evidence concerning the ability of the well-known PDE5 inhibitor SIL to reverting established cardiac hypertrophy in an animal model that clearly resembles human essential hypertension. From a clinical point of view, repurposing of an approved drug to treat severe cardiac pathologies like cardiac hypertrophy would represent an appropriate opportunity to immediately access to a novel, effective, and probably more secure antihypertrophic adjuvant therapy.

Cardiac inflammation and repair following myocardial infarction.

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Following myocardial infarction (MI), the left ventricle (LV) undergoes a series of cardiac wound healing responses that involve both the stimulation of robust inflammation to clear necrotic myocytes and tissue debris and the induction of extracellular matrix (ECM) protein synthesis to generate an infarct scar. Collectively, this process in known as LV remodeling. Matrix metalloproteinase-9 (MMP-9) is a key regulator of LV remodeling after MI, through direct effects on ECM turnover as well as indirect effects on the regulation of the major cell types that coordinate cardiac wound healing- namely the infiltrating leukocytes and the cardiac fibroblasts. We will discuss recent research that has expanded our understanding of MI LV remodeling, including recent proteomic advances focused on the ECM compartment to provide novel functional and translational insights. In summary, this talk will provide an overview of how cardiac ECM research has evolved over the last decade and will provide insight into future directions that will drive our understanding of MMP directed cardiac ECM turnover in the LV following MI.

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Vagal neuromodulation for myocardial infarction: new insights into mechanisms and future perspective.

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Dysautonomia, characterized by sympathetic hyperactivity and diminished parasympathetic activity has been implicated in the pathogenesis of many cardiovascular diseases including hypertension, myocardial ischemia, arrhythmia, and heart failure. Strong basic evidence suggested that restoring parasympathetic activity by vagal nerve stimulation (VNS) improves ventricular function, adverse myocardial remodeling, and survival in different ischemic heart failure models. However, clinical studies of VNS for heart failure with reduced ejection fraction have had mixed and unconcluded results to date. The infarct size is one of the most important determinants of the evolution of ischemic heart disease. In recent years, several experimental studies have shown that VNS reduces acute myocardial infarct size by different mechanisms. Recently we studied the effects and mechanisms of pre-ischemic VNS as a technique to improve parasympathetic activity in acute myocardial ischemia or ischemia/reperfusion injury, and its long-term effects on left ventricular remodeling and function. We also discuss the potential limitations to translate these preclinical myocardial protection results to patients. Disruptions of neural and intrinsic heart cellular pathways in patients who suffer cardiovascular diseases and comorbidities could interfere in clinical vagal protection on myocardial infarction.

Judging Arterial Hypertension: unveiling cardiac mitochondria as vital co-conspirators in pathological cardiac hypertrophy.

Irene Ennis. *Centro de Investigaciones Cardiovasculares "Dr. Horacio E. Cingolani", CONICET, Facultad de Ciencias Médicas, Universidad Nacional de La Plata, La Plata, Buenos Aires. Argentina.*

Cardiac hypertrophy (CH) defines the increase in ventricular mass, mainly due to cardiomyocyte enlargement triggered by either physiological (e. g., post-natal developmental growth, regular exercise, and pregnancy) or pathological (e. g., hypertension, myocardial infarction, etc.) hemodynamic overload. The first one preserves cardiac structure and normal or enhanced contractile function, while pathological CH is associated with increased cardiomyocyte death, fibrosis and declined pump function that frequently evolves to heart failure. Since the myocardium is a highly oxidative tissue, mitochondria, that represent approximately one-third of the cardiomyocyte's volume, are essential to provide the energy consumed mainly by the excitation-contraction coupling, myofilaments interaction and ion homeostasis. To achieve this goal, mitochondria depend on their integrity, the maintenance of the membrane potential (ΔΨm) and internal pH, as well as the appropriate arrangement in relation with the sarcomeres. Sustained hemodynamic overload, such as hypertension, derives in high energy consumption by the myocardium that jeopardize the balance between mitochondrial energy supply and heart requirements. Mitochondrial dysfunction is typical in several models of pathological CH, mainly due to increased oxidative stress and mechanical disarrays, ultimately contributing to the development of heart failure (Parmeshwar B et al. Oxidative stress in heart diseases, 2019). On the other hand, it has been shown that training might enhance antioxidant systems and, in skeletal muscle, promote the clearance of dysfunctional mitochondria (Zoladz et al. Pflügers Archiv., 2016). Moreover, we have previously demonstrated in spontaneously hypertensive rats the reversion of pathological hypertrophy into a more physiological phenotype with improved cardiac pump function by aerobic training (Garciarena et al. Hypertension, 2009). In this talk, I would like to share with you some new data from my lab regarding to the potential benefits of aerobic training in the pathological hypertrophied rat myocardium, this time specially focusing in the mitochondrial network.

SYMPOSIUM V COMMISSION OF YOUNG RESEARCHERS • The Endocannabinoid System and its modulation: from the laboratory to practice.

Neuronal sets that co-express the growth hormone secretagoge receptor and the cannabinoid receptor type-1 in the mouse brain.

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The growth hormone secretagogue receptor (GHSR) and the cannabinoid receptor type 1 (CB1R) are both G-protein coupled receptors (GPCR) highly expressed in the brain. GHSR mediates the effects of hormones (i.e.,ghrelin) and notably, acts via ligand-independent mechanisms, including constitutive activity and allostericmodulation of other GPCRs. CB1R is activated by endogenous endocannabinoids (i.e., anandamide) as well asphytocannabinoids. Interestingly, GHSR and CB1R expression have been observed within many of the samebrain nuclei, suggesting that these GPCR may act on common neuronal sets to mediate its neurobiologicaleffects. Here, we explored the extent of this putative GHSR and CB1R interaction in the mouse brain. To mapGHSR distribution, we used two complementary approaches: 1) a fluorescent variant of ghrelin (Fr-ghrelin) and2) a mutant mouse in which GHSR promoter drives the expression of GFP (GHSR-eGFP mice). In both cases, thepresence of CB1R was visualized using a validated anti- CB1R antibody. Using the Fr-ghrelin labeling together with CB1R immunolabeling, we found that cells containing both GHSRand CB1R are mainly located in the hippocampus area, where GHSR cells were also positive for CB1Rrepresenting the 45,46 ± 7,28% of all GHSR cells ($p<0.05$, one sample t-test). In brain sections of GHSR-eGFPmice inmunostained with CB1R antibody, we also found cells containing both signals located in the neocortex,amygdala and mainly in the hippocampus area where GHSR cells were also

positive for CB1R representing the40,08 ± 13,33% of all GHSR cells (p= 0.095, one sample t-test). In contrast, simultaneous presence of GHSR andCB1R was not observed elsewhere in the brain In contrast, simultaneous presence of GHSR and CB1R was notobserved elsewhere in the brain by either of these two approaches. Thus, we started to elucidate some of theneuronal populations where GHSR and CB1R may directly act.

Maternal cannabis consumption disinhibits male VTA dopamine neurons but does not affect cocaine conditioned place preference in mice.

Colleen S. Peterson, Nada Sallam, Sarah Mina, Stephanie L. Borgland.
Faculty of Medicine, University of Calgary. Department of Neuroscience, Faculty of Medicine, University of Calgary, Canada.

After alcohol, cannabis is the most commonly used psychoactive substance in pregnancy. However, the psychoactive component, delta-9-tetrahydrocannabinol (THC), is lipophilic and readily crosses the placenta, and thus may impact neurodevelopment. This is supported by human epidemiological studies which have indicated increased risks of substance use and risk of depression. Furthermore, preclinical rodent studies have similarly found increased motivation for opioid rewards and male-specific hyperexcitability of ventral tegmental area (VTA) dopamine neurons resulting from prenatal cannabis exposure. However, most animal models of cannabis use injection of pure THC or cannabinoid receptor agonists, which does not model human consumption patterns. Using a voluntary oral consumption mouse model of prenatal cannabis exposure, mouse dams received whole cannabis oil (5 mg/kg THC) in peanut butter in their home cage daily from GD1-PD10. During adolescence (PD36-49), offspring were either tested for cocaine-seeking behaviour using conditioned place preference or electrophysiological recordings of VTA dopamine neuron firing and excitatory or inhibitory synaptic transmission. We find that our voluntary oral cannabis exposure model impacts VTA dopamine neurons of male, but not female adolescent offspring. Prenatal cannabis increased spontaneous firing rates, depolarized resting membrane potential, altered NMDA decay kinetics, and decreased inhibitory drive. Despite these changes in VTA dopamine neuronal activity, we observed no changes in cocaine-induced hyperlocomotion or cocaine-seeking behaviour. Our results confirm the sex-specific impact of prenatal cannabis exposure on VTA dopamine neurons despite differences in species, dose, and route of administration. However, we find that magnitude of effects and behavioural implications for reward-seeking may differ with a maternal voluntary oral cannabis delivery model.

The "B-side" of cannabis on the heart: Cardioprotective effects.

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In Argentina, Law No. 27,350 establishes that patients with medical prescriptions may acquire medicinal cannabis products duly registered in the Cannabis Program Registry (REPROCANN). However, the efficacy and safety of cannabis products remain subjects of ongoing discussion. Specifically, there have been no studies conducted on the effects of oral cannabis oil treatment in patients with cardiac diseases. The available data is limited and pertains mainly to animal or human studies involving THC or CBD alone or synthetic cannabinoids. Additionally, research on the cardiovascular adverse effects of cannabis has primarily focused on smokers. In such cases, cannabis has been associated with myocardial infarction, the precipitation of arrhythmias, stress cardiomyopathy, and arteritis. However, these effects may be attributed to toxic substances from cigarettes or the combination of cannabis with alcohol or other drugs. Furthermore, the pharmacokinetics and pharmacodynamics of oral administration differ from those when cannabis products are inhaled. On the other hand, research on the endocannabinoid system (ECS) has shown that it plays a significant role in controlling heart rate and blood pressure in both healthy individuals and patients with various pathological conditions. It affects both cardiac and arterial performance, either directly or indirectly, by altering cardiometabolic risk factors. Although components of the ECS are detectable in the heart, the impact of endocannabinoids on cardiac hypertrophy remains unclear. In recent years, we conducted the first-ever evaluation of the cardiovascular consequences of

chronic oral cannabis oil treatment in spontaneously hypertensive rats (SHR). Our research suggests that this treatment is effective in reducing left ventricular hypertrophy, improving cardiac pump function, and beneficially influence myocardial mitochondrial function and dynamics. Thus, activation of the ECS by orally administrated cannabis oil may have therapeutic benefits. It is crucial to continue investigating the cardiovascular effects, efficacy, and safety of medicinal cannabis to determine its potential therapeutic value.

Use of cannabis sativa for the development of dermal scaffolds and Bioinks for 3D Bioprinting.
Pablo Antezana *Instituto de Química y Metabolismo del Fármaco (IQUIMEFA, Facultad de Farmacia y Bioquímica, UBA, Buenos Aires, Argentina).*
Nowadays, biomaterials with therapeutic molecules play an active role in wound healing and infection prevention. 3D printing approach has emerged to overcome several of the major deficiencies of tissue engineering. Cannabis sativa oil is known for its anti-inflammatory and analgesic effects and antioxidant activity. The aim of this work was the development of hydrogels loaded with cannabis sativa oil using different approaches. We were able to develop collagen hydrogels loaded with silver nanoparticles and Cannabis sativa oil extract. The presence of the silver nanoparticles gives interesting feature to the biomaterial such as improved mechanical properties, resistance to collagenase degradation and long-lasting antimicrobial effect. The antioxidant activity of Cannabis sativa oil successfully improved the biocompatibility and also enhances the antimicrobial activity against Gram positive and Gram negative bacteria during seven days in liquid medium of the nanocomposite. On the other hand, we developed a bioink made with gelatin and alginate that was able to be printed using an extrusion 3D bioprinter. The 3D scaffolds obtained were able to be lyophilized and when the elastic modes were assessed they show hydrogels properties. The swelling capacity of the 3D scaffolds was almost 800%. In this sense, the scaffolds were loaded with Cannabis sativa oil extract and the presence of the extract provided antimicrobial against Gram positive and Gram negative bacteria in both liquid and solid medium, and antioxidant activity to the 3D scaffolds. Altogether, these results suggest that collagen hydrogels loaded with silver nanoparticles and Cannabis sativa oil extract are a promising alternative to common treatments of wound infections and wound healing. In addition, the new biocompatible material printed with 3D technology and with the addition of Cannabis sativa oil could become an attractive alternative to common treatments of soft-tissue infections and wound repair.

SIMPOSIUM VI • Oncology and Inflammation

Histaminergic System as a Promising Target for Breast Cancer Treatment.
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Breast cancer is an heterogeneous disease and the deadliest cancer for women worldwide, highlighting the need for new effective therapy. Hence, for several years we have been conducting research on one of the most interesting and complex biological pathways involved in cancer disease, the histaminergic system. Particularly, this work focuses on the histamine H4 receptor (H4R), discovered two decades ago, which has contributed to a better understanding of the histamine roles in health and disease, opening new perspectives in cancer research. Our findings show the H4R expression in different in vitro and in vivo experimental models, demonstrating its critical role in the regulation of tumor proliferation and progression in triple negative breast cancer (TNBC). Furthermore, the administration of histamine or H4R agonists diminished the tumor growth in both immunodeficient and immune-competent TNBC preclinical experimental models, exhibiting a critical role in the antitumor immunity in TNBC for the H4R. It is interesting to highlight that, at least in TNBC, the H4R-mediated effects on cell proliferation seem to be counterbalanced by the H3R. H3R antagonists display significantly enhanced antitumoral effects, increasing apoptosis and reducing cell proliferation of TNBC both in controlled in vitro settings and in vivo experiments. Furthermore, we have demonstrated the expression of H3R and H4R in TNBC human samples, which is clinically relevant since they could represent promising therapeutic targets for this

aggressive and difficult-to-treat type of breast cancer. Immunohistochemical analysis evidenced a higher H3R expression in tumor samples when compared with histologically normal peritumoral tissue, plus a high level of H3R and a reduced level of H4R were associated with poor overall survival in TNBC patients. This work will discuss our latest findings supporting the exploitation of the histamine receptors as molecular targets for TNBC.

Ugly pro-inflammatory molecular actors helping the liver?"

Daniel Francés. *Instituto de Fisiología Experimental (IFISE-CONICET), Universidad Nacional de Rosario, Rosario. Argentina.*

The term nonalcoholic fatty liver disease (NAFLD) identifies a broad spectrum of liver disorders strongly related to dysmetabolic diseases. It is characterized by the presence of hepatic steatosis associated with type 2 diabetes mellitus and overweight/obesity, regardless of alcohol intake. It could progress to more severe and irreversible non-alcoholic steatohepatitis (NASH), cirrhosis and, eventually, hepatocellular carcinoma (HCC). Insulin resistance (IR) is associated with a state of chronic low-grade inflammation that is assumed to contribute in a major way to its development. Cyclooxygenase (COX) is the enzyme that catalyzes the rate-limiting step in prostanoid biosynthesis and plays an important role in the onset of inflammation, mitogenic responses and cancer. COX-2 is the inducible COX isoenzyme. A variety of stimuli, such as inflammation, can rapidly induce COX-2. Our group has focused its attention in the relationship between COX-2 expression and liver pathology: hepatic insulin-resistance, NAFLD, NASH and HCC using different animal experimental models and human biopsies. In this regard, we showed that the expression of COX-2 in hepatocytes protects the liver against damage induced by hyperglycemia, IR and obesity by enhanced insulin sensitivity, thermogenesis and fatty acid oxidation. Also, we corroborated that COX-2 expression ameliorates NASH progression by reducing inflammation and oxidative stress, suggesting a protective role for COX-2 induction in NASH/NAFLD progression. Also, we studied the role of proinflammatory cytokines such as tumor necrosis factor alpha (TNFα) and interleukin-1 beta (IL-1β) after a high fat diet (HFD) feeding on liver inflammation in TNF Receptor 1 (TNFR1) knock-out mice. We showed that knocking out TNFR1 induces an enhanced IL-1β plasmatic release upon HFD feed with an increased inflammatory response. TNFR1 signaling disruption upon a HFD leads to an accelerated progression from simple steatosis to a more severe phenotype with several NASH features. These results shed new insights into a possible physiological protective mechanism elicited by classical pro-immflamatory actors in the progression of different hepatic diseases.

Translational Research in Obesity and Thyroid Cancer: The start of a journey at the Translational Research Unit of the Hospital Dr Arturo Oñativia.

Marta Toscano. *Unidad de Conocimiento Traslacional Hospitalaria Dr. A. Oñativia. Hospital Dr A. Oñativia. Salta, Argentina.*

Translational health research promotes the effective and efficient integration of basic research, clinical investigation, and implementation sciences with the overall aim of improving public health in the long term. Obesity is one of the world's most prevalent diseases and is associated with an increased risk of developing at least 13 different types of cancer, including thyroid carcinoma (TC). TC is the most common endocrine neoplasia worldwide, with an incidence that has tripled in the last four decades and continues to rise. Concurrently, the prevalence of overweight and obesity has also increased. Factors such as chronic low-grade inflammation, altered cytokine levels, insulin resistance, oxidative stress, and hormonal changes observed in obese patients contribute to the initiation and progression of TC. In this regard, it is important to highlight that the Hospital Dr. Arturo Oñativia (HAO) is a regional reference center for the treatment of patients with thyroid and metabolic disorders in the province of Salta. In this context, the Translational Research Unit aims to provide new knowledge and tools for better diagnosis, follow-up, and treatment of patients with thyroid cancer and metabolic disorders associated with obesity. In this dissertation, I would like to introduce you to this new Research Unit, the projects it has undertaken and the progress that has been made in TC research in recent years.

The mRNA-binding protein TTP acts as a tumor suppressor gene and regulator of the inflammatory response in head and neck carcinogénesis.

Ana R. Raimondi. *Instituto de Fisiología, Biología Molecular y Neurociencias (IFIBYNE)CONICET-Universidad de Buenos Aires, Buenos Aires, Argentina.*

Head and neck cancers, including cancers of the oral cavity, are highly prevalent worldwide, characterized by high morbidity and limited therapeutic options. RNA-binding proteins (RNA-BPs) that impact the stability of transcripts play a key role in disease pathogenesis. Tristetraprolin (TTP) is a RNA-BP that regulates proinflammatory mediators which promote tumorigenesis. The possible role of TTP in maintaining oral epithelium homeostasis and in oral carcinogenesis has not yet been established in vivo. We have developed TTP conditional knockout mice specific for oral cavity (TTP-KO). These mice developed mild dysplastic lesions in the tongue over time along with inflammatory infiltrate in the connective tissue (mast cells: MC and CD11b cells). Following quantification we found a significant increase of MC in TTP-KO tongues, which remained increased for the 8 months follow up. We found a significant increase of CD11b positive cells. Next, we analyzed the status of NFκB pathway, finding a significant increase in the expression of p65 and a significant reduction of IκBα, suggesting an activation of the pathway. We evaluated whether interfering with TNFα activity in TTP-KO mice could revert the described phenotype. The treatment of TTP-KO mice with etanercept led to changes in the inflammatory response. 2 months after treatment we could not observe morphological differences between control and KO, however the MC were significantly reduced in treated mice. These results indicate that TNFα activity plays a relevant role recruiting MC in TTP-KO tongues. Besides, to know if TTP contributes to tumor progression we studied the effects of both TTP ablation and K-ras activation in vivo. These compound mice exhibited a complete oral phenotype and presented a significant reduction in survival time. Next, we tested the role of TNFα in this model. Etanercept treatment significantly increased the survival of TTP-KO/K-ras mice, increasing by 40% the life expectancy of the treated vs the untreated. Interestingly, the inflammatory infiltrates in tongues treated with etanercept exhibited a significant reduction in MC compared to the control however without epithelial changes. Altogether, the results indicate that TTP expression would have a protective role against oral carcinogenesis by regulating signaling pathways associated with oral tumorigenesis, such as the NFκB pathway.

JOINT SYMPOSIUM HYPERTENSION COUNCIL OF THE ARGENTINEAN SOCIETY OF CARDIOLOGY AND THE ARGENTINEAN SOCIETY OF PHYSIOLOGY • Renal Physiology and Hypertension.

Impact of chloride anion in the development of arterial hypertension.

Nicolás Kouyoumdzian. *Instituto de Investigaciones cardiológicas Dr. Alberto Taquini, Universidad de Buenos Aires, Buenos Aires. Argentina.*

The present lecture will focus on the role of chloride anion in cardiovascular disease, with special emphasis in the development of hypertension and renal infammation. It is well established that an acute and chronic overload of sodium chloride increase blood pressure and exert proinfammatory and profibrotic effects on diferent target organs, but it is unknown how chloride may infuence these processes. Chloride anion is the predominant anion in the extracellular fuid and its intracellular concentration is dynamically regulated. As the queen of the electrolytes, it is of crucial importance to understand the physiological mechanisms that regulate the cellular handling of this anion including the diferent transporters and cellular chloride channels, which exert a variety of functions, such as regulation of cellular proliferation, diferentiation, migration, apoptosis, intracellular pH and cellular redox state. The different sources of chloride in the organism (such as dietary, serum and intracellular chloride) are affected in hypertension and have a strong impact on cardiovascular disease. Additionally, it must be taken into consideration the approach of potential strategies that may afect chloride handling, as well as its potential effect on cardiovascular system, including pharmacological blockade of chloride channels and non-pharmacological interventions such as replacing chloride by another anion.

Renal Autoregulation and Hypertension.

Cesar Romero. *Renal Division, Department of Medicine, Emory University, Atlanta, GA, USA.*

In the kidney, in addition to the myogenic response present in every capillary bed, two additional mechanisms exist to regulate renal blood flow based on the intratubular content of water and salt. These mechanisms are intrinsically regulated within the kidney through feedback communication (autoregulatory). One of these mechanisms, known as tubule-glomerular feedback (TGF), induces vasoconstriction in the afferent arteriole, resulting in a reduction in glomerular filtration when a high salt content is detected in the macula densa. The primary goal of TGF is to prevent a massive loss of salt and water. The second mechanism, which is the opposite of TGF, is called connecting tubule-glomerular feedback (CNTGF). CNTGF aims to increase the excretion of sodium by enhancing glomerular filtration when the body needs to eliminate excess salt. CNTGF is initiated in the connecting tubule when the epithelial sodium channel (ENaC) detects an excessive amount of sodium and finally inducing afferent arteriole vasodilation. These two feedback mechanisms interact with each other through a common effector, which is the afferent arteriole. Under normal conditions, the presence of these feedback mechanisms contributes to salt homeostasis. However, an imbalance in this equilibrium can lead to salt and water retention, ultimately resulting in an increase in blood pressure. In this presentation, we will delve into the effects of an imbalanced autoregulation system in the development of hypertension.

Role of Natriuretic Peptide in SRH and normotensive rats.

Carolina Canifi. *Instituto de la Química y Metabolismo del Fármaco (UBA-CONICET). Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Buenos Aires. Argentina. Consejo Argentino de HTA de la Sociedad Argentina de Cardiología.*

Natriuretic peptides, including atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), and C-type natriuretic peptide (CNP), play pivotal roles in the regulation of renal function. These peptides are primarily synthesized and released by cardiac myocytes and endothelial cells in response to various stimuli, such as atrial stretch, volume expansion, and sympathetic activation. Once released into the circulation, natriuretic peptides exert their effects on the kidney, leading to diuresis, natriuresis, and vasodilation. ANP and BNP primarily target the kidney to promote diuresis and natriuresis. They bind to natriuretic peptide receptors (NPR-A) located on the renal tubular cells, particularly in the distal nephron segments. Activation of NPR-A initiates a cascade of intracellular events that ultimately lead to increased sodium excretion and urine production. Specifically, ANP and BNP stimulate the production of cyclic guanosine monophosphate (cGMP), which in turn inhibits sodium reabsorption in the renal tubules. This mechanism aids in reducing blood volume and blood pressure, making natriuretic peptides crucial players in the regulation of cardiovascular homeostasis. CNP, on the other hand, differs in its target and function within the kidney. It predominantly influences the glomerular and vascular compartments, promoting vasodilation and enhancing glomerular filtration. CNP acts through NPR-B receptors, which are abundant in the renal vasculature and glomerulus. Activation of NPR-B by CNP results in increased cGMP levels, leading to relaxation of vascular smooth muscle and enhanced renal blood flow. This vasodilatory effect is essential for maintaining adequate renal perfusion, particularly in conditions of reduced cardiac output. Furthermore, natriuretic peptides exhibit renoprotective properties. By counteracting the actions of the renin-angiotensin-aldosterone system (RAAS), they contribute to the regulation of blood pressure and the prevention of sodium and water retention. This antagonistic relationship with RAAS highlights the importance of natriuretic peptides in renal and cardiovascular health. In summary, natriuretic peptides, including ANP, BNP, and CNP, are pivotal in regulating renal function and maintaining cardiovascular homeostasis. Understanding the mechanisms by which natriuretic peptides modulate renal physiology provides valuable insights into their therapeutic potential in managing conditions such as hypertension, heart failure, and renal dysfunction.

MAS receptor regulation in hypertension.

Mariela Gironacci. *Cátedra de Química biológica de la Facultad de Farmacia y Bioquimica, Universidad de Buenos Aires. Buenos Aires. Argentina.*

G protein-coupled receptors (GPCRs) are a remarkably multifaceted family of transmembrane proteins that exert a variety of physiological effects. They are targets for around one third of currently approved and clinical prescribed drugs and represent the largest and most structurally diverse family of transmembrane signaling proteins, with almost 1000 members identified in the human genome. Mas receptor is a GPCR that belongs to the renin-angiotensin system and mediates cardiovascular protective responses. GPCR trafficking is a fundamental process that regulates receptor function and the ultimate cellular responses. The association of signaling with the route of endocytosis and trafficking is specific to each receptor and determines the final net signaling output. Alterations in receptor trafficking have been associated with several diseases. But GPCRs function is also regulated by interaction with other receptors. GPCRs form and function as heterodimers/heteromers that exhibit distinct pharmacological, trafficking and functional properties as compared to their parent monomeric or homodimeric/homomeric GPCRs. In our lab we investigate how the functional properties of Mas receptor are influenced by its trafficking and by interaction with other GPCRs. GPCRs heteromerization not only brings forth a plethora of drug target combinations, but also gives an opportunity to carefully tweak the structure and function of one or more GPCRs involved in the complex, with the final goal of improving therapeutic strategies.

SCIENCE ROUNDTABLE DEBATE ABSTRACTS

SCIENCE ROUNDTABLE DEBATE • How to communicate science in the era of fake news

Moderator: Leonardo Lacoa. *Scientific Journalist, National University of La Matanza, Buenos Aires, Argentina*

Fighting fake news with a "multi-dose" of science

Soledad Gori. *Instituto de Química Biológica. Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Buenos Aires. Argentina.*

In 2016 and 2017, the terms "Post-truth" and "Fake News" were selected by the Oxford Dictionary as word of the year. The surge of social media, often referred to as the new fourth estate, played a significant role in the proliferation of false information. This impact quickly extended to the field of science, and its growth saw a sharp rise at the onset of the COVID-19 pandemic. In this presentation, we will explore the four sources of health misinformation, examine the public perception of science in our society, and delve into the influence of sustained negative anti-vaccine campaigns on social media. Furthermore, we will contemplate how we, as scientists, can engage in the battle against fake news. In response to this question, we will share some experiences related to the democratization of scientific knowledge on social media and in mass media. We will emphasize the creation of "Ciencia Anti Fake News," a team comprising scientists from CONICE, that has shed scientific light on a context as uncertain as the COVID-19 pandemic.

Understanding and debunking misinformation about science and health

Florencia Ballarino. *Chequeado ciencia, Buenos Aires. Argentina.*

We are witnessing an alarming global phenomenon that is becoming increasingly concerning: the emergence of science denial movements and the proliferation of misinformation about science and health that gain acceptance in certain sectors of society. This is not a new phenomenon, but its likelihood of spreading increased significantly, especially after the coronavirus pandemic. There are several strategies that have proven effective in tackling false narratives, such as fact-checking content on social media and debunking misinformation by exposing the deceptive tactics employed or the hidden motives of the misinformation authors. Media and information literacy (MIL) also play a key role.

Preprints' and misinterpretation of scientific studies as a source of misinformation

María González Dionis. *Newport, España.*

In recent years, hoaxes have arisen from non-specialised public access to preprints, (scientific studies that have not yet been peer-reviewed) and to complex studies that have already been reviewed, but are misinterpreted. This was already happening before the pandemic, but has been a major source of misinformation about covid vaccines. This type of misinformation generates sensationalist claims that appeal to emotion and are attributed a false authority, as they provide supposed scientific evidence. They use technical and complex language, as they are aimed at professionals in the field, and are easily misinterpreted by an outsider with a preconceived idea of what they want to find in the study

Communicating science to non-expert audiences

Ma. Soledad Casasola. *Directora de Comunicación de la Ciencia. Universidad Nacional de Rosario, Argentina.*

Public communication of science is one of the ways in which science and society are linked. Societies with democratic postulates require mechanisms of access to information, transparency and participation that enable their members to become creators and

protagonists of the collective and individual choices that will shape their future. From this perspective, access to information and knowledge, together with the possibility of critical and reflective discussion, enhances the possibility of citizen participation and decision-making. The participation of experts willing to share and debate in public communication and education spaces is necessary for them to be able to participate in the collective decisions that concern them, with certain knowledge and information, and at the same time to ask questions and express fears and hopes. Knowledge-producing institutions, through their communities of scientific experts, researchers and communicators, have an essential role to play in the task of communicating on issues on the scientific and technological agenda, not only what they themselves want to communicate and disseminate, but also what citizens need to know.

VII MEETING OF PHYSIOLOGY AND BIOLOGICAL PHYSICS TEACHERS 2023

Organized by the SAFIS Education Committee

CONFERENCES

BLOCK I
Non-traditional technologies in the teaching of physiology.

Artificial intelligence and immersive techniques as teaching tools.

José Campusano (*Asociación Docente de la Universidad de Buenos Aires, Argentina),* Lic. Dolores Tezanos *(Universidad del Museo Social Argentino)* y Pablo Wahnon *(Innovación, Tecnología y Salud en Forbes)*

BLOCK II
Emerging trends in university teaching: from Big Data to AI and evaluation

Fernando Salvatierra *(Departamento de Pedagogía, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Buenos Aires, Argentina)* y Marilina Lipsman *(Departamento de Pedagogía, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Buenos Aires, Argentina)*

25/10 Screen 1 • VII MEETING OF PHYSIOLOGY AND BIOLOGICAL PHYSICS TEACHERS (ENCUENTRO DOCENTE) 15.30 - 17.30 HS

1. FACILITADORES EN EL ACCESO A LA EDUCACIÓN MÉDICA (R16)

Said M[1], Ciancio C[1], Ferreiro N[1], Godoy Coto J[1], Gonano L[1], Surace F[1], Ibañez A[1], Quiroga A[1], Valverde CA[1], Velez Rueda O[1], Zavala M[1], Ennis I[1], Caldiz C[1].

[1]. Cátedra de Fisiología y Física Biológica, Facultad de Ciencias Médicas, Universidad Nacional de La Plata. La Plata, Argentina.

Introducción: La diversidad de perfiles y trayectorias educativas puede representar un desafío en la apropiación de contenidos en materias básicas de la carrera de Medicina. En las últimas décadas, han tomado fuerza programas de apoyo como herramientas para alumnos reincidentes que requieren un soporte adicional para superar las dificultades que han tenido en el pasado. Estos espacios, crean un ambiente de estudio inclusivo y amigable, donde los estudiantes se sienten seguros de expresar sus dificultades y pueden recibir ayuda sin temor a la crítica. A su vez, fomentan el trabajo en equipo y la colaboración entre compañeros, lo que resulta ser una excelente manera de promover el aprendizaje mutuo. Desarrollo: Con este fin, la Cátedra de Fisiología y Física Biológica implementa un Programa de Acompañamiento (PA) voluntario para las y los estudiantes recursantes que, desde el año 2020 cuenta con el aval de la UNLP. El PA consta de encuentros presenciales semanales, con grupos de 10-15 alumnos donde un docente actúa como guía. Las actividades consisten entre otras en: revisión de conocimientos previos; identificación de áreas de dificultad; direccionamiento hacia conceptos relevantes; búsqueda de ambientes colaborativos entre compañeros para compartir experiencias y estrategias de estudio; simulacros de evaluación oral. Al finalizar el primer semestre de 2023, se compararon los resultados de 2 grupos de alumnos: los alumnos recursantes que adhirieron al PA (n=150) y los alumnos recursantes (AR) que no adhirieron al mismo (n=267). El 80% del grupo PA aprobó el 1er. parcial de la materia, mientras que del grupo AR, solo aprobó el 61% ($p<0.05$). Además, el 27% del grupo PA obtuvo una calificación suficiente para aspirar al régimen de promoción respecto al 22% del grupo AR. Conclusión: Consideramos que el PA como estrategia de intervención sistemática para acompañar a estudiantes que recursan la asignatura, facilita su proceso cognitivo y fomenta la adquisición de habilidades que lo ayudarán en su profesión. Es muy alentador que los estudiantes que se mantienen en el PA logren, calificaciones que los habilitan para ingresar al régimen de promoción sin examen final.

2. DESARROLLO E IMPLEMENTACIÓN DE ACTIVIDADES PRACTICAS BASADAS EN DISPOSITIVOS ELECTRÓNICOS CON PLACAS DE DESARROLLO PROGRAMABLES Y SENSORES DE BAJO COSTO (R28)

Speranza ED[1,2], Ferreira AC[1,2]

[1.] Cátedra de Fisiología Animal, Facultad de Ciencias Naturales y Museo (FCNyM), Universidad Nacional de La Plata (UNLP) [2.] Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET)

El alto costo y la complejidad de los procesos de compra plantea un serio desafío para el uso de instrumentación electrónica en actividades prácticas, una herramienta clave para la enseñanza de la Fisiología. En el contexto del proceso de reemplazo de actividades practicas con sacrificio de animales, en la Cátedra de Fisiología Animal (FCNyM, UNLP) se crearon, en los últimos 2 años, equipos en base placas de desarrollo y sensores de bajo costo para su aplicación en experiencias no invasivas.

Enmarcadas en las unidades temáticas de Electrofisiología, Sistema Cardiovascular y Sistema Respiratorio se desarrollaron los siguientes trabajos prácticos: simulación de las propiedades eléctricas de la membrana con modelo electrónico (con fuente variable externa), monitoreo de actividad cardiorespiratoria en reposo y ejercicio con un medidor de pulsioximetria y ritmo cardíaco (con sensor MAX30100), análisis de la actividad cardíaca con un electrocardiógrafo (con amplificador AD8232), análisis de dinámica respiratoria en reposo y forzada con un espirómetro (con sensor MPX5010DP) y análisis del contenido de CO2 exhalado mediante la producción de ácido carbónico en agua (con sensor Yf-s201 y modulo 4502C). Los dispositivos se elaboraron con placas Arduino compatibles y Raspberry Pi Pico programadas en C y Python respectivamente. El costo de los dispositivos oscilo entre 10 (pulsioximetria) y 100 U$S (análisis de CO2), siendo 5-20 veces menor que el de equipos comerciales.

El desarrollo de estos equipos constituye una valiosa herramienta para la enseñanza de la Fisiología y ofreció las siguientes ventajas: 1. Elaboración con muy bajo costo de componentes y disponibilidad inmediata en el mercado nacional, 2. Hardware y software se desarrolló a medida de las necesidades de la actividad práctica, 3. Los alumnos pudieron comprender mejor los principios de funcionamiento, usualmente difíciles de apreciar en equipos comerciales, 4. Los alumnos pudieron interactuar con los circuitos sin riesgo de daño de instrumental costoso, 5. Los dispositivos permitieron familiarizarlos con el uso de placas de desarrollo programables, una tecnología creciente en la investigación científica.

TOPIC AREA: EDUCATION. Subarea: Experiences in the use of technologies in teaching Physiology

3. LUNGOVAX: UN SIMULADOR INTERACTIVO DEL MODELO DE COMPARTIMIENTO SIMPLE PARA LA MECÁNICA VENTILATORIA (R34)

Agustín Luna Simondi[1], Gonzalo Andrés Grau[1], Josue Francisco Laszeski[1]
Instituto Tecnológico de Buenos Aires (ITBA)

Introducción: LungoVax es un simulador de procesos respiratorios que implementa el modelo de mecánica ventilatoria de dos o tres elementos. Se trata de un software desarrollado en Python, con una amigable interfaz gráfica, creado como trabajo práctico final para la cátedra de Fisiología Cuantitativa del Instituto Tecnológico de Buenos Aires (ITBA). Desarrollo: El modelo de compartimiento simple de la mecánica ventilatoria plantea la siguiente ecuación movimiento:

donde ΔP es la presión transpulmonar, *PEEP* es la presión positiva después de la espiración, *V* es el volumen en los pulmones, *F* es el flujo de aire, *R* es la resistencia de las vías aéreas, y *C* es la compliancia pulmonar. Si se asumen como constantes los parámetros *PEEP, R, y C*, esta ecuación diferencial permite describir la evolución temporal del volumen, el flujo, y la presión transpulmonar conociendo tan solo una de estas variables. A través de un motor de simulación programado en Python, LungoVax implementa este modelo, permitiendo al usuario modificar los parámetros y elegir un estímulo inicial aplicado al flujo aéreo o a la presión. A partir de estas entradas, el programa genera automáticamente las curvas de ΔP(*t*), F(*t*), y V(*t*) a lo largo de un ciclo respiratorio, además de graficar el bucle flujo/volumen. En su versión interactiva, el usuario puede generar un estímulo pulsátil matemáticamente ideal (función rectangular), uno continuo de morfología suave (fisiológicamente realizable), o uno con ruido sinusoidal (eléctricamente realizable). También se puede optar entre modelos de dos y tres elementos, planteando la compliancia del sistema como una combinación de compliancia pulmonar y compliancia torácica.

$$\Delta P(t) = \frac{V(t)}{C} + F(t).R + PEEP$$

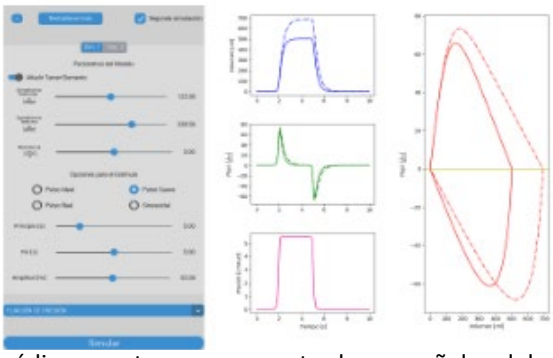

Figura 1: a través de una interfaz gráfica, el usuario puede ingresar los parámetros y estímulos para hasta dos simulaciones en simultáneo. La visualización de estos grupos de curvas se da en forma superpuesta sobre un mismo conjunto de ejes. Ver y descargar proyecto en GitHub

Conclusión: Este programa busca ser de uso educativo, brindando una herramienta didáctica que los docentes podrían sumar a sus clases y así mejorar la experiencia de sus alumnos. Se fomenta la curiosidad del usuario, ya que se permite variar de manera dinámica y totalmente libre los parámetros del sistema y de su entrada. Por otra parte, existe una potencialidad para aplicar este modelo a estudios clínicos, adaptando el código para tomar como entrada una señal real de un espirómetro o ventilador mecánico.

4. FISIOLOGÍA 2.0: POTENCIANDO EL APRENDIZAJE A TRAVÉS DE ESTRATEGIAS MEDIADAS POR TECNOLOGÍAS (R38)

Mestre Cordero, VE[1]; Fernández Pazos, MdlM[1]; Hermann, R[1]; Reznik, FJ[1]; Vatta, M[1]; Marina Prendes, MG[1].
Cátedra de Fisiología, Departamento de Ciencias Biológicas, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires. Buenos Aires, Argentina. vmestrecordero@docente.ffyb.uba.ar

Resumen: En la búsqueda constante de mejorar la calidad de la enseñanza y el proceso de aprendizaje, los recursos digitales han adquirido una relevancia creciente en los últimos años. En la asignatura Fisiología de las carreras de Farmacia y Bioquímica (FFyB-UBA), implementamos estrategias basadas en tecnología con el fin de estimular activamente la participación y el compromiso de los estudiantes, contribuyendo así a enriquecer su aprendizaje de una forma interesante, motivadora y atractiva. Con el fin de estimular la participación de los estudiantes durante las clases, empleamos preguntas de opción múltiple mediante plataformas en línea como Strawpoll, Socrative y Google Forms, utilizadas como disparadores o para evaluar la comprensión de los contenidos. Otra de las estrategias pedagógicas implementadas se basó en la utilización de la plataforma Spotify. A través de esta plataforma se planteó la creación de una lista de reproducción colaborativa, con la finalidad de que los alumnos pudieran seleccionar, agregar y/o, adicionalmente, elaborar su propio contenido que le permitiera evocar, afianzar e incluso integrar los temas trabajados en las clases. Además, como herramientas de autoevaluación y repaso, diseñamos cuestionarios de opción múltiple para cada unidad temática, los cuales informaban a los alumnos su desempeño y les permitían obtener devoluciones sobre la respuesta de cada pregunta. También desarrollamos Juegos de Escape en la plataforma Genial.ly, como espacio para la autoevaluación y aplicación de los conocimientos adquiridos. Como cierre de la cursada, organizamos un "Concurso de Memes", desafiando a los alumnos a aplicar los conceptos aprendidos elaborando de forma grupal un meme a partir de imágenes o recursos disponibles en la Web. Esta propuesta, realizada en la última clase, se convirtió en un espacio lúdico para consolidar los conocimientos adquiridos. En conclusión, las estrategias tecnológicas implementadas incrementaron

el interés de los alumnos por la materia, fomentando su participación activa en clase. Además, nos han permitido realizar un seguimiento continuo del progreso de los estudiantes a lo largo de la cursada, complementando así las evaluaciones formales.
TOPIC AREA: EDUCATION. Subarea: Experiences in the use of technologies in teaching Physiology

5. ARTIMOTION: A SIMPLE JOINT MODEL (R50)

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Introduction: We developed a visualization tool that provides representative graphs of angular displacement as a function of time for the myotatic reflex at the elbow joint. We used a theoretical foundation based on the Hill model and the neuromuscular reflex, modeled by the following block diagram.

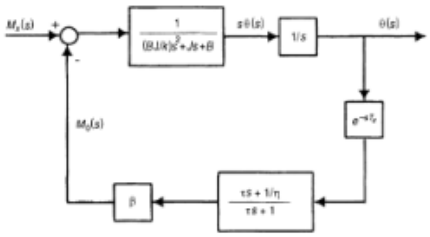

Figure 1: Block diagram.

We obtained the corresponding transfer function:

$$H(s) = \frac{\tau \cdot s + 1}{(\frac{\tau BJ}{K} - \frac{\beta\tau T_d^3}{6}) \cdot s^4 + (\frac{BJ}{K} + \tau J + \frac{\tau\beta T_d^2}{2} - \frac{\beta T_d^3}{6\eta}) \cdot s^3 + (J + \tau B - \beta\tau T_d + \frac{\beta T_d^2}{2\eta}) \cdot s^2 + (B + \beta\tau - \frac{T_d}{\eta}) \cdot s + \frac{\beta}{\eta}}$$

where,
B: Contractile component of the muscle.
J: Moment of inertia around the rotational axis of the elbow.
Td: Temporal delay of feedback.
k: Elastic component of the muscle.
β: Reflex gain.
Objectives: We aimed to design a user-friendly interface that displays the step response of the transfer function. Physiologically, the myotatic reflex response to a constant stimulus. Methods: We developed a Python program that allows the user to input physiological parameters to obtain a response from the neuromuscular reflex system. This response is observed in a graph that records the angular displacement of the joint over time after the stimulus is applied.

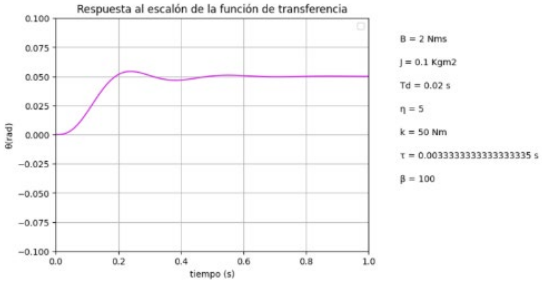

Figure 2: Graph obtained after running the program with standard physiological parameters.

Results: We examined the effects of varying parameters on the system. Lower values of B offered higher recorded amplitudes. When varying k we found greater oscillations for lower values. Changing the value of showed that for higher values the system became unstable. Conclusions: Changes in B show that higher oscillation amplitudes indicate greater limb elasticity and broader movements, corresponding to lower muscle tone (hypotonia). Conversely, lower amplitudes reflected higher muscle tone (hypertonia). Thinking of the elastic component as a spring with constant k helped us understand muscle stiffness and its resistance to deformation. Altering J gave us insights into joint displacement amplitude. Modifying affected system stability and myotatic reflex function, indicating the importance of reflex gain in recruiting muscle fibers and influencing system behavior.
TOPIC AREA: EDUCATION. Subarea: Experiences in the use of technologies in teaching Physiology
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6. UN PROYECTO DE EXTENSIÓN, UNA HERRAMIENTA PARA MEJORAR EL APRENDIZAJE Y ACERCAR EL CONOCIMIENTO A LA COMUNIDAD (R63)

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Introducción: Las enfermedades cardiovasculares son la principal causa de muerte en nuestro país. Para integrar conocimientos aprendidos en la unidad "sistema cardiovascular", diseñamos un proyecto de extensión involucrando a un equipo docente interdisciplinario. El objetivo fue concientizar a estudiantes de nivel primario y secundario de La Plata y alrededores, acerca de los beneficios de controlar la presión arterial (PA) y adoptar hábitos saludables. Desarrollo: articulamos el proyecto con la función docente incorporando como partícipes a estudiantes del profesorado de Biología que cursan Fisiología, para que logren integrar el contenido aprendido con una práctica real vinculada al contexto del aula. Estas acciones permiten la transferencia de conocimiento, la toma de decisiones respecto a las actividades a realizar y la puesta en práctica de saberes adquiridos en el ámbito académico. La propuesta contó con el aval de la UNLP, el Centro de Investigaciones Cardiovasculares y la Sociedad Argentina de Hipertensión (SAHA), y fue supervisada por docentes investigadores de la UNLP y una Lic. en Nutrición. Participaron más de 500 alumnos de 6 instituciones escolares, quienes fueron encuestados acerca de su actividad física, hábitos tabáquicos y de alimentación y conocimientos sobre hipertensión. Además, se capacitaron en toma autónoma de PA, realizaron un taller de nutrición, y una actividad lúdica con desafíos que ponían a prueba sus conocimientos sobre el tema. Se observó un bajo conocimiento sobre PA, alto índice de tabaquismo en nivel secundario, y baja actividad física en nivel primario. Los resultados estadísticos fueron presentados en congreso de SAHA 2023. Conclusión: respecto al impacto pedagógico, los estudiantes participantes califican la experiencia como muy enriquecedora para su trayectoria académica. En cuanto a su desempeño lograron posicionarse como agentes promotores de hábitos saludables, hacer un recorte del contenido a partir de una problemática en contexto y diseñar actividades específicas. Todo esto les permitió resignificar el contenido teórico adquirido, aproximarse a la investigación y adquirir competencias para su futuro quehacer profesional.

TOPIC AREA: EDUCATION. Subarea: Scientific research and the teaching-learning process of Physiology.

7. FORMACIÓN DE FORMADORES: CURSO DE CAPACITACIÓN DE AUXILIARES DOCENTES DE FISIOLOGÍA EN LA FACULTAD DE FARMACIA Y BIOQUÍMICA DE LA UNIVERSIDAD DE BUENOS AIRES (R67)

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La formación docente nos permite comprender, analizar y transformar nuestras prácticas en el aula, mejorando la enseñanza y calidad académica en la Universidad. El curso de formación y capacitación docente de los auxiliares de Fisiología de las carreras de Farmacia y Bioquímica de la Universidad de Buenos Aires tiene como finalidad profundizar los conocimientos teórico-prácticos, incorporar conocimientos didácticos de los aspirantes a incorporarse al equipo docente y promueve una formación continua de los docentes con más experiencia mejorando sus prácticas. Se brindan diferentes herramientas pedagógicas y didácticas que favorecen el proceso de enseñanza-aprendizaje, así como también los principios básicos para el trabajo con animales de laboratorio (investigación básica de Fisiología). Durante el primer cuatrimestre del curso, semanalmente dos de los aspirantes, mediante el juego de roles asumen el rol de docentes y el resto, de estudiantes. De esta manera, los aspirantes aprenden a organizar las actividades del trabajo práctico (TP) en cuanto a la distribución de los tiempos y el uso de recursos didácticos (pizarrón, simuladores online, carpetas con resultados de experimentos), así como también a promover la participación de los alumnos que deben resolver actividades bajo la supervisión del docente (modalidad taller). Finalizado el primer cuatrimestre, se realiza una evaluación de los aspirantes que consiste en la explicación de una actividad de TP que se le asigna en un tiempo determinado utilizando el pizarrón frente a sus pares. En el siguiente cuatrimestre, durante la cursada de Fisiología, los aspirantes participan del dictado de los TPs, siendo tutoreados por docentes más experimentados y los encargados de TP. Considerando el desempeño de los aspirantes durante todo el curso, se determinará su incorporación al plantel docente. Concluimos que esta propuesta de formación de docentes de Fisiología resulta muy útil para capacitar a los aspirantes de forma integral a través del intercambio con sus pares y superiores promoviendo el trabajo colaborativo que garantiza la transmisión efectiva del conocimiento.

TOPIC AREA: EDUCATION. Subarea: Scientific research and the teaching-learning process of Physiology.

8. CALAMAX: PRINCIPIOS DE LA NEUROCIENCIA, MODELO DE HODGKIN Y HUXLEY (R70)

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Introducción: Este trabajo tiene como objetivo ser utilizado como una herramienta educativa para el estudio de las variaciones del potencial de membrana de las neuronas. Calamax es una herramienta computacional que implementa el modelo de Hodgkin y Huxley para simular potenciales de acción neuronal. Se trabajó con corrientes de iones de Na+, K+ y Cl-, cada uno con sus respectivas conductancia y permeabilidad a través de la membrana; también se consideró el estado (activado/desactivado) de los canales n, m y h de la membrana. Este trabajo fue desarrollado como trabajo final para la cátedra de Fisiología Cuantitativa del Instituto Tecnológico de Buenos Aires (ITBA). Desarrollo: El programa ofrece un menú con seis opciones para simular distintas condiciones fisiológicas o patológicas y graficar el potencial de acción de una neurona. Las distintas opciones son: basal de referencia, bloqueantes, capacitancia, corriente, concentraciones y canalopatías. Para cada una de las situaciones mencionadas anteriormente, el programa graficará tanto el caso seleccionado como el caso basal de referencia, de forma que la diferencia entre ambos pueda observarse con mayor facilidad. Además, se mostrará un texto donde se mencionan los cambios y se explica la razón por la que ocurren, para que el usuario pueda entender mejor el efecto que tiene la modificación del parámetro seleccionado.

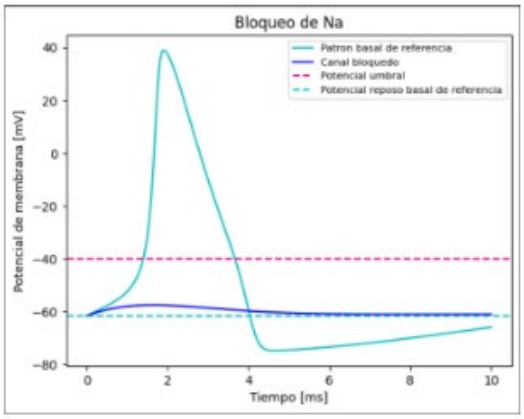

Figura: Patrón basal de referencia (en celeste) y canales de sodio bloqueados (en azul)

Conclusión: Como herramienta educativa, este trabajo busca presentar de forma sencilla las situaciones planteadas anteriormente, a expensas de la precisión. De esta manera, el usuario no tendrá control sobre los valores exactos de los distintos parámetros, sino que podrá elegir entre las diferentes opciones que se le presentan en pantalla. Esto permite que la herramienta pueda ser aprovechada por individuos cuyo conocimiento sobre el tema no les permite manipular de forma efectiva los distintos parámetros para obtener el resultado deseado, posiblemente por no conocer los valores normales o los detalles de la técnica.

TOPIC AREA: EDUCATION. Subarea: Experiences in the use of technologies in teaching Physiology

9. HEMODYNAMIX: INTERFAZ GRÁFICA INTERACTIVA PARA EL ESTUDIO DEL SISTEMA CIRCULATORIO SEGÚN EL MODELO DE WINDKESSEL (R85)

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Introducción: HemodynamiX es un programa que se basa en el Modelo de Windkessel de tres elementos para permitir hacer un estudio de las presiones del sistema circulatorio al cambiar valores de parámetros claves como el ritmo cardíaco, el volumen de eyección sistólica y el radio arterial. Se trata de un software desarrollado en Python, con una amigable interfaz gráfica, creado como trabajo práctico final para la cátedra de Fisiología Cuantitativa del Instituto Tecnológico de Buenos Aires (ITBA). Desarrollo: El fundamento del proyecto radica en el Modelo de Windkessel y la analogía entre el sistema circulatorio y un circuito eléctrico. La misma postula que el corazón se comporta como la fuente de tensión del circuito compuesto por los distintos sistemas del cuerpo, compuestos por el flujo sanguíneo, la distensibilidad de los vasos, y la inertancia. La tensión se representa con la presión vascular; la corriente eléctrica, con el flujo; y la carga, con el volumen. Se propone el ventrículo izquierdo (VI) como bomba de la circulación sistémica. Su presión se determina entendiendo el comportamiento ventricular como el de un capacitor: su volumen varía en base a las funciones de carga y descarga del capacitor, y su presión se define por su relación carga-tensión:

$$P(t) = E(t) \cdot (V_{full} - V(t))$$

En lo que respecta a la elastancia, se modela su variación en el tiempo mediante un Modelo de Hill doble. Luego, la presión aórtica se modela en base al comportamiento rectificador de la aorta, que transforma la presión pulsátil proveniente del VI a una semicontinua. Se plantea que mientras la válvula aórtica está abierta la presión aórtica y la ventricular son idénticas. Cuando la válvula se cierra, la distensibilidad aórtica provoca que la excursión de la presión arterial simule la de la tensión provista por un capacitor en descarga. Las presiones arteriales se calculan según el Modelo de Windkessel de tres elementos: sabiendo que cada vaso sanguíneo se comporta como un sistema RLC, se calcula la función de transferencia, se anti transforma y se obtiene la convolución entre la presión aórtica y la respuesta al impulso del sistema. Como resultado, a través de un motor de simulación

programado en Python, se obtienen gráficas que muestran las morfologías de las distintas presiones sistémicas, sus desfasajes y las variaciones que se presentan cuando el usuario modifica parámetros clave, como se observa en la siguiente figura.

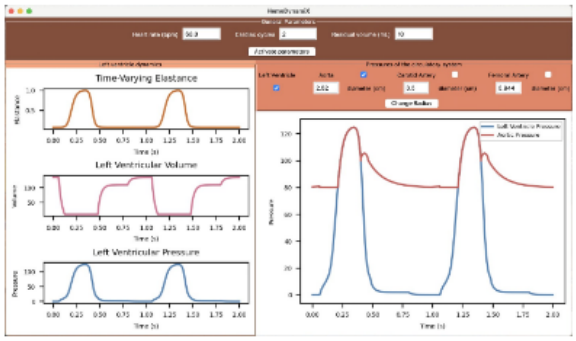

Figura 1: Prototipo de la interfaz gráfica diseñada. A la izquierda, las dinámicas ventriculares. A la derecha, la presión ventricular izquierda y la aórtica, superpuestas.

Conclusión: Este programa busca ser de uso educativo, brindando una herramienta didáctica que los docentes podrían sumar a sus clases y así mejorar la experiencia de sus alumnos. Se fomenta la curiosidad del usuario, ya que se permite variar de manera dinámica los parámetros del sistema. Por otra parte, existe una potencialidad para profundizar el uso de este modelo y visualizar la morfología de las presiones vasculares a nivel capilar y venoso.

TOPIC AREA: EDUCATION. Subarea: Experiences in the use of technologies in teaching Physiology

10. UTILIZAR ESTRATEGIAS DIDÁCTICAS INNOVADORAS PARA INCENTIVAR A LOS ALUMNOS AL ESTUDIO (R93)

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Departamento de Química y Bioquímica. Fac. Cs. Exactas y Naturales. UNMDP

La clase invertida, también conocida o Flipped Classroom, es una metodología de aprendizaje que se propuso al detectar un gran porcentaje de ausentismo en sus clases. El aula invertida es una metodología educativa disruptiva que cuestiona los métodos tradicionales al intercambiar los roles entre docentes y alumnos. Por un lado, el docente se convierte en acompañante de los alumnos. Para ello, se le demanda que proponga temas de debate, reformule y resuelva dudas o busque nuevos modos de aprendizaje atractivos y no tan tradicionales. Por el otro, los alumnos son los que dirigen la formación en la clase invertida mediante preguntas y la generación de debates o propuestas de aprendizaje. De este modo, se pone el foco en los intereses más habituales del alumnado, lo que fomenta la motivación, la participación y la cooperación entre ellos para resolver dudas comunes. En definitiva, el objetivo de esta metodología educativa es fomentar la participación directa de los alumnos en el transcurso de las clases, así como salir fuera del aula para experimentar con conocimientos más prácticos.

Esta metodología la utilizamos en un curso optativo con una carga horaria de 50 horas y 12 alumnos. Se emplearon diversas herramientas didácticas tales como: Aprendizaje basado en problemas. a) búsqueda, selección y lectura de bibliografía científica actualizada en idioma inglés, b) interpretación y presentación de un determinado trabajo y casos, c) diseño de PowerPoint como material de apoyo. d) autoevaluación y coevaluación en cada una de las actividades desarrolladas. Se aplicó una encuesta anónima al final de la cursada, con la finalidad de evaluar el grado de empatía con las estrategias didácticas utilizadas. Se observó una crítica general, preferían que el docente explique los temas y luego se evalúen esos contenidos. Consideramos que los alumnos están condicionados a la enseñanza centrada en el profesor y al cambiar las estrategias didácticas si bien les cuesta acostumbrarse, fomentamos el aprendizaje autónomo y la capacidad de autoevaluación de los alumnos. Obteniendo finalmente, como resultado un elevado interés por parte de los alumnos.

TOPIC AREA: EDUCATION. Subarea: Scientific research and the teaching-learning process of Physiology.

11. ENSEÑANZA DE HERRAMIENTAS DE LA FARMACIA OFICINAL EN LA MATERIA DE FISIOLOGÍA COMO INTRODUCCIÓN A LAS INCUMBENCIAS PROFESIONALES DEL FARMACÉUTICO (R92)

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Introducción: La biotecnología y la inteligencia artificial impulsan la producción de fármacos innovadores y la investigación de nuevos blancos terapéuticos en base a datos en búsqueda de una mayor personalización de los tratamientos. Los farmacéuticos desempeñan un rol esencial como expertos en medicamentos destinados a la curación, alivio, prevención y diagnóstico. Su capacitación les permite no solo dispensar medicamentos, sino también asesorar sobre su uso adecuado, garantizar la seguridad del paciente y optimizar la farmacoterapia. En este contexto, la materia de Fisiología, impartida en el quinto cuatrimestre de la carrera, se erige como un pilar fundamental para conectar la formación farmacéutica con la promoción del uso racional del fármaco. Desarrollo: Esta iniciativa surge del interés manifestado por los estudiantes en comprender su futuro rol y responsabilidades desde el inicio de su formación universitaria y aborda cómo la Fisiología, que se centra en el funcionamiento normal de los organismos vivos, es crucial en la resolución de las consultas en la farmacia. Los pacientes comparten sus

síntomas y dolencias, y los farmacéuticos pueden utilizar su conocimiento fisiológico para identificar el medicamento de venta libre adecuado, interpretar prescripciones en función de la patología o derivar al paciente a un médico. Es imperativo comprender que la base de esta capacidad radica en la fisiología como ciencia integradora, desde la comprensión profunda de la función de los órganos y sistemas en situaciones normales y patológicas, esencial para que los farmacéuticos puedan colaborar eficazmente en la educación del paciente sobre su diagnóstico y fomentar su adherencia al tratamiento.
Conclusión: El enfoque pedagógico propuesto integra a la Fisiología con la práctica diaria de la farmacia oficinal. Esto proporciona a los estudiantes, herramientas para abordar de manera efectiva las demandas de los pacientes y garantizar un uso responsable de los medicamentos de venta libre. Además, se abre la puerta a la posibilidad de extender esta integración del conocimiento a otras materias y a diversas responsabilidades profesionales dentro del campo farmacéutico.

25/10 Screen 2 • I MEETING OF UNDERGRADUATE STUDENT INVESTIGATORS (ENCUENTRO DE ESTUDIANTES DE GRADO)
15.30 - 17.30 HS

12. DIFERENTIAL EFFECT OF CHRONIC STRESS EXPOSURE ON DIABETES INCIDENCE ON MALE AND FEMALE NOD/ShiLtJ MICE. PARTICIPATION OF GUT MICROBIOTA AND INTESTINAL PERMEABILITY (R25)

Wydra L[1], Gonzalez Murano MR[1], Bianchi MS[2], Wald MR[1], Genaro AM[1], Rubinstein MR[1]
[1] Instituto de investigaciones Biomédicas (UCA-CONICET). [2]Instituto de Biología y Medicina Experimental (CONICET).

Introduction: Type 1 diabetes (T1D) is an autoimmune disease characterized by impaired insulin secretion. It has been recognized the contribution of psychosocial factors (including chronic stress exposure) in T1D in its development. Gut microbiota is the group of microorganisms (commensal, symbiotic and pathogenic) that we find in our gut. It participates in multiple functions and an association between unbalanced microbiota and several diseases, including diabetes, has been reported. NOD/ShiLtJ mice are a model for autoimmune T1D, where the animals spontaneously became diabetic. There is a marked sexual dimorphism: a higher T1D diabetes incidence in females than in males. Objectives: the aim of the present work was to study the effect of chronic stress in diabetes development and to characterize the microbiota alterations in NOD/ShiLtJ mice in females and males. Methods: Two-month old NOD/ShiLtJ mice were subject to chronic stress (CS) by the application of aleatory and unpredictable stressors. Results: females NOD/ShiLtJ mice exposed to CS showed an increase in diabetes incidence ($p<0.05$) while CS did not have any effect on males. To determine microbiota alterations, fecal samples were collected and genomic DNA was extracted in female mice. 16s total bacteria, 16s Bacteroidetes and 16s Firmicutes (the most abundant components of the microbiota) were measured by qPCR using specific primers. At 30 days of CS, no changes were detected in 16s total bacteria and 16s Bacteroidetes but an increase in 16s Firmicutes in mice not exposed to CS that later became diabetic was found compared to those who did not ($p<0.05$). At 90 days of CS, only a decrease in 16s Bacteroidetes was observed in animals exposed to CS regardless of later diabetic development ($p<0.05$). In female mice exposed to CS that became diabetic, an increase in intestinal permeability was found ($p<0.05$). Conclusion: These results show that CS has a role in type 1 diabetes development probably through altering intestinal permeability and CS alters gut microbial composition.
TOPIC AREA: ENDOCRINOLOGY – METABOLISM – REPRODUCTION

13. EFFECT OF UROLITHINS ON ENDOMETRIOSIS AND ANGIOGENESIS IN VIVO (R73)

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Introduction: Endometriosis is a chronic disease defined by the growth of endometrial tissue outside the uterine cavity. Current therapeutic options are limited, often failing to alleviate symptoms and having several side effects. Urolithins are natural compounds generated by the human intestinal microbiota from ellagitannins and ellagic acid. In previous studies, we demonstrated that Urolithin A (UA) and B (UB) have anti-proliferative, anti-migratory, anti-invasive, and pro-apoptotic effects, and downregulate the expression of angiogenesis-promoting genes in endometriosis in-vitro. Objectives: Since angiogenesis is one of the major processes involved in endometriotic lesion establishment and progression, the aim of the present work was to evaluate the effect of UA and UB on angiogenesis in endometriosis in-vivo. Methods: Endometriosis was surgically induced in female BALB/c mice. Fifteen days post-surgery mice were treated with a daily i.p. injection of UA, UB, or PBS (Control, C). After 28 days, animals were sacrificed, peritoneal fluid (PF) was collected, and endometriotic-like lesions were counted, measured,

removed, and fixed. Vascularized area was assessed by CD31 immunohistochemistry. The in-vivo angiogenic potential of PF from UA, UB, and C mice, or UA and UB alone, was evaluated using the quail chorioallantoic membrane (CAM) bioassay. Results: UA completely prevented the development of endometriotic-like lesions ($p<0.001$), while UB significantly reduced implants size ($p<0.05$). Preliminary results indicate that urolithins have anti-angiogenic effects on endometriosis. UB reduced the percentage of vascularized area in lesions. Besides PF from UA and UB-treated mice affected angiogenesis by decreasing the number of blood vessel branch points compared to PF from C. However, UA directly assayed on CAM increased the number of blood vessel branch points ($p<0.05$), while UB had no effect. Conclusion: UA and UB proved effective as treatments for endometriosis in our mouse model. Furthermore, UA completely prevented the development of the disease. Nevertheless, further studies are required to fully assess urolithins anti-angiogenic potential in endometriosis.
TOPIC AREA: ENDOCRINOLOGY – METABOLISM – REPRODUCTION

14. INTERPLAY BETWEEN CORTICOSTERONE AND BDNF SIGNALING IN ANIMALS FED WITH HIGH FAT DIET AND EXPOSED TO CHRONIC STRESS (R26)

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Introduction: Adaptation to stress requires adjustments through interrelations between the hypothalamus–pituitary–adrenal axis (HPA), sympathetic efferent pathways and chemical messengers (like hormones and neurotrophins). Prolonged stress can produce severe consequences that affect the hippocampus, leading to behavioral alterations. On the other hand, stress exposure and poor eating habits are interconnected to each other, leading to an increased in obesity and becoming a serious health problem. We have previously shown that a high fat diet (HFD) increased body weight, but chronic stress exposure (CS) induced a decrease in body weight. Objectives: the aim of the present work was to study the effect of HFD and CS exposure on cognitive performance and to investigate the participation of the HPA axis, sympathetic nervous system and neurotrophins in hippocampus in C57Bl/6J male mice. Methods: C57Bl/6J male mice were fed a standard diet (SD) or a HFD. After eight weeks, some animals were also exposed to CS by the application of aleatory and unpredictable stressors for 20 more weeks. This resulted in 4 groups of animals: SD, SD + CS, HFD and HFD + CS. To study the cognitive performance, the spontaneous alternation in the Y-maze task and Barnes maze task were performed. Results: In the Y-maze task CS, HFD and HFD + CS groups showed a decreased the spontaneous alternation. In Barnes maze task, the results showed that the percentage of time spent in the target quadrant was similar for all experimental groups, but the latency to find the target hole was increased in HFD +CS group. Plasma corticosterone levels showed an increase in HFD group. In contrast, in HFD + CS group this increase was impaired. Glucocorticoid receptor expression in hippocampus was increased in HFD and HFD + CS and similar results were found for beta2-adrenergic receptor. A decreased in hippocampal BDNF expression was found in HFD and HFD + CS groups measured by qPCR. Conclusion: These results show an interplay between corticosterone, BDNF and beta2-adrenergic signaling in animals fed with HFD and HFD + CMS. More studies are necessary to elucidate the mechanism involved.
TOPIC AREA: IMMUNOLOGY – NEUROIMMUNOENDOCRINOLOGY

15. STUDY OF THE EFFECTS OF LEUKOTRIENE A4 HYDROLASE INHIBITION ON THE HEPATOCELLULAR CARCINOMA CELL LINE HUH7 (R13)

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Introduction: Leukotriene B4 (LTB4) is a lipid mediator synthesized from the hydrolysis of the precursor LTA4 by the action of the enzyme leukotriene A4 hydrolase (LTA4H). Our group demonstrated that the LTB4 signaling pathway plays an important role in controlled proliferation during liver regeneration after partial hepatectomy; thus, it is also likely for it to play a role in uncontrolled proliferation occurring in liver cancer. Indeed, an overproduction of LTB4 has been shown in other types of human cancers, leading to proliferation of cancer cells. Objectives: To analyze the effects on specific behavior associated with malignancy of tumor liver cancer cells, upon inhibition of the enzyme LTA4H. Methods: Human hepatocellular carcinoma (HCC) Huh7 cells were treated with the LTA4H inhibitors bestatin (BST) and SC-57461A (SC) or left untreated (control, C). Studies in 2D cultures (C, BST and SC groups): cell viability, migration, clonogenicity, invasion, flow cytometry and the expression of key proteins for proliferation and apoptosis. Studies in 3D cultures (C and SC groups): viability, proliferation and migration.

Results: After performing dose-response curves, the established working concentrations were: BST 200 µM, SC 265 µM (2D), and SC 600 µM (3D). Viability: BST -26,4%**; SC -25,0%*. Migration: BST -25%**; SC -25%**. Clonogenicity: BST -28%*; SC -34%**. Invasion: BST -25,7%*; SC -28,9%**. Proliferation markers: PCNA: BST -48% *; SC +95%; Cyclin D1: BST -30%*; SC -66%*, (results are expressed as %C; *p<0,01; **p<0,001). Apoptosis was not the mechanism by which cell viability was reduced. Studies in 3D cultures showed that SC treatment reduced cell viability, proliferation and migration in a dose-dependent manner. Conclusion: Inhibition of LTA4H by using BST or SC affects the specific behavior of Huh7 cells associated with malignancy. 3D cultures confirmed that LTA4H inhibition has an antiproliferative effect on Huh7 cells. Although preliminary, these studies support the participation of the LTB4 pathway in HCC. These results are relevant since they demonstrate the importance of the inhibition of this pathway as a strategy for the treatment of liver cancer.
TOPIC AREA: ONCOLOGY - INFLAMMATION

16. OSTEOPONTIN REGULATES AQUAPORIN-4 EXPRESSION AFFECTING CELL VOLUME REGULATION AND MIGRATION OF RETINAL MÜLLER CELLS (R55)

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Introduction: During the intense neuronal activity in the retina, Müller cells are exposed to a hypotonic environment leading to cell swelling followed by a regulatory volume decrease (RVD) response, which depends on Aquaporin-4 (AQP4) and the calcium channel TRPV4. It was recently reported that Osteopontin (OPN), a component of the extracellular matrix (ECM), may modulate the RVD of Müller cells. OPN also participates in cell survival and migration, as well as in neuronal regeneration after injury. Since migration and differentiation of Müller cells into different retinal cell types are neuroprotective and volume dependent, OPN may participate in these processes. Moreover, in astrocytes, AQP4 membrane localization and cell morphology depend on AQP4 interaction with ECM components, which also modulates cell migration. Objective: The aim of this work was to study the putative crosstalk of OPN with AQP4 and/or TRPV4 in the regulation of cell volume, morphology and migration of Müller cells. Methods: Cell volume and osmotic water permeability (Pf) during an osmotic swelling were measured by fluorescent videomicroscopy. AQP4 expression was evaluated by immunocitochemistry and Western Blot. Cell migration was evaluated by wound healing assay and cell shape, ramification index (Sholl Analysis) and F-actin fibers organization by immunocitochemistry. Results: OPN induced a 50% reduction of Pf and RVD by downregulation of AQP4 expression, which was prevented by TRPV4 inhibition. OPN also induced significant changes in cell shape, with an increase in nucleus to cytoplasm ratio and ramification index. No changes were observed in the amount of F-actin or its degree of organization after OPN treatment. However, OPN reduced migration of Müller cells by 20%, being this effect also dependent on TRPV4. Conclusion: We propose that OPN modulates water permeability, cell volume regulation and migration of Müller cells by TRPV4 activation and AQP4 downregulation. The increase in cytoplasmic projections of Müller cells induced by OPN are independent of changes in F-actin cytoskeleton, but may be related to AQP4 downregulation, as it may be a strategy to increase membrane water flux.
TOPIC AREA: CELLULAR PHYSIOLOGY AND SIGNAL TRANSDUCTION

17. BOOSTING SERCA2A FUNCTION WITH ISTAROXIME TO PREVENT PACING-INDUCED CALCIUM ALTERNANS IN CARDIOMYOCYTES FROM HYPERTENSIVE RATS (R14)

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Introduction: Istaroxime is an inotropic-lusitropic steroid capable of both inhibiting Na+/K+ ATPase (NKA) and potentiating SERCA function. However, low concentrations in the range of 100-500 nM can target SERCA without affecting NKA and inotropy1. Given that Ca2+ alternans rely on the kinetics of Ca2+ cycling and that Spontaneously Hypertensive Rats (SHR) are prone to developing Ca2+ alternans with age2, we propose the following objective. Objective: To test if Istaroxime 400 nM can reduce the propensity and magnitude of Ca2+ alternans in cardiomyocytes from SHR. Methods: Ventricular cardiomyocytes were obtained from 6 to 7 months old from SHR, after collagenase-based heart digestion. These cells were loaded with Fluo-4 for 10 minutes and then separated in control and istaroxime groups to allow 30 minutes of incubation with 400 nM istaroxime. Cytosolic Ca2+ was recorded in a confocal microscope by line scanning across the cell length and the following pacing protocol was performed: 1, 4, 5 and 6 Hz, 1 minute at each frequency. Data was analyzed with T-test and 2-way ANOVA as appropriate, P≤0.05 was considered significant. Results: The amplitude of Ca2+ transients elicited at basal pacing (1 Hz) was compared between groups and we did not observe a significant difference, supporting that incubation with istaroxime 400 nM does not

affect the inotropic state of the cells. To characterize the occurrence of Ca2+ alternans we calculated both alternans threshold (minimum pacing frequency at which the consecutive Ca2+ transients exhibit a difference in amplitude higher than 10%) and alternans ratio at each pacing frequency (AR= the substraction of Ca2+ transient amplitude between consecutive transients divided by the amplitude of the highest of the pair). The group of cardiomyocytes treated with istaroxime presented a significantly higher alternans threshold (5,5±0,11 Istaroxime vs 4,4±0,13 Control) and a lower AR (significantly lower at 4 and 5 Hz) compared to untreated cells (22-24 cells from 6 hearts per group). Conclusion: The use of istaroxime at a concentration that is reported to target SERCA reduces alternans propensity and magnitude in cardiomyocytes from SHR.

Torre E et al. SERCA2a stimulation by istaroxime improves intracellular Ca2+ handling and diastolic dysfunction in a model of diabetic cardiomyopathy. Cardiovasc Res. 2022. doi: 10.1093/cvr/cvab123.

Mariángelo JIE, Di Marzio GD, Gonano LA, Said M, Mundiña-Weilenmann C. Prolonged Ca2+ release refractoriness and T-tubule disruption as determinants of increased propensity to cardiac alternans in hypertensive heart disease. Acta Physiol (Oxf). 2023 Jun;238(2): e13969. doi: 10.1111/apha.13969. Epub 2023 Apr 3. PMID: 36971744.

TOPIC AREA: CARDIOVASCULAR PHYSIOLOGY - HYPERTENSION

18. PROTECTIVE ROLE OF OUABAIN AGAINST PREMATURE CARDIAC ALTERNANS IN SPONTANEOUSLY HYPERTENSIVE RATS (SHR) (R37)

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Introduction: It is well established that cardiac glycosides, such as ouabain (OUA), inhibit the Na+-K+ pump leading to an increase in [Na+]i, which via Na+/ Ca2+ exchanger (NCX1), reduce the net Ca2+ efflux rate with the consequent increase in sarcoplasmic reticulum (SR) Ca2+ load and contractility. However, excessive pump inhibition leads to Ca2+ overload and toxic effects. Cardiac alternans, recognized as an arrhythmogenic substrate, is a beat-to-beat oscillation in action potential duration, strength of contraction or amplitude of Ca+2 transient at constant heart rate. Experimental evidence suggests that alternans is ultimately caused by intracellular Ca+2 mishandling. We have demonstrated that the hypertrophic myocardium of spontaneously hypertensive rats (SHR) is more prone to alternans. Objectives. To examine if non-toxic OUA concentrations delay the appearance of alternans development in the myocardium of SHR. Methods. Frequency-induced Ca2+ alternans was measured by epifluorescence microscopy in myocytes isolated from 6 mo-old SHR hearts loaded with Fura-2, in the absence or presence of 10µM OUA. Sarcoplasmic reticulum (SR) Ca2+ load was assessed by a caffeine pulse. Results. OUA treatment diminished the propensity to Ca2+ alternans (frequency threshold 4.50±0.18 and 3.75±0.21Hz for SHR+OUA and SHR myocytes respectively, n=11-12 cells/4 hearts, $p<0.05$). As expected, OUA increased SR Ca+2 content (SHR+OUA: 0.717±0.042 vs. SHR: 0.592±0.034a.u.) and prolonged the time constant (Tau) of the caffeine-induced Ca+2 transient decay (SHR+OUA: 4631±858 vs. SHR: 3144±276 msec, n=12-21 cells/2-7 hearts, $p<0.05$), indicative of the inhibition of NCX1-mediated Ca+2 efflux. Conclusion. Our data suggest that OUA, by enhancing SR Ca+2 content, is able to decrease the susceptibility to cardiac alternans in SHR myocytes. These results demonstrate, new beneficial effects of OUA on the susceptibility to cardiac alternans.

TOPIC AREA: CARDIOVASCULAR PHYSIOLOGY-HYPERTENSION

19. THE LACK OF GALECTIN-3 REDUCES ACUTE CARDIAC TOXICITY AND DYSFUNCTION BY INCREASING OXIDATIVE STRESS AND FIBROSIS IN DOXORUBICIN-TREATED MICE (R62)

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Background: Doxorubicin (DOX) leads to cardiovascular toxicity through direct cardiomyocyte injury and inflammation. We aimed to study the role of Galectin-3 (Gal-3), a β-galactosidase binding lectin associated with inflammation and fibrosis in DOX-induced acute cardiotoxicity in mice. Methods: Male C57 and Gal-3 knockout (KO) mice were given a single dose of DOX (15 mg/kg, i.p) or placebo. Serum creatine phosphokinase (CPK) and lactate dehydrogenase (LDH) as well as TBARS were measured at 3 days to assess cardiac injury. Cardiac function was studied by catheterization at 7 days. Myocardial fibrosis was quantified in picrosirius red stained slices. Results: All variables were similar in both genotypes treated with saline. Expression of cardiac Gal-3 was significantly increased in treated C57 mice. CPK was 3169±573 and 573±54 UI/L in C57+DOX and Gal-3 KO+DOX mice

respectively (p=0.03). TBARS (mM/mg of protein) was increased from 14±0.8 in C57 saline to 31±6 in C57+DOX (P<0,05) and this increase was attenuated in Gal-3+DOX mice (12±1.8; p<0.05 vs C57+DOX). LV-systolic pressure was significantly reduced in C57+DOX to 61±10 mmHg (p<0.05 vs C57 saline) and that reduction was attenuated in Gal-3KO+DOX (96±14 mmHg, p=0.02 vs C57+DOX; n=5/group). In addition, diastolic dysfunction was prevented in Gal-3KO+DOX since the LV-end diastolic pressure was reduced from 9±2 mmHg in C57+DOX to 2±1 mmHg Gal3KO+DOX (p=0.02). At histology, myocardial fibrosis was also reduced in Gal-3KO+DOX mice (3.5±0.5 % vs 4.6±0.2% in C57+DOX, p=0.03). Conclusion: genetic deletion of Gal-3 prevented cardiac damage and dysfunction associated with reduced cardiac oxidative stress. Understanding the contribution of Gal-3 to doxorubicin-induced cardiac toxicity reinforces its potential use as a therapeutic target in patients with several cancer types.
TOPIC AREA: CARDIOVASCULAR PHYSIOLOGY-HYPERTENSION

20. INSULIN RESISTANCE AT THE HEART OF RECENTLY ESTABLISHED MENOPAUSE: PARTIAL ROLE OF ESTROGENS (R77)

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Introduction: According to previous reports, both from our laboratory and from others, male rats and mice, after a fructose-rich diet (FRD) showed insulin-resistance (IR) and cardiac alterations, such as hypertrophy, systolic dysfunction, arrhythmias, apoptosis and ultrastructural remodeling. Moreover, canonical knowledge agrees to accept the protective effect of estrogens in the reproductive period of females. However, hormone therapy replacement after menopause, is nowadays, controversial, twisting the past concept. Objectives: Identify the beneficial effects, if there are some, of estrogens in IR context on heart function. Methods: Female Wistar rats and C57 mice were fed for 3-4 weeks with control diet (CD) or with a high-fructose diet (FRD), 10% fructose in drinking water. After treatment, the animals were metabolically characterized (weight, intraperitoneal glucose tolerance test (IpGTT), lipid profile) and cardiac function (echocardiography) and morphology were studied. Another group of female C57 mice underwent a total ovariectomy (OVX) or a placebo operation (SHAM), and after a month an echocardiography and an IpGTT were performed. Then, the mice were subjected to the same FRD as the first group. Subsequently, echocardiography and IpGTT were performed again and they were sacrificed in order to study them morphometry. Intracellular Ca2+ in enzymatically isolated myocytes loaded with the fluorophore Fura2-AM, were measured in the mice groups. The data were compared by t-test, and the p<0.05 was considered a significant difference. Results: FRD did not induce insulin resistance, fat gain, or echocardiographic abnormalities in females at reproductive age: In the presence of ovaries, the animals fed with FRD or CD did not present either difference in IR indexes (glycemia/triglycerides index, or the area under the curve after an IpGTT), morphometric parameters or in echocardiographic structural and functional parameters. Estrogens are involved in insulin resistance but not in cardiac alterations: Thinking about the protective effects of estrogens, we removed the ovaries and fed them with FRD or CD. OVX-FRD mice showed a significant difference in the area under the curve after an IpGTT with respect to SHAM-FRD, indicating an IR condition. However, the morphometric and echocardiographic parameters did not reveal any difference between the groups. Estrogens are not involved in Isoproterenol and CaiT-Frequency response: OVX-FRD isolated myocytes showed the same Ca2+ handling response (Ca2+ transient amplitude and dynamics) to 100nM Isoproterenol or an increase in stimulation frequency with respect to SHAM-FRD. Conclusion: The absence of estrogens would be linked to the development of IR, but are not involved in cardiac alterations that the same diet induces in males. We could suggest that female hearts, under IR conditions, are protected by other feminine molecules which not derived from the ovaries.
TOPIC AREA: CARDIOVASCULAR PHYSIOLOGY-HYPERTENSION

21. GENDER-AFFIRMING HORMONE THERAPY (GHT) IN HYPERTENSIVE RATS: CHARACTERIZATION OF ESTROGEN EFFECTS ON CARDIAC HYPERTROPHY (R78)

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Introduction: GHT, aimed to align the characteristics of people with gender dysphoria with their identity, have been studied in terms of sexual hormone effects and main adverse effects. However, some concerns about its cardiovascular (CV) consequences remained unresolved, due to incongruence of the reported clinical evidence and the existing sex differences in CV functions. Transgender females (TGF) have major CV risk, but it is unclear if estrogen therapy (ET) per se or testosterone deprivation is related to the higher CV risk. In addition, it is even less clear whether preexistent hypertension in these populations can worsen CV condition. Aims: The aim of present work is to characterize the cardiac effect of ET in a model of TGF in spontaneously

hypertensive rats (SHR). Methods: 3 month-old male SHR were assigned to 3 groups: GDX (gonadectomized for 3 months), TGF (GDX plus 1-month treatment with ET), SHAM. Dose and frequency of ET were selected according to replacement therapy, and simulating estrogen estrous peaks. Blood Pressure (BP) was monitored along the treatment, echocardiography was assessed previous to sacrifice. After sacrifice, collagen content, oxidative stress and protein expression were determined. Results: Testosterone deprivation prevented the increase in LV mass observed in SHAM, an effect that was canceled by ET (mg/mm: SHAM 27.7±1.4; GDX 21±4.2; TGF 27.1±0.8). No changes in BP or h/r ratio were found suggesting that the remodeling pattern remained unaltered. GDX presented a diminution in cardiac fibrosis that was partially reverted by ET (% of total collagen: SHAM 1.07±0.01; GDX 0.53±0.003; TGF 0.66±0.001). Both GDX and TGF hearts showed reduced reactive oxygen species (IF/ug protein: SHAM 15.3±12.2; GDX 9.2±4.6; TGF 3.2±0.5) together with an increased catalase expression (%: SHAM 100± 20; GDX 158±37; TGF 188±24), and an increased eNOS expression only in TGF (%: SHAM 100±35; GDX 106±33; TGF 157±26), that would indicate a lesser oxidative stress compared to SHAM. Body weight decreased in GDX, effect that was restored by ET (g: SHAM 358±10; GDX 339±9, TGF 344±7), while white adipose tissue index increased in GDX and increased more in TGF (WAT in g/g: SHAM 4.4±0.04; GDX 5.5±0.3; TGF 6.2±0.5). Conclusion: These preliminary results suggest that ET therapy in TGF comprises a complex scenario that deserves further investigation.
TOPIC AREA: CARDIOVASCULAR PHYSIOLOGY-HYPERTENSION

22. ADENOSINE-LOADED CHITOSAN NANOPARTICLES IMPROVE MITOCHONDRIAL MEMBRANE POTENTIAL IN CHRONIC ß-ADRENERGIC STIMULATED CARDIAC MYOBLASTS (R90)

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Introduction: Sustained ß-adrenergic overstimulation impairs both function and structure in cardiomyocytes, ultimately leading to cardiac failure. Chitosan is obtained from crustaceans and is useful for biomedical uses due to its capability to form nanoparticles that can transport drugs within. Adenosine is a widely used cardioprotective drug, but its short half-life is a limiting factor for long-term treatments. Objective: To develop an efficient adenosine drug delivery system and explore if adenosine-loaded nanoparticles can prevent mitochondrial damage produced by ß-adrenergic chronic overstimulation in H9c2 cells. Methods: Blank and adenosine-loaded chitosan nanoparticles (B-CNP and A-CNP) were synthesized by ionic gelation. Their size and entrapment efficiency was assessed. Spectrophotometry was used to obtain chitosan (CS), adenosine and CNP absorbance spectra. Mitochondrial membrane potential was assessed with TMRE probe. H9c2 cells were cultured for 48 h with 10 mM Isoproterenol (ISO). 24 h prior experiments cells were treated with adenosine 10 µM (Ade), B-CNP or A-CNP. Results are shown as mean ± SEM (n) and considered statistically different, otherwise p-value is stated. Normality was assessed and t-tests or two-way ANOVA were carried out. Results: CS absorbance spectra showed a linear behavior at 330nm (r2=0.972), wavelength used to assess synthesis CS yield. CNP showed a similar spectra and behavior at 330 nm. Adenosine absorbance spectra showed a peak ~260nm and linear behavior in that wavelength (r2=0.974). Hydrodynamic radius of B-CNP and A-CNP were similar (d.nm, B-CNP: 302.3±77.8 (5), A-CNP: 342.6±57.8 (4)). Entrapment efficiency (%) of A-CNP was in 42.91±5.80 (8). FCCP and ISO treatment depolarized H9c2 mitochondria (%C, FCCP: 76.6±4.7 (4), ISO: 85.4±4.6 (12)). A-CNP treatment prevented mitochondrial depolarization both in FCCP and ISO treatments (%C, FCCP: 84.2±5.7 (2), ISO: 106.0±10.2 (3)). Conclusion: We developed a system to quantify both CS synthesis yield and adenosine loading in the CNP. Adenosine loading did not change CNP size. A-CNP may protect the mitochondria against chemically depolarization and ß-adrenergic chronic overstimulation. Further experiments are needed to elucidate the exact mechanisms involved and other potential beneficial effects.
TOPIC AREA: CARDIOVASCULAR PHYSIOLOGY-HYPERTENSION

26/10 Screen 1 • CELLULAR PHYSIOLOGY AND SIGNAL TRANSDUCTION
8:00 - 10:00 HS

23. DOWN-REGULATION OF MULTIDRUG RESISTANCE-ASSOCIATED PROTEIN 2 (MRP2) BY LONG-TERM OXIDATIVE STRESS IN A MODEL OF HUMAN INTESTINAL EPITHELIUM AND BENEFICIAL EFFECT OF N-ACETYL-L-CYSTEINE (NAC) (R11)

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Introduction: Oxidative stress (OS) produced by continuous exposure to dietary additives and contaminants, is a key factor in the development of gastrointestinal disorders, in which the intestinal barrier is altered. Post-translational down-regulation of rat Mrp2, an essential component of the intestinal transcellular barrier in the disposition of environmental toxicants and therapeutic drugs, by short-term OS, was recently demonstrated. N-acetylcysteine (NAC) is a potent antioxidant, which exerts its effect as GSH precursor and effective ROS scavenger. Objectives: To evaluate the long-term effect of OS on the expression and activity of human MRP2 by TBH 250 µM and its prevention by NAC 1mM, as well as the probable molecular underlying pathway, in Caco-2 cell. Methods: MRP2 expression was evaluated by western blot in total cell membranes and plasma membranes (PM). Real time RTq-PCR was performed for evaluate changes in RNAm expression. MRP2 activity was determined by quantifying the efflux of dinitrophenyl-S-glutathione into the incubation medium by HPLC, using 1-chloro-2,4-dinitrobenzene 100 µM as precursor substrate. Statistical analyses were performed using one-way ANOVA followed by the post hoc Tukey-test and results expressed as a % difference with respect to control (C). Results: We confirmed that TBH generated OS at 24 h, as indicated by increased lipid peroxidation end products (+140%) and reduced SOD activity (-29%) ($p<0.05$, N=6). Protein expression and activity of MRP2 were decreased significantly in TBH group (-42% and -55% respectively) respect to C ($p<0.05$, N=3), without change in RNAm levels; while these returned to C values in cells with NAC co-treatment. In addition, TBH short-term treatment resulted in a decrease of expression in PM and activity of MRP2 (-38% and -55% respectively) ($p<0.05$, N=3), and the aggregate of cPKC pathway inhibitor GÖ6976 1 µM returned them to C values. Conclusion: MRP2 is down-regulated at post-transcriptional level in OS conditions, demonstrating cPKC pathway participation. NAC was able to reestablish MRP2 expression and activity alteration by OS, so it could be proposed to preserve MRP2 function under OS conditions.

24. TRPV4 MODULATION ELICITS DIFFERENT CALCIUM AND CELL GROWTH RESPONSES IN NORMAL AND CANCER DERIVED RENAL CELLS (R27)

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Introduction: The transient receptor potential vanilloid (TRPV) channel family has been recognized to exert a significant influence on a wide range of pathological processes in various cancer types. It has been proposed that specific TRPVs may serve as prognostic biomarkers in patients with clear cell renal cell carcinoma (ccRCC). However, the exact biological functions of TRPVs in ccRCC remain largely unexplored. Objectives: This study aims to investigate the role of the calcium channel TRPV4 in calcium signaling and cell growth in both normal and renal cancer-derived cells. Methods: To achieve our objectives, we utilized three cell lines: HK2, serving as a model for normal proximal tubule epithelial cells, and two derived from human ccRCC: 786-O, as a primary ccRCC model, and CAKI-1, as a metastatic ccRCC model. Using fluorescent probe techniques (FURA-2), we examined intracellular calcium concentration ([Ca2+]i) in response to TRPV4 activation (4α-PDD, 4 µM). Cell growth studies were conducted in HK2 and 786-O cells in the presence of a TRPV4 inhibitor (HC-067047, 1 µM). Results: All cell lines responded to TRPV4 activation by increasing intracellular calcium levels, but renal cancer-derived cell lines reached a maximum in a shorter time (Tmax) compared to the HK2 cell line (Tmax, in min.: CAKITmax: 3.11 ± 0.63, n=8; 786-OTmax: 3.50 ± 0.58, n=9; HK2Tmax: 6.65 ± 0.78, n=12; $p<0.01$). In cell growth studies, we found that the cell doubling time for 786-O cells (26.9 ± 0.26 hours, n=4) was significantly shorter than that of HK2 cells (45.1 ± 2.1 hours, n=6), $p < 0.001$. Furthermore, incubation with the TRPV4 inhibitor resulted in a significant reduction in 786-O cell growth but not in HK2 cells. Conclusion: We found that TRPV4 modulation elicited distinct responses in cancer cells when compared to normal cells. The observation that TRPV4 inhibition affects cell growth solely in cancer-derived cells may offer a promising opportunity for ccRCC therapies. However, we must

discern whether the reduction is attributable to alterations in cell proliferation or cell death and investigate whether the faster calcium response upon activating TRPV4 influences these processes.

25. EXPRESSION OF NRF2 ANTIOXIDANTS TARGET GENES DURING METABOLIC DYSFUNCTION-ASSOCIATED FATTY LIVER DISEASE (MAFLD) IN TUMOR NECROSIS FACTOR ALPHA RECEPTOR 1 (TNFR1) KNOCKOUT MICE (R33)

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Introduction: Previously, we demonstrated that disruption of TNFR1 signaling pathway decreases NRF2 nuclear translocation, enhancing hepatocyte oxidative stress in a High Fat Diet (HFD) murine model. The reactive oxygen species are involved in liver inflammation and HFD-derived hepatic injury. Objectives: The aim of this work was to evaluate the NRF2 targets genes and other antioxidant genes response in our mouse model of obesity and insulin resistance associated to TNFR1 signaling disruption. Methods: C57BL/6J wild type (WT) and C57BL/6-Tnfrsf1atm1Imx/J knockout (TNFR1 KO) mice (n=6) were fed with regular chow diet (CHOW) or a 40% high-fat diet for 16 weeks (HFD). Results: NRF2 nuclear protein expression determined by Western Blot showed a decrease in HFD WT (-48%; $p<0.05$) and HFD KO (-66%, $p<0.05$) when compared to paired CHOW fed groups. The gene expression levels were determinate by RT-PCR: Hmox-1decreased in both, HFD WT (-49%; $p<0.05$) and HFD KO (-56%;$p<0.05$) when compared with their respective CHOW feed mice. Sod2 and Catalase mRNA levels showed a statistically significative decrement only in HFD KO (-32%; $p<0.05$ and -35%; $p<0.05$ respectively) mice when compared to CHOW KO. In the other hand, Sod1and Gsr (encoding for glutathione reductase)did not show differences between groups. Consistently,HMOX-1 expression analyzed by Western Blot, showed a drop in both genotypes feed with HFD (HFD WT -39%; $p<0.05$ and HFD KO-53%; $p<0.05$). In addition, SOD-2 expression also showed a similar behavior (HFD WT-65%; $p<0.05$ and HFD KO -81%; $p<0.05$). Conclusion: Based on these results, we propose that the impaired NRF2 nuclear translocation observed in HFD KO group, mainly leads to lowerHMOX-1 and SOD2 gene and protein expression, enhancing hepatocyte oxidative stress and cell injury.

TOPIC AREA: CELL PHYSIOLOGY AND SIGNAL TRANSDUCTION

26. MICROTUBULES STABILIZATION AT THE trans-GOLGI IS NECESSARY FOR IMMUNE SYNAPSE MATURATION (R45)

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Introduction: Natural Killer Cells (NK) are cytotoxic cells from the innate immune system. They form a specialized junction with their target cells, the immune synapse (IS). Along the IS formation, one of the key events is the polarization of the centrosome (Ct) and the Golgi apparatus (GA). We have previously demonstrated that the GA participates in the leukocyte functional antigen (LFA)-1 organization at NK-IS. Besides those studies, GA role in IS maturation in NK cells has hardly been investigated. CLASP1/2 are plus-end binding proteins that are associated with the trans-GA, where they stabilize microtubules (Mt). Objective: To analyze the participation of GA associated proteins,CLASP1/2, in the IS maturation in NK cells. Methods: NK-YTS cells with decreased expression of CLASP1/2 (CLASPKD) were prepared by transduction with lentiviral particles for expression of CLASP1 and CLASP2 specific shRNAs. YTS were exposed to erythroleukemia KT86 cells (2:1 ratio) for 30 minutes. LFA-1 clustering at IS and Ct and GA polarization were analyzed by confocal microscopy. Relative distance to the IS (RD) was estimated as the difference between the distance from Ct to the IS and the distance from the cell centroid to the IS, related to the latter. The Area Weighted Distance (AWD) was estimated as the average distance of GA particles to the IS, weighted by each particle area. At least 10 IS were analyzed for each experiment. Results are expressed as media±standard error. Results: Our results revealed the inhibition of LFA-1 accumulation at the IS in CLASPKD cells (Control: 54±8%, CLASPKD: 37±9%, $p < 0.05$). Furthermore, the AWD of the GA was increased in CLASPKD cells (Control: 3,6±0,9, CLASPKD:6,9±1,3, $p < 0.05$). Concomitantly, CLASPKD cells showed an impaired Ct polarization (Control: -0,35±0,2, CLASPKD:0,2±0,1, $p < 0.05$). Conclusion: These results point out the importance of GA mt stabilization by CLASP1/2 in the organization of LFA-1 during the IS formation and brings out the first evidence that, similarly to what has been demonstrated in migratory cells, the GA acts as a regulator of Ct polarization throughout the course of SI formation in NK cells.

27. REGULATION OF THE ASSOCIATION OF CDC42 INTERACTING PROTEIN 4 (CIP4) WITH MICROTUBULES DURING THE ESTABLISHMENT OF THE IMMUNE SYNAPSE IN NATURAL KILLER CELLS: INVOLVEMENT OF PKA (R47)

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Introduction: Natural killer (NK) cell cytotoxicity requires extensive actin and microtubule (MT) remodeling at the NK-target cell immune synapse. CIP4 is a CDC42 effector that scaffolds proteins involved in actin remodeling, which has a prominent role in NK-immune synapse maturation. CIP4 also contains an amino-terminal domain that allows CIP4 interaction with MT, and is phosphorylated by PKA in CIP4T225. Our previous results indicate that YTS-NK cell activation leads to decreased CIP4 association with MT. Objectives: We aimed to assess the relevance of our previous findings in ex vivo NK (eNK) cells and to analyze the involvement of PKA in the regulatory mechanism. Methods: eNK cells from volunteer blood donors were purified by negative selection and cultured in IL-2-supplemented medium. To evaluate the role of PKA, YTS-NK cells were cultivated in the presence of PKA activator forskolin (Fk, 10 µM) or PKA inhibitor H89 (1 µM). Both, eNK and YTS-NK cells, were exposed to erythroleukemia KT-86 cells for 30 min at 2:1 or 6:1 ratios for immunofluorescence (IF) or Western blot (WB) studies. CIP4 interaction with MT was assessed by IF confocal microscopy. An antibody that specifically recognizes residues phosphorylated by PKA was used to assess PKA activity in NK-cells exposed to target cells (activated, Ac) and in isolated NK cells (resting, R) by WB. Results are expressed as media ± SE. Results: IF studies showed that the fraction of MT associated CIP4 was reduced in Ac-eNK (MT-CIP4 (%), R: 17 ± 2; Ac: 13 ± 3; n=3; $p<0.05$). WB analysis indicated an increase of phosphorylation of PKA substrates in Ac-YTS-NK cells (% of R: 164 ± 24; n=3; $p<0.01$). IF studies of those cells showed that H89 increases CIP4 association to MT both in Ac-cells (MT-CIP4 (%), control: 10 ± 0.1; H89: 17 ± 1.6; n=3; $p<0.05$) and in R-cells (MT-CIP4 (%), control: 12 ± 1; H89: 15 ± 1; n=3; $p<0.01$). Conversely, Fk decreases CIP4 association to MT in R-cells (MT-CIP4 (%), control: 12 ± 1; Fk: 10 ± 1; n=3; $p<0.05$). Conclusion: Our results confirm that NK cell activation induces a significant decrease in CIP4 interaction with MT, and indicate that the mechanism involves PKA activation.

28. EFFECTS OF THE CFTR MODULATORS LUMACAFTOR AND IVACAFTOR ON MITOCHONDRIAL DYNAMICS (R53)

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Introduction: Mutations in the CFTR gene, responsible for cystic fibrosis (CF), are associated with mitochondrial abnormalities, including changes in mitochondrial dynamics. Previously, we reported increased mitochondrial fission with cAMP stimulation of CFTR activity in IB3-1 (CF) cells. In addition, lumacaftor (VX-809) and ivacaftor (VX-770), the initial CFTR modulators approved for CF therapy, are known to impact CFTR localization and activation. Objectives: This study aims to explore the effects of VX-809 and VX-770 on both mitochondrial morphology and function while considering the hypothesis that CFTR activity may modulate mitochondrial dynamics. Methods: IB3-1 (CF) cells (ΔF508/W1282X) were treated with VX-809 (10 µM) and VX-770 (0.1 µM) for 48h. Mitochondrial morphology was analyzed using confocal microscopy with Mitotraker Orange-labeled cells and analyzed with the MiNA plugin in Fiji and Micro-P softwares. Also, MFN1 and DRP1 levels were measured in IB3-1 (CF), S9 (IB3-1 expressing wt-CFTR), and C38 (IB3-1 expressing a truncated functional CFTR) cells. Cellular and mitochondrial ROS levels were quantified by flow cytometry using DCFH-DA and MitoSOX probes, respectively. Mitochondrial membrane potential (Ψm) was measured using TMRE or JC-1 probes by flow cytometry. Results: The combination of VX-809 and VX-770 for 48h increased several indicators of mitochondrial fission ($p<0.05$, n=7), whereas individual drug treatments did not significantly affect mitochondrial morphology in IB3-1 cells. VX-809 treatment for 48h increased cellular ROS levels ($p<0.05$, n=7). In contrast, treatment with different concentrations of VX-770 led to an increase in Ψm at 1 µM in both C38 and IB3-1 cells ($p<0.05$, n=4), suggesting potential off-target effects on mitochondria. The expression of mitochondrial dynamics proteins DRP1 and MFN1 was also modulated by these modulators. Conclusion: These results highlight the potential of CFTR modulators to induce CFTR-dependent and non-target effects, potentially affecting mitochondrial function. Further research is required to assess whether these changes in mitochondrial function could have adverse implications for the drug's activity.

29. GHRELIN TRANSPORT DIRECTION AND DYNAMICS IN HYPOTHALAMIC TANYCYTES: POSSIBLE ROLE IN GHRELIN CSF CLEARANCE (R56)

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Introduction: hypothalamic tanycytes are polarized ependymoglial cells that line the ventral part of the third ventricle (V3) and send processes through the hypothalamic parenchyma and median eminence contacting blood vessels, neurons and other glial cells. Thus, they form an anatomical interface for the transport of molecules between blood and CSF. We recently described that tanycytes internalize the orexigenic hormone ghrelin trough clathrin-mediated endocytosis. Objectives: Here, we study the uptake and transport direction of ghrelin in these cells with a fluorescent ghrelin tracer (Fr-ghrelin) using in vivo, ex vivo and in vitro strategies. Methods: For in vivo experiments, we centrally injected mice with Fr-ghrelin and quantified fluorescence

intensity in tanycytes after 15, 30, 60 and 90 minutes. For ex vivo studies we used mouse hypothalamic explants incubated with Fr-ghrelin only on their outer side (contacting terminals) or within the V3 (contacting somas) and analyzed the presence of fluorescent signal in tanycytes. For in vitro experiments, we incubated primary cultures of rat hypothalamic tanycytes with a 5-min pulse of Fr-ghrelin, then washed with fresh medium and incubated for an additional 10 to 25 min. Subsequently, we fixed the cells and quantified the fluorescence in somas, processes and terminals of each cell. Results: In mice centrally injected with Fr-ghrelin, we observed that the fluorescent signal was highest at 15 min post-injection, then was reduced by ~87% at 30 min, as compared to 15 min post-injection, and returned to control values at 60 min. In hypothalamic explants we observed fluorescence within tanycytes exclusively when the explants were incubated within the 3V. In turn, quantification of the intracellular redistribution of fluorescent signal over time in cultured tanycytes indicated that the signal was mostly found in somas after the 5 min pulse, and significantly increased in processes and terminals after 10 min. After 30 min, fluorescence decreased in the whole cell.Conclusion: This evidence indicates that tanycytes internalize ghrelin in their CSF-contacting soma and transport it to their terminals, possibly playing a role in CSF ghrelin clearance.

30. ROSUVASTATIN REDUCES CHOLESTEROL LEVELS IN HUMAN RHABDOMYOSARCOMA CELLS IN CULTURE (R65)

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Introduction: Rosuvastatin is currently the most prescribed statin worldwide. It has been demonstrated to be effective in reducing serum low density lipoprotein (LDL)-cholesterol and vascular inflammation, effects that contribute to diminish the risk of cardiovascular disease. Some of the main side effects of the statins are experienced in the muscles, where they produce weakness and pain, leading in extreme cases to rhabdomyolysis. Objectives: The aim of this work was to generate an in vitro model to study the statins´ muscle effects at the molecular level. Methods: We analyzed different ways to induce the differentiation of a human rhabdomyosarcoma cell line (hRD). So far, we observed that the starvation of these cells with 1% fetal bovine serum (FBS) for 48 hours leads to morphological changes that resemble a muscle phenotype. After differentiation, we treated these cells with 20 µM rosuvastatin for 48 or 72 hours and analyzed the cellular and secreted cholesterol levels by high performance liquid chromatography (HPLC). Results: In agreement with the expected cholesterol synthesis inhibition, we observed a significant cholesterol reduction in the whole extracts of the differentiated cells upon 48 or 72 hours of rosuvastatin treatment. Nonetheless, the content of this lipid in the secreted extracellular vesicles did not show a significant decay. In non-differentiated hRD cells grown with 10% FBS and treated with 20 µM rosuvastatin for 48 hours the cellular cholesterol also showed a significant decrease, but the amount of this sterol in the secreted extracellular vesicles remained constant. Conclusions: Our study demonstrates that a non-lipophilic statin like rosuvastatin can inhibit cholesterol synthesis in hRD cells, meaning that these cells express a transporter with affinity for rosuvastatin. Further investigation is needed to identify the molecule implicated in rosuvastatin internalization, as well as the molecular mechanisms leading to the functional deterioration of these muscle-like cells.

31. NHE1 MODULATION BY pH IS DIFFERENT IN NORMAL AND CANCER-DERIVED RENAL CELLS (R69)

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Introduction: Even though pH homeostasis is critical for cell survival, extracellular acidosis is a hallmark of cancers. Our previous studies in a renal carcinoma model (ccRCC) showed that plasma-membrane expression of NHE1 isoform of Na+/H+ exchanger is higher in ccRCC than in normal cells. After 72h alkali, while healthy cells raise NHE1 plasma-membrane expression cancer ones reduce it. Objectives: This study aimed to investigate whether NHE1-pH regulation changes in ccRCC. Methods: We used three cell lines: HK2 (model for normal proximal tubule epithelial cells), 786-O (primary ccRCC model), and CAKI-1 (metastatic ccRCC model). We used fluorescent probe techniques (BCECF) to examine NHE1 activity at different extracellular pH (pHe). Results: At pHe=7.4, ccRCC derived cells exhibited a higher NHE1 function (NHE1, 10-5 pH units. s-1, HK2: 11 ± 2; 786-O: 290 ± 16; Caki-1: 284 ± 30. HK2 vs 786-O $p<0.001$ n=725. HK2 vs Caki-1 $p<0.001$ n=371). Only Caki-1 cells have less Buffering power, β (β, mM pH units, HK2: 4.99 ± 0.15; 786-O: 4.97 ± 0.12; Caki-1: 4.11 ± 0.04. HK2 vs Caki-1 $p<0.05$ n=371). Altogether, even though both cancer models had similar NHE1 function, the net H+ flux they elicited was different (Flux mM s-1. 10-5: 786-O: 1398 ± 76; Caki-1: 729 ± 95. $p<0.001$ n=506). We then evaluated if NHE1 activity was affected by alkali after a short (5 min) or long (3 days) exposure. In healthy cells, NHE1-dependent H+ flux was unaffected after 5 min alkali. However, it was higher after 72h exposure

(HK2 Flux at 7.5 mM s-1. 10-5: 5 min: 0.33 ± 0.33; 3 days: 260 ± 40. p<0.001 n=450). In cancer-derived cells alkali inhibited H+ Flux, independent of exposure time (786-O Flux at 7.5 mM s-1. 10-5: 5 min: 209 ± 28; 3 days: 223 ± 20. ns n=387). Conclusion: As healthy cells need longer alkaline exposure to raise NHE1 activity, this activation is probably due to the changes in plasma-membrane protein expression induced by the alkali that we previously described. In contrast in cancer cells, as inhibition of NHE1 occurs after a short exposure, it is likely due to pH modulation.

32. AQUAPORIN-4 FACILITATES CELL MIGRATION IN RETINAL MÜLLER CELLS: IMPLICATIONS IN NEUROMYELITIS OPTICA (R72)

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Introduction: Müller cells are involved in controlling extracellular homeostasis in the retina, activating a regulatory volume decrease (RVD) response, which depends on the efflux of solutes and water through Aquaporin-4 (AQP4). AQP4 is also the target of the autoantibody AQP4-IgG present in the sera of patients with Neuromyelitis Optica (NMO). Müller cells are also important for retinal integrity, as their activation in certain types of injuries leads to their proliferation, migration and differentiation to different neuronal cells. In astrocytes, AQP4 contributes to cell proliferation and migration and we recently demonstrated that AQP4 facilitates Müller cell proliferation. Objectives: The aim of this study was to evaluate the role of AQP4 in migration of Müller cells. Methods: MIO-M1 human Müller cells were exposed to 0.5 µM TNG-020 (AQP4 inhibitor) or vehicle or to 1/50 dilution of AQP4-IgG positive or control sera. Cell volume, osmotic water permeability (Pf) and RVD during an osmotic swelling were measured by fluorescent videomicroscopy. AQP4 expression and F-actin fibers organization were evaluated by immunocitochemistry. Cell migration was evaluated by wound healing assay. Results: Pf and RVD in migrating MIO-M1 cells were reduced in comparison with non-migrating cells. AQP4 inhibition by TGN-020 did not modify the decreased Pf or RVD observed in migrating cells, but increased the time to reach the maximum swelling volume. TGN-020 did not change AQP4 abundance or plasma membrane localization in migrating MIO-M1 cells. AQP4 inhibition induced a reduction of the anisotropy of F-actin fibers, which indicates its degree of organization, and decreased MIO-M1 cell migration by 30% in comparison to control. Cell treatment with AQP4-IgG positive sera decreased AQP4 plasma membrane expression in MIO-M1 cells and reduced cell migration by 20% in comparison to control sera. Conclusion: We propose that AQP4 participates in Müller cell migration by facilitating cytoskeleton organization. This is of particular importance in NMO, as the decreased ability of Müller cells to migrate may affect retinal tissue repair in vivo.

33. EFFECT OF ALKALI EXPOSITION AND NHE1 INHIBITION IN NORMAL AND TUMOR RENAL CELLS ADHESION AND MIGRATION (R74)

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Introduction: Extracellular acidity is a hallmark of tumor cells. Also, it has been described that the isoform 1 of the Na+/H+ exchanger (NHE1) would be associated with the adaptation of tumor cells to this acidic extracellular environment. Therefore, we have hypothesized that modulation of both pHe and NHE1 may affect distinctively normal and tumor cells. Our previous studies showed that cells derived from clear cell renal cell carcinoma (ccRCC) were more susceptible to death than normal cells after 72h exposition to mild alkalosis. Moreover, the combination of alkali plus inhibition of NHE1 improved the damage induced by alkali in normal cells. Objective: The aim of our work was to investigate if the exposition to mild alkalosis and/or inhibition of NHE1 would also affect distinctively the adhesion and migration of normal and ccRCC-derived cells. Methods: We used two renal cell models: HK-2, derived from normal human proximal epithelial cells, and 786-O, derived from human ccRCC cells. Cell adhesion was evaluated with adhesion assays after 72h exposition to mild alkalosis (NaOH 9.6mM), in the presence of NHE1 inhibitor (HOE, 1µM), or with the combination of both treatments. Cell migration was evaluated in the same conditions with wound healing assays. Results: Our results showed that exposure to mild alkalosis, NHE1 inhibition, and the combination of treatments reduced cell adhesion (Cell number, HK2: Control 85±5, NaOH 55±2, HOE 65±1, NaOH+HOE 59±2, n=3-9, p<0.001; 786-O: Control 122±7, NaOH 73±6, HOE 79±3, NaOH+HOE 87±7, n=6-14, p<0.001) without changing cell migration (%, HK2: Control 15.41±0.58, NaOH 14.47±0.99, HOE 13.93±1.00, NaOH+HOE 13.19±1.83, n=8-17; 786-O: Control 46.30±0.87, NaOH 47.43±3.14, HOE 43.88±1.79, NaOH+HOE 48.25±3.59, n=8-37) in both in HK-2 and 786-O cell lines. Conclusion: Results demonstrate that, although mild alkalosis affected ccRCC-derived cells and our combination of treatments seemed to be

protective to normal cells, this is not the case with cell adhesion and cell migration processes. Further experiments are needed to evaluate if the alkalosis/NHE1 interplay results in an effective therapeutic proposal.

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8:00 - 10:00 HS

34. POSTTRANSLATIONAL REGULATION OF INTESTINAL MULTIDRUG RESISTANCE-ASSOCIATED PROTEIN 2 (MRP2) BY THE PRO-INFLAMMATORY CYTOKINE IL-1B IN A MODEL OF HUMAN INTESTINAL EPITHELIUM (R12)

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Introduction: Multidrug resistance protein 2 (MRP2) is an ABC transporter (ATP-binding Cassette) of great relevance in the intestinal barrier function, acting to prevent the absorption of not only environmental and food toxicants but also therapeutic drugs. Expression and activity of rat intestinal Mrp2 is downregulated in LPS-induced endotoxemia, demonstrating transcriptional and posttranscriptional regulation. We also showed that IL-1β, an initial mediator of LPS-inflammatory responses, induces early internalization of intestinal Mrp2 in simultaneous with alteration of its transport activity. Objectives: To evaluate the effect of LPS, as well as its primary mediator IL-1β, on MRP2 expression and activity in Caco-2 cell culture, a model of human intestinal epithelium. Methods: MRP2 expression was evaluated by western blot in total cell membranes. Immunodetection and confocal studies were carried out utilizing specific antibodies and performing densitometry analyses along the Z axis. MRP2 activity was determined by quantifying the efflux of dinitrophenyl-S-glutathione (DNP-SG) into the incubation medium by HPLC, using 1-chloro-2,4-dinitrobenzene 100 µM as precursor substrate. Statistical analyses were performed using one-way ANOVA followed by the post hoc Tukey-test for multiple comparisons and results expressed as % difference with respect to control (C). Results: LPS 10 µg/ml treatment for 24 h had no effect on MRP2 expression. IL-1β 10 ng/ml treatment at 24 h demonstrated a decrease in the MRP2 expression (-62% of C, N=3, $p<0.05$), which was counteracted with the aggregate of the lysosomal inhibitors pepstatin A (100 µM) and leupeptin (250 µM). IL-1β treatments at 30 min revealed internalization of MRP2 from plasma membrane into intracellular compartments and loss of MRP2 activity (-45% of C, N=4, $p<0.05$). Conclusion: The results suggest that LPS would not have a direct effect on the MRP2 expression of human origin. Conversely, IL-1β induced loss of MRP2 from the plasma membrane and relocalization to intracellular compartments resulting in significant impairment of its transport activity; lysosomal degradation is also suggested after sustained internalization.
TOPIC AREA: GASTROENTEROLOGY

35. FENOFIBRATE IMPROVES HEPATIC AND RENAL EXCRETION OF BILE ACIDS IN ESTROGEN-INDUCED CHOLESTASIS (R19)

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Introduction: Estrogens are causal agents of pregnancy- and oral contraceptive-induced cholestasis in susceptible women. 17α-ethinylestradiol (EE) is a prototypical estrogen used to mimic this disease in rats. EE impairs bile formation by inhibiting expression of bile salt (BS) heparocellular transporters. Fenofibrate (FF) is a potent PPARα agonist used to treat some human cholestatic hepatopathies, since it can upregulate several of these transporters.
Objetives: To ascertain whether FF improves elimination of accumulated bile salts (BS) in EE-induced cholestasis, by counteracting the drop in the expression of BS hepatic transporters and/or by stimulating alternative renal BS excretion.
Methods: Male Wistar rats were randomly divided into the following groups: i) Control (C), ii) EE (5 mg/kg/day, i.d., 5 days), iii) FF (200 mg/kg/day, p.o., 7 days), and iv) EE+FF. Next, serum alkaline phosphatase (ALP), a surrogate marker of hepatic retention of BS in cholestasis, and the maximal cumulative biliary output of the model BS taurocholate (TCBO) were assessed. The expression of Bsep (main apical BS transporter) and Mrp3 (main basolateral BS efflux pump) were evaluated by Western blot and real-time PCR, respectively. The alternative renal route of BS excretion was evaluated by determining total BS concentration in plasma and urine. Results: (*$p<0.05$ vs. control; #$p<0.05$ vs. EE). FF normalized serum ALP (U/L), which had been elevated (+64%*) by EE, and improved TCBO (mmol/ g liver wt) (C: 486 ± 40; EE: 214 ± 31*, EE+FF: 403 ± 34#). This improvement in BS biliary output was associated with an increase (+42%#) in Bsep expression, compared to that in the EE group (31%* lower than C). FF also increased expression of Mrp3 (+336%#) in EE-treated rats, thus potentiating the inducing effect that EE had per se

(131%*). The marked increase in Mrp3 was associated with higher BS blood (+652%#) and urine (+302%#) levels in the EE+FF group. Conclusions: FF has anticholestatic effects in EE-induced cholestasis by improving BS elimination through both biliary and urinary routes, via induction of the apical and basolateral efflux transporters Bsep and Mrp3, respectively.
TOPIC AREA: GASTROENTEROLOGY

36. EFFICACY OF THE INTRAEPITHELIAL LYMPHOGRAM IN THE DIAGNOSIS OF CELIAC DISEASE (R22)

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Introduction: Celiac disease (CD) is a chronic autoimmune systemic disorder induced by the intake of gluten protein contained in wheat, oat, barley and rye in genetically predisposed individuals [HLA DQ2(+) and/or DQ8(+)] and is characterized by the presence of antibodies and proximal small intestine mucosal injury: villous atrophy, crypt hyperplasia, and increase in intraepithelial lymphocytes (IEL). The IEL pattern is relevant as a marker of CD; an intraepithelial lymphogram (IL) presents a celiac pattern (CP, present in 95% of the patients) when an increase in total IEL, an increase in TCRgd+ subpopulation and an almost complete disappearance of the CD3-/CD103+ subpopulation are found. Objectives: To evaluate the utility of IL in the diagnosis of CD. Methods: Patients attending the Gastroenterology Service of the Hospital Provincial del Centenario, who had signed the informed consent were included in the study. Control Group (CG): patients subjected to upper endoscopy, in whom CD had been ruled out by clinical and serological criteria and whose intestinal biopsy at the time of the study was 0-2 (Marsh-Oberhuber classification, n=21). Study group (SG): patients positive for clinical and/or serological markers of CD and whose intestinal biopsy at the time of the study was compatible with CD (grade≥3, Marsh-Oberhuber classification, n=11) or patients with confirmed diagnosis of CD and under a gluten-free diet, subjected to control intestinal biopsy (n=5). Flow cytometric analysis of IEL subpopulations was performed in an intestinal biopsy sample and the presence of a CP was assessed. Results: None of the patients in the CG present CP; 15 patients of the SG presented a CP and 1 did not. Sensitivity (Se) and specificity (Sp) of IL for CD diagnosis were 94% and 100% respectively; positive predictive value (PPV) and negative predictive value (NPV) were 100% and 95%, respectively. Conclusion: The elevated Se and Sp of IL, together with its high PPV, allow us to postulate that applying the IL to the current diagnostic process would provide the clinician with an alternative algorithm in cases of unclear clinical manifestations and discrepancy with other markers of CD.

37. DEXAMETHASONE PROTECTION IN A MICE ESTROGEN-INDUCED CHOLESTASIS MODEL (R31)

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Introduction. Estrogen-induced cholestasis is a common pathology in susceptible pregnant women. A murine model of this pathology consists in a 5-days administration of 17-ethinylestradiol (EE) to rats or mice. EE administration reduces bile flow and bile salt synthesis by altering transporters and enzymes responsible for maintaining the normal bile salt excretion. Cholestatic murine models have exhibited substantial infiltration of innate immune cells and elevated levels of proinflammatory cytokines such as TNFα and IL-1β in the liver. These cytokines can exacerbate the cholestatic effects triggered by estrogens. This study proposes the use of Dexamethasone (Dex) due to its potent anti-inflammatory properties.
Aim. To evaluate whether the administration of Dex in a mice EE-induced cholestasis model improves bile flow, bilirubin biliary excretion rate (ER) and plasma levels of liver markers enzymes. Methods. Male C57BL/6 mice were randomly divided into four groups: (a) Control (vehicles); (b) EE (10 mg/kg/day, s.c., 5 days); (c) Dex (1 mg/kg/day, i.p. 5 days); and (d) EE+Dex. The common bile duct was catheterized via a trans duodenal surgery and bile was collected for 30 min. Plasma samples were used to measure the enzymes: Alkaline Phosphatase (ALP), Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST). Also, the concentration of bilirubin was determined in bile samples. Results. (Mean±SEM. n=6. * $p<0.05$ vs. Control; # $p<0.05$ vs. EE.). Dex prevented significantly the decrease in bile flow (µl/min/g liver) in EE-induced cholestatic animals (Control: 1.10±0.07; EE: 0.68±0.04*; EE+Dex: 0.97±0.06#; Dex: 1.00±0.12). Also, Dex partially normalized plasma ALP levels (U/L), which had been elevated by EE (Control: 166±20; EE: 392±90*; EE+Dex: 199±31#; Dex: 119±24). In ALT and AST, no significant difference was seen between groups. Finally, EE decreased bilirubin ER (nmol/min/g liver) and DEX prevented in part this effect (Control: 0.29±0.01; EE: 0.06±0.02*; EE+Dex: 0.16±0.06*#; Dex: 0.14±0.01). Conclusion. The anti-inflammatory agent Dex improves the EE-induced cholestasis mice by normalizing bile flow, cholestasis marker ALP and bilirubin ER.
TOPIC AREA: GASTROENTEROLOGY.

38. Decreased Mrp2 expression by fructose in the primary culture model in sandwich-cultured rat hepatocytes (SCRHs) (R40)

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The increase in fructose consumption in the global diet contributes to an increase in total caloric intake and it is related to an increase in the incidence of Metabolic Syndrome (MS). Previously, we demonstrated that administration of 10% fructose in drinking water for 8 weeks to normal rats, a model of MS, reduced bile flow and decreased the expression of canalicular transporters Mrp2 (multidrug resistance-associated protein 2). and Bsep (export of bile salts bomb). Aim: To develop a model to study the signaling pathways involved in the actions of fructose, we evaluated whether incubation of a primary culture of rat hepatocytes in collagen sandwich configuration (SCRHs) with fructose can mimic the effect on Mrp2 observed in vivo. Methods: Once the hepatocytes were polarized, they were treated with 22 mM fructose for 24 hours. LDH (lactate dehydrogenase) release in the media was measured by a detention kit (Wienner Lab). Mrp2 activity was evaluated by secretion of fluorescent glutathione methylfluorescein (GMF) using the index BEI (biliary excretion index). For that, treatments were performed duplicated, in one the experiment was performed with standard buffer whereas in the other a Ca2+/Mg2+ free buffer was used. The BEI of GMF was calculated as: BEI = (fluorescenceCa2+/Mg2+ - fluorescenceCa2+/Mg2+ -free) / fluorescence Ca2+/Mg2+ x100%. Mrp2 expression was evaluated by SDS-polyacrylamide gel electrophoresis and transfer to PVDF membranes of cell homogenates from both groups using primary antibodies directed against Mrp2. Results: (Average ± SEM). Fructose 24 mM did not affect LDH (C: 100±5%; F: 105±3%; n=3) and produced a decrease in BEI by 60% with respect to the Control (C: 24±4; F: 9±4; n=4; *p<0.05 vs Control) and in Mrp2 expression (C:100±16% F: 36±12%; n=5 * p<0.05 vs Control). Conclusions: These preliminary results suggest that Fructose decreases the expression of Mrp2 leading to a decrease in functionality. This in vitro model would serve to study the signaling pathways activated by the cellular effects of fructose.

39. INULIN REVERTS INTESTINAL MULTIDRUG RESISTANCE ASSOCIATED PROTEIN 2 DOWN-REGULATION IN HIGH-FAT DIET-INDUCED OBESE MICE (R42)

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Introduction. Intestinal multidrug resistance associated protein 2 (Mrp2) is an ABC transporter that limits the absorption of xenobiotics and drugs orally ingested, thus acting as a biochemical barrier. This function may be disrupted under pathophysiological conditions such as obesity, where the intestinal microbiota is altered. In previous studies we observed that standard diet enriched with 40% kcal (HFD) for 8 weeks, reduced the expression and activity of intestinal Mrp2 in mice. Objectives. Thus, we here evaluated the potential effect of inulin administration (5 % w/w), a prebiotic well-known gut modulator, in reverting HFD induced Mrp2 alterations. Methods. Proximal jejunum from C57BL/6 male mice was removed to study Mrp2 expression by western blot, and its transport activity, by using the everted intestinal sacs model. Results. After obese-like conditions were evoked, animals cotreated with inulin for another 2 weeks, showed a marked decrease in plasma triglycerides and glycemia levels, as well as an improvement in glucose homeostasis and fat deposition. Prebiotic supplementation was able to revert downregulation of Mrp2 protein expression (-57%, p<0.05) induced by diet. Also, efflux of the Mrp2 substrate dinitrophenyl-S-glutathione (DNP-SG), generated from its precursor 1-chloro-2,4-dinitrobenzene (CDNB), decreased in obese animals (-53%, p<0.05) respect to controls, returned to normal values after inulin administration. Concomitantly, cotreated group reverted alterations in parameters of oxidative stress by decreasing lipid peroxidation end products and reactive oxygen species induced by fat consumption (+330% and +54%, respectively, p<0.05). Conclusion. Our study demonstrated that inulin was able to re-establish the expression and functionality of intestinal Mrp2, which represents an additional beneficial effect of this prebiotic, used as a positive modulator of gut microbiota in metabolic disorders, where diverse therapeutic drugs are administered.

40. IN VIVO MITOCHONDRIAL AQUAPORIN-8 KNOCKWDOWN DECREASED BILIARY CHOLESTEROL EXCRETION (R49)

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Introduction: Hepatocyte mitochondrial aquaporin-8 (mtAQP8) is able to work as a peroxiporin. Our previous results suggest that mtAQP8 is involved in the hepatic metabolism of cholesterol, by means of hydrogen peroxide signaling. Biliary excretion is a key step in liver cholesterol processing. Cholesterol is excreted into bile directly in its unesterified form, via the canalicular cholesterol transporter ABCG5/8, or after conversion into bile acids, via the bile salt export pump, BSEP/ABCB11. Objectives: to study the role of hepatic mtAQP8 knockdown in the biliary excretion of cholesterol. Methods: The aquaporin-8 gene-knockdown was made by using a short hairpin RNA (shRNA)-expressing adenovirus vector (Adshaqp8). Adshaqp8 was administrated by retrograde intrabiliary infusion to male C57BL/6 mice. Control mice received a scrambled adenovector. After 72 h, the gallbladder was ligated, the common bile duct was cannulated and bile was collected. Hepatic mtAQP8 knockdown expression was confirmed by immunoblotting with specific antibodies. Results: Adshaqp8 downregulated hepatic mtAQP8 expression by about 65% ($p<0.001$). The biliary excretion of cholesterol was decreased (~40%, $p<0.001$), whereas that of bile acids was not significantly altered. Biliary cholesterol excretion (nmol/min/100g bodyweight) was: 9.33±0.28 (controls) vs.5.87±0.22 (Adshaqp8-treated) ($n=4$; $p< 0.001$). Biliary bile acid excretion (nmol/min/100gbody weight) was: 129.4 ±11.1 (controls) vs 106.4 ±4.0 (Adshaqp8-treated) ($n=4$; ns). Adshaqp8 delivery caused no alteration in serum hepatic enzymes indicating absent of toxic effects. We found that hepatic mtAQP8 knockdown decreased ABCG5/8 expression by around 60%, whereas BSEP/ABCB11 expression was not significantly changed. Conclusion: Our data suggest that hepatic mtAQP8 is involved in the biliary elimination of cholesterol, likely by modulating the canalicular expression of ABCG5/8, and further supports a key regulatory role for AQP8 in liver cholesterol processing.

41. TAUROURSODEOXYCHOLATE PREVENTS ENDOCYTIC INTERNALIZATION AND FURTHER PROTEASOMAL DEGRADATION OF THE CANALICULAR TRANSPORTER MRP2 IN ESTRADIOL 17B-D-GLUCURONIDE-INDUCED CHOLESTASIS (R59)

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Introduction: We have shown (Hepatology 35:1409, 2002) that exacerbated endocytosis of the canalicular transporter Mrp2 is involved in cholestasis induced by estradiol 17β-D-glucuronide (E217G), a causal agent of intrahepatic cholestasis of pregnancy (ICP). It has been suggested, but not probed, that Mrp2 sustained endocytosis leads to its exacerbated degradation. Ursodeoxycholic acid (UDCA) is the first-line therapy for ICP, but its therapeutic mechanims are unknown. However, UDCA anti-endocytic properties are likely, since we have shown this effects in sepsis-induced cholestasis (Biochem Pharmacol 168:48, 2019). Objectives: To ascertain whether E217G-induced Mrp2 endocytosis leads to its accelerated proteosomal degradation, and whether tauroursodeoxycholate (TUDC), the main UDCA metabolite, can prevents this phenomenon by halting endocytosis and further degradation of Mrp2 in sandwich-cultured rat hepatocytes (SCRH). Methods: Cycloheximide (1.5 mg/ml)-treated SCRH were preincubated with the proteosomal inhibitor MG132 (10 µM, 30 min) or TUDC (100 µM, 30min), and then exposed to E217G (200 µM, 24h), or its vehicle (DMSO) in controls (C). Protein expression of Mrp2 was assessed by Western blot, its localization by immunostaining followed by confocal microscopy, and its transport function by quantifying the initial transport rate (ITR) of its fluorescent substrate GSH-S-methylfluorescein (GS-MF). Results: (*$p<0.05$ vs. C, MG132 or TUDC, as appropriated; #$p<0.05$ vs. E217G). E217G induced a significant decrease (-60±12%*) of Mrp2 protein content compared with C, and pretreatment with MG132 (-12±11%#) and TUDC (-6±7%#) fully prevented this decrease. ITR was also decreased by E217G (-70±6%*), and this was fully and partially prevented by MG132 (-22±4%#) and TUDC (-32±15%*#), respectively. A similar protection pattern of MG132 and TUDC against E217G-induced endocytosis and further degradation was apparent when Mrp2 was visualized by confocal microscopy. Conclusions: Sustained endocytosis of Mrp2 in E217G-induced hepatocellular secretory failure leads to its exacerbated proteosomal degradation, and this phenomenon is fully prevented by blocking Mrp2 endocytosis with TUDC.

26/10 Screen 2 • NEUROLOGY
8:00 - 10:00 HS

42. EFFECTS OF RHYTHMIC AUDITORY STIMULATIONS ON FUNCTIONAL MOBILITY AND GAIT IN PEOPLE WITH PARKINSON DISEASE AT COMMUNITY ENVIRONMENT (R1)

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Introduction: The external auditory cues in rehabilitation programs have shown benefit in gait disturbances in patients with Parkinson's disease (PD). These provide an external reference system, partially replacing deficient structures responsible for automaticity, leading to more voluntary control of gait. The cues effects are not well determined in unpredictable environments. Objective: The aim of our study was to determine the effects of Rhythmic Auditory Stimulations (RAS) during gait on PD people at community environment (CE). Methods: Patients expressed their agreement through an informed consent by Biomedical Research Ethics Committee (IRB). In this context, 27 patients with an average age of 71, 10.5 year average of PD length -phase levodopa "on"- and 1 - 3 Hoehn & Yahr stages were recruited. Mini mental test: ≥26 points. Patients were tested using Timed Up & Go test (TUG) and 10 Meter Walk Test (10MWT). The TUG time (s) and 10 MWT velocity (m/s) was measured in three conditions: 1) baseline (gym), 2) CE without RAS, 3) CE with RAS by metronome dosed at the basal cadence of the patient, synchronizes steps with the RAS rhythm. Data were analyzed comparing different groups (mean ± SD) by ANOVA. Results: TUG at the CE with RAS (11.69 ± 4.28) decrease time required to complete the test compare to TUG without RAS in the same condition (13.47 ± 4.65, ***$p < 0.001$). Furthermore, TUG with RAS at the CE was minor respect to the baseline condition (13.39 ± 4.33, *$p < 0.05$). Regarding to 10 MWT, the use of RAS at CE increased significantly the gait speed (1.18 ± 0.35), compared to walk at CE without RAS (1.06 ± 0.31, **$p < 0.01$), and to baseline condition (1.12 ± 3.24). Conclusion: These findings show that the functional mobility and gait of PD patients could be improved with the application of RAS at CE. The differences on behavior between different conditions could be compensated by the use of RAS. These sensory inputs may be considered as useful tools to improve the mobility of people with PD.

43. IMPACT OF MICROCYSTIN IN DIFFERENT RAT BRAIN AREAS AFTER CHRONIC EXPOSURE.OXIDATIVE STRESS AND ANTIOXIDANT RESPONSES (R8)

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Introduction: Freshwater cyanobacteria produce microcystins (MCs), which pose a significant threat to drinking water quality worldwide. While MCs are primarily known as hepatotoxins and tumor promoters, their potential neurotoxicity is gaining increasing attention. Consequently, MCs have the capacity to induce substantial behavioral and morphological changes, neuronal loss, and oxidative stress at the brain level. Objectives: The aim of this study was to assess the impact of D-Leu1MC-LR on various areas of the rat brain (striatum, cortex, cerebellum, and hippocampus) in terms of oxidative stress. Methods: In two independent experiments, a single dose of D-Leu1 MC-LR at 10 and 75 µg/kg (total dose, administered intraperitoneally) was given every 4 days for 21 days in acute exposures. Results: When low MC doses were administered, a notable reduction in lipid damage, as determined by thiobarbituric acid reactive species (TBARS), was observed in both the striatum and cortex. However, no significant differences were found compared to the control for the striatum with high MC doses. Additionally, there were no significant differences in lipid damage in the cerebellum and hippocampus at any MC dose. An increase in reactive species, as determined by the oxidation of dichlorofluorescein diacetate (DCFH-DA), was observed in all areas except the cerebellum when exposed to low MC doses. For these doses, a significant decrease in catalase (CAT, an enzymatic antioxidant) was observed in all areas except the hippocampus. In both doses, a differential accumulation of MCs was determined, with cerebellum and hippocampus showing significantly higher levels than the control. Conclusion: In summary, increased levels of MCs were observed in the cerebellum and hippocampus without associated lipid damage, possibly due to catalase consumption or other unidentified antioxidants. Conversely, in both the cortex and striatum, lipid damage was lower compared to the control, which

coincided with decreased catalase activity and increased reactive species in the presence of MCs. These findings collectively emphasize the necessity for further research on MC neurotoxin toxicity.
Keywords: cyanobacteria – microcystin – hippocampus – cerebellum - oxidative stress – rat brain

26/10 Screen 2 • GENETICS - GENE THERAPY
15:30 - 17:30 HS

44. EFFICIENCY OF BACULOVIRAL TRANSDUCTION IN CHEMICALLY MATURED HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED CARDIOMYOCYTES AS A MODEL FOR CARDIOREGENERATIVE GENE THERAPY (R6)

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Introduction: Cardiovascular disease is the leading cause of mortality worldwide. From this perspective, cardiomyocytes (CMs) derived from human induced pluripotent stem cells (hiPSC-CMs) have emerged as a powerful tool for modelling cardiopathies and evaluating therapeutic strategies. Although their immature phenotype is a major limitation, many approaches are being developed to improve their maturation and turn bench discoveries into potential therapeutic strategies.
Objective: To determine the optimal multiplicity of infection (MOI) of baculoviral (Bv) transduction in a culture of chemically matured hiPSC-CMs for subsequent screening of gene therapies for cardiac regeneration. Methods: hiPSC were cultured and differentiated into pure immature CMs (day 21). They were then cultured until day 37 in low glucose medium supplemented with hormone T3, dexamethasone, PPARα agonist and palmitic acid (mature) or in RPMI medium supplemented with B27 (control). RNA samples were obtained and gene expression analysis of CMs molecular markers was assessed by RT-qPCR. Mature and control hiPSC-CMs were transduced with a GFP reporter Bv (BAC-GFP) at different MOIs; 0, 250, 500, 750 for both conditions and 1000 for mature only. GFP expression was monitored by fluorescence microscopy every 24 h until 96 h post-transduction and assessed by flow cytometry at 48 and 96 h post-transduction. Results: In contrast to the control, mature hiPSC-CMs had significantly increased expression of metabolic (COX6A2, CPT1B) and ion transport (RYR2, ATP2A2, CX43) genes, and an enhanced switch of structural protein isoforms from immature to mature (MYL7 to MYL2 and TNNI1 to TNNI3). Furthermore, they required MOIs ≥ 750 to achieve transduction percentages of around 80%, whereas under control conditions this was achieved with a MOI of 250. Conclusion: Using these culture conditions, we obtained a robust model of hiPSC-CMs with a high maturation gene profile within 40 days of culture and with a low transduction efficiency, consistent with adult CMs behaviour. We conclude that this is a suitable model for screening potential cardiac regeneration therapies in the adult heart.

26/10 Screen 1 • CARDIOVACULAR PHYSIOLOGY AND HYPERTENSION SESSION I
15:30 - 17:30 HS

45. ROLE OF AMP-ACTIVATED PROTEIN KINASE (AMPK) IN CARDIOPROTECTION MEDIATED BY ISCHEMIC POSTCONDITIONING (R20)

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Introduction: Ischemic postconditioning (IPostC) has been established as a cardioprotective strategy to reduce ischemia (I)-reperfusion (R) injury. The role of AMPK, a key enzyme in the regulation of cellular metabolic homeostasis, in the beneficial effects of IPostC has not been fully elucidated. Objectives: The aim of the present study was to investigate the role of AMPK in the cardioprotective effects exerted by IPostC, in Langendorff perfused rat hearts subjected to I-R. Methods: Isolated hearts from female Wistar rats (220-270 g) fed ad libitum were perfused and subjected to 25 min I and 60 min R. IPostC (7 cycles of 5 sec R-I) was induced at the onset of R. In order to inhibit AMPK, Compound C (CC 20µM) was added during the first 5 min of R. To evaluate contractile function, left ventricular developed pressure (LVDP), rate-pressure product (RPP), peak rate of contraction and relaxation (±dP/dt), and left ventricular end-diastolic pressure (LVEDP) were measured. Infarct size was determined by TTC method. AMPK activation (pThr172-αAMPK/total-αAMPK ratio), GSK-3ß inactivation (pSer9-GSK3ß/total-GSK3ß ratio), and PGC-1α expression were studied by Western Blot. Mitochondrial structure was analyzed by electron microscopy and mitochondrial isolated function was evaluated by rate of ATP synthesis, respiratory complexes I-III, II-III and IV

activities, and Calcium retention capacity (CRC). ANOVA, n=8/group. Results: Contractile function was improved by IPostC and infarct size was reduced (p<0,05 vs Control). IPostC increased AMPK activation, mitochondrial ATP synthesis rate, tissular ATP content (p<0,05 vs Control), and preserved mitochondrial structure. IPostC also improved CRC, and complex I-III activity (p<0,05 vs Control). GSK-3ß inactivation was increased by IPostC (p<0,05 vs Control). These beneficial effects were all reversed by AMPK inhibitor, CC. Expression of PGC-1α, an indicator of mitochondrial biogenesis, showed no significant differences between groups. Conclusion: These findings suggest that AMPK could be involved in the beneficial effects of IPostC, contributing to the preservation of contractile function, as well as mitochondrial structure and function.
TOPIC AREA: CARDIOVASCULAR PHYSIOLOGY-HYPERTENSION

46. NHE1 AND MITOCHONDRIAL DYSFUNCTION (R41)

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Fisiología cardiovascular – Hipertensión
Introduction: Mitochondria play vital roles in both energy production and pathogenesis. Their dysfunction is linked to various diseases, such as heart failure and diabetes. NHE1, a key alkalinizing mechanism in the heart, is hyperactive in heart conditions like diabetic cardiomyopathy. In obese and diabetic mice (ob/ob), mitochondrial NHE1 expression increases despite similar cardiac levels, along with disrupted calcium handling, which can be restored with an NHE1 inhibitor. Objectives: understanding the consequences of increased mitochondrial NHE1 expression. Methods: Mitochondria were isolated from ob/ob mice. Δψm was measured with Rhodamine 123. PTP opening was assessed by monitoring Ca+2 release through the CRC assay with CsA as an inhibitor. Mitochondrial Ca+2 content was determined using Fluo-3 and a chemical assay. For the in vitro model HEK293T cells were transfected with pmitoNHE1, and Δψm was quantified as JC-1 aggregate/monomer fluorescence. PTP opening was measured by the release of calcein. ATP levels and mitochondrial ROS production were evaluated in both transfected cells and isolated mitochondria using a commercial ATP assay kit and H2DCFDA indicator, respectively. Data were analyzed with Student's t-test (means ± SEM). Results: In isolated mitochondria from ob/ob mice, membrane hyperpolarization and reduced calcium content were observed, accompanied by a decrease in ATP content and an increase in ROS. These changes came together with an increased sensitivity for PTP opening. In the in vitro model, mitochondrial NHE1 overexpression resulted in mitochondrial membrane hyperpolarization, accompanied with an increased ROS production and more sensibility for PTP opening. These cells also presented lower ATP levels. Conclusion: our results show a correlation between increased mitochondrial NHE1 expression with altered mitochondrial membrane potential, ROS production and ATP levels.

47. EFFECTS OF ACUTE ISOSTEVIOL ON MITOCHONDRIAL STATUS FROM HEARTS SUBJECTED TO ISCHEMIA-REPERFUSION: IMPLICATION OF PROTEIN KINASE B (AKT). (R48)

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Introduction: Recent investigations have highlighted the cardioprotective properties of isosteviol (I), although the underlying mechanisms remain unclear. Previous studies carried out in our laboratory have shown that acute pre-ischemic administration of I (5 µM), promoted Akt activation and enhanced certain functional aspects and mitochondrial morphology in rat hearts exposed to IR. Objectives: The aim of the present work was to investigate the mitochondrial dynamics and some aspects of the functionality of mitochondria from hearts subjected to IR, treated with I; using succinate as a metabolic substrate. Methods: Langendorff perfused hearts from female Wistar rats (200-250g) fed ad libitum were used. Wortmannin (W, 100 nM), a PI3K/Akt inhibitor, was administered before ischemia and administration of I. The function of isolated mitochondria (ATP synthesis rate, O2 consumption, and membrane potential) was evaluated using succinate as metabolic substrate. Calcium retention capacity (CRC) was analyzed with Calcium Green-5N. Using Western blot, the amounts of PGC1α, TFAM, S-OPA/L-OPA ratio and mitochondrial DRP were examined. ANOVA n=5. Results: Pre-ischemic mitochondria treated with I exhibited a higher ATP synthesis rate (p<0.05 vs. control and W), which was reversed with the addition of W. Likewise, a lower O2 consumption was observed with succinate in mitochondria treated with I both preischemic and subjected to IR (p<0.05 vs control and W). This, with better preserved respiratory control post IR in groups I and I+W (p<0.05 vs control and W) and a greater mitochondrial

membrane potential in state 4 (p<0.05 vs control). CRC was significantly higher in group I post IR (p<0.05 vs. Control and W), being only partially reversed with the addition of W. An increase in mitochondrial DRP was observed in group I and S-OPA/L-OPA ratio increased with the addition of W (p<0.05 vs control and I). Conclusion: These results demonstrate that acute I could intervene in mitochondrial dynamics and preserve mitochondrial respiration after IR with increased mitochondrial membrane potential and CRC when using succinate. These effects could be only partially mediated by Akt.
TOPIC AREA: CARDIOVASCULAR PHYSIOLOGY-HYPERTENSION.

48. EFFECT OF EARLY MATERNAL SEPARATION ON BLOOD PRESSURE REGULATION IN MALE AND FEMALE MICE (R76)

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Introduction: Evidence in the experimental and clinical field have shown that during sensitive periods of ontogeny, certain perinatal stimuli may induce “differential programming effect” on homeostatic systems, altering their response even during adulthood. Objectives: We sought to analyze the influence of sex and early maternal separation (EMS) programming on mean arterial pressure (MAP) regulation in response to intravenous vasopressin and hypertonic NaCl continuous infusion in adult MF1 male (M), female in diestrus and in female in proestrus. Methods: In the EMS group, litters were daily separated from their dams for 3 hours each day between postnatal day 2 until day 14, while in the control groups (CON) the offspring remained with their dams. In adult male, female in diestrus and in proestrus mice aged 73 to 83 days old (from EMS and CON groups), changes in blood pressure induced by intravenous 30 min-continuous infusion of vasopressin (1; 00µl 0.01 IU/100ul) and hypertonic NaCl solution (100µl, 3.4 M NaCl) were analyzed. Results: The statistical analysis showed that, although vasopressin continuous infusion induced the expected increase in MAP in all groups, no differences among EMS and CON groups were observed in males, however significant differences attributable to the interaction of estrous cycle (proestrus and diestrus), treatment and time were observed in females {F(8,192)= 2.535, p=0.02}; with females in diestrus-CON group presenting a smaller increase in MAP when compared to female in diestrus-EMS and female in proestrus-CON groups. Furthermore, the continuous infusion of hypertonic NaCl in female in proestrus resulted in a significant effect of the interaction of the time and treatment factors {F(12,103)=3.1444, p=0.00073}, with a decrease in MAP in the EMS group from 15 minutes after ending NaCl hypertonic infusion when compared to the CON group. However, in male and female in diestrus, no differences among EMS and CON groups were observed. Conclusion: These results demonstrate sex differences in blood pressure regulation in CON and EMS groups, with an influence of the estrous cycle in females on MAP response to both AVP infusion and osmotic challenge.
TOPIC AREA: CARDIOVASCULAR PHYSIOLOGY-HYPERTENSION

49. MYOCARDIAL IGF-1 TARGETS IN ACUTE AND CHRONIC TREATMENT OF HYPERTENSIVE RATS (R88)

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Introduction: Essential hypertension promotes oxidative stress and mitochondrial dysfunction. Exercise promotes cardioprotection in part due to increased IGF-1 plasma levels. Whether IGF-1 affects the mitochondria phenotype in an acute/chronic way is still unknown. Objectives: To determine the beneficial effects of exercise/IGF-1 on the mitochondria and reactive oxygen species production in the hypertensive myocardium in both chronic and acute models. Methods: Spontaneously hypertensive rats (SHR) were randomized into sedentary (S) or trained by 8-week swimming routine (T) groups. Also, SHR cardiomyocytes were exposed for 15 min to 10 nM IGF-1 with/without an IGF1R antagonist (100nM AG1024). mRNA and phosphorylation levels, ROS production, and mitochondrial membrane potential (ΔΨm) were quantified in left ventricle, isolated mitochondria, and cardiomyocytes. Results are shown as mean±SEM (n) and statistically different, otherwise p-value is stated. Normality was assessed and t-tests or two-way ANOVA were performed. Results: Chronic model. Training increased IGF1R (%S, T: 144.4±18.5 (6)), but not IGF-1 mRNA; diminished ROS production both in left ventricle ((%S, T: 63.74±7.26 (11)) and energized mitochondria (F/min*mg, S: 0.0090±0.0006 (5), T: 0.0038±0.0009 (5)); enhanced ΔΨm (mV, S: -157.6±9.2 (5), T: -183.6±2.9 (5)); and it also increased the phosphorylation of Akt (%S, T: 123.4±6.3 (5)) and its downstream target GSK3β (%S, T: 159.1±22.1 (10)). Acute model. Basal ROS production was not changed by IGF-1 with/without AG1024. H2O2-induced ROS production was prevented by IGF-1 (F/min, 0.0017±0.0006 (6)) but not in the presence of AG1024 (F/min, 0.0025±0.008(6)). Mild mitochondrial depolarization (FCCP 500 nM) did not change ROS production. Mild oxidative stress had no effect on ΔΨm. Mitochondrial depolarization was not avoided by acute IGF-1 treatment. Conclusion: Training -probably through IGF-1- improved oxidative stress and ΔΨm in SHR myocardium. Acute IGF-1 treatment prevented cardiomyocyte ROS damage but not

mitochondrial insult. IGF1R/Akt/GSK3β pathway seems to be the responsible for ROS protection, but other pathways might be involved in mitochondrial improvement.
TOPIC AREA: CARDIOVASCULAR PHYSIOLOGY-HYPERTENSION

26/10 Screen 1 • RENAL PHYSIOLOGY
15:30 - 17:30 HS

50. MECHANISMS INVOLVED IN ANGIOTENSIN TYPE 2 RECEPTOR (AT2R) NEPHROPROTECTIVE ROLE DURING ISCHEMIA/REPERFUSION KIDNEY INJURY (R17)

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Introduction: Ischemia/reperfusion (IR) kidney injury is associated to microtubule alterations in proximal tubular cells. AT2R stimulation elicits nephroprotective effects in IR. Using a tubular cell model, we found that pre-stimulation of AT2R for 24h increased the relative abundance of acetylated α-tubulin (ac-tub) and partially prevented cell damage in IR. We further found that the increase in ac-tub levels induced by inhibition of Histone Deacetylase 6 (HDAC6) elicited similar protective effects. Therefore, we hypothesized that AT2R stimulation induces a preconditioning effect by increasing ac-tub levels in renal tubular cells. Protein Kinase B (AKT) is involved in the regulation of cell survival. Activated AKT (pAKT) inhibits Glycogen Synthase Kinase 3 (GSK3), which is a HDAC6 activator. Objectives: To evaluate the effect of AT2R agonist C21 on ac-tub levels in the rat and to investigate the role of AKT in C21 signaling during IR. Methods: Wistar male rats were pretreated with AT2R agonist C21 0,3 mg/kg/day or vehicle for 24 h and kidneys homogenates prepared in sucrose 0.25 M. MDCK cells were incubated in serum free/ATP depletion media for 90 min and re-incubated with full media for 2 h (IR) at 37 °C. 24 h before IR, cells were pre-incubated with AT2R agonist C21 (1 µM) or GSK3 inhibitor SB216361 (20 µM, SB). Relative ac-tub and pAKT levels were analyzed by western blot. Cell viability was tested by Trypan Blue exclusion. Results are expressed as media ± SE. *p<0.05 vs control (C) not subjected to IR (basal, B), #p<0.05 vs C-IR. Results: In vivo experiments showed that AT2R stimulation increased ac-tub levels in kidney homogenates (C-B: 0.42±0.02; C21-B: 1.05±0.05*; n=3). In MDCK cells, C21 treatment increased pAKT levels (C-B: 0.51±0.07; C21-B: 0.84±0.09*; n=8). GSK3 inhibition partially prevented IR-induced loss of cell viability (C-B: 88±1; C-IR: 55±2*; SB-B: 82±3; SB-IR: 67±3*#; n=5) and increased ac-tub levels (C-B: 0.67±0.03; SB-B 1.12±0.03*; n=3). Conclusion: Our results confirm that AT2R stimulation increases α-tubulin acetylation in renal tissue, and suggest that AT2R preconditioning effect in renal tubular cells is mediated by AKT/GSK3 pathway.
TOPIC AREA: RENAL PHYSIOLOGY

51. ON THE ROAD TO THE PREVENTION OF HEMOLYTIC UREMIC SYNDROME: DISCOVERY, SYNTHESIS AND TESTING OF CANDIDATE MOLECULES TO INHIBIT THE ACTION OF SHIGA TOXIN TYPE 2. IN SILLICO AND IN VITRO RESULTS (R79)

Gioia D.S[1], Casal J.J[1], Mollo M.C[2], Beltramone N[1], Bollini M[2], Roitberg A[3] and Toriano R[1].
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Introduction: The main cause of Hemolytic Uremic Syndrome (HUS), an endemic disease in Argentina clinically characterized by microangiopathic, hemolytic anemia, thrombocytopenia and acute kidney injury, is infection by Shiga toxin (Stx)-producing E. coli (STEC). HUS is mainly associated with Stx2, which inhibits protein synthesis triggering proapoptotic mechanisms and causing the disease. Currently, there is no specific treatment to prevent the progression of HUS. The objectives of our work were i) to obtain molecules with anti-Stx2 activity that can be transformed into drugs and ii) to test the ability of these molecules to decrease Stx2 cytotoxicity in HK2 and VERO cells. Methods and Results: We started from the crystallized structure of Stx2 (id:1R4P, PDB) and used a fast and inexpensive methodology, structure-based drug design (SBD), to select molecules from different databases. From the molecular docking score and subsequent molecular dynamics refinement performed with the Stx2-drug complex, a total of 20105 molecules were analyzed and ranked. Among them, we chose the best candidate molecules, in terms of binding free energy (ΔGu) values calculated with the PBSA method and synthesized 4 drugs from the in-house database (Group A) and acquired 2 drugs from the FDA-approved database (Group B). For these drugs, the ΔGu values (kcal/mol) were A1:-14.62; A3:-12.60; A16:-16.67; A24:-19.45; B3:-7.96 and B18:-18.13. When in vitro assays of inhibition of

Stx2 cytotoxicity by these drugs were performed, the results obtained showed significant differences between the viability of cells treated only with Stx2 (IC50= 1.0 - 2.5 ng/ml for HK2 and VERO cells) vs Stx2 in the presence of drugs, with the following detail: A3 (10-2µM $p<0.05$, n=8, HK2) and B18 (10-3µM in HK2, $p<0.05$, n=6, and 10-1µM in VERO, $p<0.0001$, n=8). Furthermore, when we tested the effect of prior co-incubation of Stx2 with B18 (20min, 37°C) and subsequently exposed VERO cells to the Stx2-B18 mixture, we observed a significant increase in cell viability over cells exposed to Stx2 alone. In conclusion, our results demonstrate antiStx2 activity for at least two of the tested and SBD-selected drugs.
TOPIC AREA: RENAL PHYSIOLOGY

52. THE USE OF COMPUTATIONAL METHODS TO DESIGN STRATEGIES FOR THE PREVENTION OF HEMOLYTIC UREMIC SYNDROME (R81)

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Introduction: Hemolytic Uremic Syndrome (HUS) is a severe, acute-onset systemic disease. It is endemic in Argentina, with more than 300 new cases per year. It is the leading cause of acute kidney disease in pediatric patients, causing renal and neurological complications. Shiga toxin-producing E. coli (STEC), in particular serotype O157:H7, is the main etiological agent. HUS is mainly associated with STEC producing Shiga toxin type 2 (Stx2). Stx2 inhibits protein synthesis and triggers proapoptotic mechanisms, which subsequently cause disease. Currently, there is no specific treatment to prevent the progression of HUS. Objective: Our aim is to find Stx2 inhibitor molecules that can be transformed into drugs to prevent the development of the disease and its sequelae. Methods and Results: Starting from the described structure of Stx2 (id:1R4P, PDB) and applying the structure-based drug design method combined with molecular docking and molecular dynamics strategies, we found three good candidate compounds, named A3, A24 and B18, which showed significant effects on the viability of cells exposed to the toxin. Based on the promising results of A3 in renal cell lines, and to improve the structure of the molecule -which has a 2,4-diphenyl quinoline core- we designed eight compounds for possible synthesis in our laboratory, using currently available reagents. In addition, we performed a search in the PubChem database, employing Lipinski's rules as a filter and using a search box centered on the amino acid glu 167, strategically located in the active site of the toxin. This search yielded a set of 2408 molecules. All these structures, both designed and selected, were subjected to rigorous virtual screening using VINA 1.2.3 software. We ranked the results according to their binding free energy (ΔGu). For the 8 designed compounds, the mean±SD and median values were (-9.4 ± 0.5) kcal/mol and -9.3 kcal/mol respectively. For the 2408 molecules, the values were (-9.6 ± 0.8) kcal/mol and -9.5 kcal/mol respectively. Conclusions: Using in sillico strategies, we have obtained a set of improved molecules, to test their ability to inhibit Stx2 in human and green monkey renal cells. TOPIC AREA: RENAL PHYSIOLOGY

53. SEX DIFFERENCES IN THE URINARY BIOMARKERS IN RESPONSE TO ISCHEMIC ACUTE KIDNEY INJURY (R84

Fussi MF[1,2,3], Rivabella-Maknis T[3], Bulacio RP[1,2], Hazzelhoff MH[1], Marquez SB[4], Campagno R[1], Molinas JL[5], Brandoni A[1,2], Monasterolo LA[1,2], Larocca MC[3], Molinas SM[1,2,3]
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Introduction: Ischemia reperfusion (IR) is one of the major causes of acute kidney injury (AKI). In rats, female sex provides protection against IR. Neutrophil gelatinase-associated lipocalin (NGAL) is a promising urinary biomarker that is secreted by tubular cells during AKI. Previously, we found that female rats had increased urinary NGAL excretion (uNGAL) levels, even though they had less damage by IR. Further studies were necessary since there is limited research on female animals and the differences in urinary biomarker excretion during AKI are unclear. Objectives: Our goal was to examine the renal expression of NGAL in male and female rats following IR damage and to investigate another urinary biomarker such as kidney injury molecule-1 (uKIM-1) in the same experimental model. Methods: Male (MIR) and female (HIR) Wistar rats (n=6 per group) underwent 40 min of unilateral renal ischemia followed by 1 day of reperfusion. Controls underwent sham operations (MC and HC). Renal NGAL was evaluated by western blot and immunohistochemistry (IH). uKIM-1 was studied by ELISA. Results: IH studies indicated that IR damage produced an increase in NGAL expression in renal cortex, that was greater in HIR than in MIR (+2232%# HIR vs HC; +1433%# MIR vs MC; +169%* HIR vs MIR). In the renal medulla, NGAL expression was higher in MIR (+4133%# vs MC), while in HIR it was not statistically different compared to HC. Similar results were obtained by western blot. uKIM-1 levels were higher in HIR than in MIR (+1101%f HIR vs HC; +262%f HIR vs MIR). *$p<0.05$ #$p<0.01$, fp< 0.001. Conclusion: We found that renal NGAL expression and localization in response to IR is different according to sex, which could be responsible for the differences in

uNGAL observed in response to IR damage. Similarly to uNGAL, the minor damage found in female rats does not correlate with their increased uKIM-1 levels, which suggest a sexual dimorphism in these biomarkers excretion in response to IR kidney damage and reveals the need of further studies for its better clinical application as biomarkers.
TOPIC AREA: RENAL PHYSIOLOGY

26/10 Screen 2 • ENDOCRINOLOGY, METABOLISM AND REPRODUCTION SESSION I
15:30 - 17:30 HS

54. FRIED SUNFLOWER INTAKE DURING GROWTH PROMOTES STEATOSIS AND OXIDATIVE STRESS IN LIVER.(R7)

Touceda V[1,2], Macri E[1], Wiszniewski M[1,3], Cacciagiú L[1,4], Ramos C[1], Morales C[5], Miksztowicz V[1,2], Friedman S[1].
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Introduction: Our previous studies in growing male rats indicated that a diet rich in fried sunflower oil (SFOx) affected the liver oxidative stress in terms of increased oxidation of 2',7' dichloro-fluorescein diacetate (DCFH-DA) levels and DCFH-DA/catalase index, concomitant with reduced amounts of catalase. Moreover, the hepato-somatic index (HSI) advised about liver damage. Objective: In this study we evaluated the effect of a SFOx diet on hepatic histology as a risk of liver disease. Methods: Male weaning Wistar rats (n=21) were fed the following three diets for 8 weeks. A standard chow diet (C), SFO or SFOx diet. SFO and SFOx were mixed with commercial rat chow. SFO was repeatedly heated for a total of 40 hours (SFOx). Food and water were offered ad libitum; total body weight and food consumption were recorded weekly. At wk.=8, animals were euthanized, blood samples for serum lipids (T-Chol and triglycerides; mg/dL) were obtained by cardiac puncture and livers were removed and weighed for hepato-somatic index (HSI%; organ mass(g)/body mass(g)%); also liver tissues samples were fixed in 10% buffered formalin for paraffin preparation for Heamtoxilin & eosine (H&E) tinction to evaluate the grade of steatosis. Substances reactive to thiobarbituric acid (TBARS; uM/mgprot) and oxidized proteins content (nmoles/mg prot) were determined. Results: SFOx showed increase in HIS% (C=3.66±0,33; SFO=3.64±0,27; SFOx= 4.17±0,34;p<0.016), the content of oxidized proteins (C=0,06±0,01; SFO=0,02±0,01;SFOx=0,15±0,03;p<0.001) and T-Chol serum levels (C=51.8±4.0;SFO= 46.0±3.9; SFOx=63.2±4.1;p<0.001). Triglycerides concentrations and TBARS were similar (p=0.058 and p=0.154, respectively); however, TBARS were higher in SFOx rats than in SFO group (5.72±0.98 vs.4.56±1.03; p=0.04). In SFO, mild (60%) and moderate (20%) steatosis was detected while in SFOx a major prevalence of moderate steatosis (50%) was observed. Conclusion: adverse effects of SFOx consumption may alter liver function as consequence of hepatic steatosis and increase in oxidative stress.
TOPIC AREA: ENDOCRINOLOGY- METABOLISM - REPRODUCTION

55. HIGH SALT DIET INTAKE ALTERS ADIPOSE TISSUE PROPERTIES. LIRAGLUTIDE AS THERAPEUTIC OPTION (R23)

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Introduction: High salt intake (NaCl) is associated with hypertension and cardiovascular disease; however it has been proposed that chronic salt consumption would also alter adipose tissue (AT) behavior. Liraglutide (LGT), a GLP-1 agonist with direct effects on expanded AT, could be a potential therapeutic option. Objectives: to evaluate the impact of high salt (HS) intake on visceral AT (VAT) remodeling and the effect of LGT treatment in an animal model. Methods: Male C57BL/6 mice (8 weeks old) were divided in Control (C, n= 15) fed with standard diet and HS diet group (HSD, n=18) fed a diet with NaCl 8% during 15 weeks. Then, both groups were subdivided according to the subcutaneous administration of LGT (200ug/kg/day) or vehicle (equivalent volume) for 5 weeks. Body weight, food and water consumption were registered weekly and arterial pressure (AP) was

evaluated by plethysmography. Epididymal AT (EAT) was removed and weighed. Histological characteristics were determined in hematoxilin-eosin stained sections and fibrosis by picrosirius red staining. Total collagen content was measured by hydroxyproline assay. Results: HSD group presented a higher water ($p<0.0001$) and caloric ($p<0.05$) intake compared to C and decreased in HSD+LGT group ($p<0.0001$ and $p<0.05$, vs HSD respectively). In HSD and HSD+LGT, body weight gain ($p<0.01$) and EAT mass ($p<0.001$) were significantly lower than C. Adipocyte size was smaller in HSD and HSD+L vs C ($p<0.05$ and $p<0.01$ respectively). HSD presented lower vascular density compared to C (80±13 vs 111±21 vessels/mm2, $p=0.003$) while in HSD+LGT an increase in vascularity was observed (105±16 vessels/mm2, $p=0.04$ vs HSD). HSD group presented an increase in total collagen content in comparison to C (13.9±2.7 vs 6.4±3.4 ug collagen/mg dry tissue, $p<0.01$) and it decreased in HSD+L group (8.6±4.2 ug collagen/mg dry tissue, $p<0.05$ vs HSD). Conclusion: Chronic salt intake alters VAT properties by increasing collagen deposit and decreasing vascular density. LGT administration would improve VAT behavior by reducing fibrosis and improving vascularity modified by high salt consumption. The study was approved by the Ethic Committee of BIOMED.
TOPIC AREA: ENDOCRINOLOGY- METABOLISM - REPRODUCTION

56. AQUAPORIN-9 EXPRESSION IN THE MITOCHONDRIA OF PREECLAMPTIC PLACENTAS (R29)

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Introduction: Preeclampsia is a gestational hypertensive disorder where increased oxidative stress leads to mitochondrial dysfunction. During the syncialization, trophoblast mitochondria undergo morphological and functional changes resulting in two mitochondria subpopulations the "heavy/large" fraction and the "light/small" one. Previously, we described an increase in AQP9 expression in the trophoblast membranes of preeclamptic placentas. However, its function is unknown. Emerging evidence shows that AQP9 may act as peroxyporin. Recently, we found that AQP9 also localizes in the trophoblast mitochondria of normal placentas with a high expression in the “heavy/large” mitochondrial fraction whereas it was almost undetectable in the "light/small" one. Here, we aimed to study the localization of AQP9 in the trophoblasts from preeclamptic placenta.
Materials and methods: Placentas were obtained from healthy-term (n=3) and preeclamptic (n=3) pregnancies. Microsomal and the two mitochondrial fractions were isolated from placental tissue by differential centrifugation. The presence of functional mitochondria was evaluated by MitoTracker. AQP9 expression was analyzed by Western blot. Co-localization of AQP9 and cytochrome-C was assessed by double-immunofluorescence and confocal microscopy. Results: AQP9 expression was significantly increased in the microsomal and in heavy/large mitochondrial fraction ($p<0.05$; n=3) from preeclamptic placentas compared to the normal one. Interestingly, we detected a high AQP9 expression in the light/small mitochondrial fraction from preeclamptic placentas ($p<0.001$; n=3). We also found that AQP9 co-localized with cytochrome-C, in both normal and preeclamptic placentas. Conclusion: Our findings suggest that AQP9 expression rises not just in the plasma membrane but also in both mitochondrial fractions in preeclamptic trophoblasts. Although the role of AQP9 in the placenta remains unknown, its increased expression in preeclampsia may be related to oxidative stress. Further studies are required to determine its function and the consequences of its dysregulation.
TOPIC AREA: ENDOCRINOLOGY- METABOLISM - REPRODUCTION.

57. DETECTION OF AQUAPORIN 3 IN EXTRACELLULAR VESICLES OF PLACENTAL EXPLANTS AND IN MATERNAL PLASMA AS A POTENTIAL BIOMARKER OF PREECLAMPSIA (R35)

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Introduction: Preeclampsia (PE) is a human gestational syndrome associated with placental insufficiency and an increased release of extracellular vesicles (EVs) from the syncytiotrophoblast into maternal circulation. Aquaporin 3 (AQP3) is involved in trophoblast migration and its expression is decreased in placentas with PE. Objectives: To validate a method to detect AQP3 in EVs from maternal plasma and placental explant culture supernatant to evaluate the potential utility of AQP3 as a biomarker of PE. Methods: This study was approved by the Ethics Committee of the Hospital Naval de la Ciudad de Buenos Aires. EDTA-anticoagulated maternal blood and placentas from normal and PE pregnancies were collected under informed consent.

Placentas were obtained immediately and processed within one hour after cesarean section. Explants of normal and PE placentas were prepared, cultured 18 h at 37°C, and the culture medium was collected. Plasma and explant EVs were obtained by differential centrifugation, filtration and ultracentrifugation. Samples enriched in EVs were characterized by DLS, NTA, transmission electron microscopy and western blot to analyze the presence of CD63 and HSP70. Protein expression of AQP3 was determined in all cases. Placental alkaline phosphatase (PLAP), syncytiotrophoblast marker, was then analyzed to confirm the presence of EVs of placental origin in plasma EV samples. Results: Preliminary results show that samples enriched in EVs were obtained, EVs of placental origin were present in plasma EVs and that AQP3 was detectable in both plasma and explant EVs samples. In addition, AQP3 content was increased in EVs from PE explants. Conclusion: This work lays the foundations to evaluate whether AQP3 is differentially expressed in placental-released EVs under normal and pathological conditions. If these changes are in turn reflected in the AQP3 content of placental EVs in maternal plasma, AQP3 could be a potential candidate PE biomarker.
TOPIC AREA: ENDOCRINOLOGÍA, METABOLISMO - REPRODUCCIÓN

58. EFFECT OF MELATONIN-TREATED TROPHOBLAST EXTRACELLULAR VESICLES ON PLACENTAL ENDOTHELIAL CELL MIGRATION (R57)

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Introduction: Placental angiogenesis is indispensable for successful gestation and it is regulated by trophoblast paracrine signaling. In this sense, placental extracellular vesicles (EVs) have been recognized as a major mediator of feto-maternal communication and have been involved in placental angiogenesis regulation. Trophoblast cells synthesize large amounts of melatonin and express its receptors. Melatonin acts in an autocrine and paracrine manner in this organ, prevents the injury produced by oxidative stress and regulates the expression of vascular endothelial growth factor (VEGF). Here, we investigated whether treatment of trophoblast with melatonin can change the content of released placental EVs and modify the biological functions of placental endothelial cells. Materials and methods: This study was approved by the Ethics Committee of Naval Hospital. Placentas were obtained from healthy-term pregnancies (n=3). Placental explants were cultured with and without melatonin (1-20 µM). Tissue viability was evaluated by the MTT assay. Placental EVs were isolated by differential centrifugation, filtration, and ultracentrifugation. EVs were characterized by DLS, NTA, TEM, and western blot for CD63 and HSP70. The EVs-VEGF expression was evaluated by western blot. EA.hy926 placental endothelial cells were treated with placental EVs for 24h and cell migration was assessed by wound healing assays. Results: Trophoblast viability was not altered by the different melatonin concentrations analyzed. VEGF expression was significantly decreased in EVs cultured with 20 µM of melatonin ($p<0.05$; n=3). EVs cultured with 20 µM of melatonin significantly reduced endothelial cell migration ($p<0.05$, n=3). Conclusion:
This study demonstrates that melatonin reduces the amount of VEGF carried by EVs released from trophoblast cells and modulates the placental endothelial cells migration.
TOPIC AREA: ENDOCRINOLOGY- METABOLISM - REPRODUCTION.

59. IMPACT OF HYPEROSMOLARITY ON PLACENTAL ANGIOGENESIS AND CAVEOLIN-1 EXPRESSION (R64)

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Introduction: The establishment of a successful pregnancy requires proper development of placental vasculature, which includes macrovasculature and microvasculature, and the coordinated regulation of vascular processes. In this context, the placenta can act as a sensor of fetal metabolic demands, inducing changes in its vasculature to ensure fetal growth and well-being. Cellular stress during pregnancy, including hyperosmolar stress, can impact the normal development of placental vasculature. Caveolin-1 (Cav-1), a constitutive protein of caveolae, plays a pivotal role in cell signaling. Our hypothesis is that hyperosmolar stress disrupts placental angiogenesis, and Cav-1 participates in this process. Objective: Our objective was to

assess the effect of hyperosmolarity on placental migration and tubulogenesis, along with the expression of Cav-1 under these conditions. Materials and Methods: This study received approval from the ethics committee of the Hospital Nacional Prof. Dr. A. Posadas. Placental microvascular endothelial cells (hPMEC) and the EA.hy926 cell line (ATCC® CRL-2922™) were used. Cells were treated with a sucrose solution (100 mM) to induce hyperosmolarity. Cav-1 expression was evaluated through RT-qPCR and western blot analysis. Cell migration was assessed via wound healing assays, and angiogenesis was evaluated through tube formation assays. Results: Hyperosmolar stress significantly reduced cell migration ($p<0.05$, n=4) and tubulogenesis ($p<0.05$; n=3) in hPMEC and EA.hy926 cells compared to iso-osmolar controls. Gene and protein expression of Cav-1 significantly decreased under hyperosmotic conditions in hPMEC cells ($p<0.05$; n=3), while it increased significantly in hyperosmolarity in EA.hy926 cells ($p<0.01$, n=3). Conclusion: These findings suggest that hyperosmolarity diminishes placental angiogenesis and alters the expression of Cav-1, consequently affecting caveolae structure. This, in turn, may impact several processes and signaling pathways present within these structures.
TOPIC AREA: ENDOCRINOLOGY- METABOLISM - REPRODUCTION.

60. ANANDAMIDE MODULATES OXYTOCIN RECEPTOR EXPRESSION IN HUMAN PLACENTA (R66)

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Introduction: Oxytocin exerts its effects through the oxytocin receptor (OTR), playing a pivotal role in the onset of labor. However, the intricate relationship between oxytocin (OT) and its receptor in the placenta remains poorly understood. Recent evidence points towards the involvement of anandamide (AEA), a key endocannabinoid, in the regulation of labor. AEA is synthesized within the placenta and exhibits a significant rise in plasma levels during labor, contrasting those undergoing elective cesarean sections. Objectives: The specific objetives were: To investigate OTR expression in normal placentas obtained from vaginal deliveries (VD) and cesarean sections (CS), as well as placentas from women with preeclampsia (PE) at term.
To examine the effect of AEA on oxytocin receptor (OTR) expression within the placenta.
Methods: Placental tissue samples were collected from women at term (37-40 weeks of gestation) from three distinct groups:
VD (n=8) with uncomplicated pregnancies, CS (n=9) without uterine contractile activity, PE placentas (n=8) from women with elevated maternal blood pressure and proteinuria after 20 weeks of gestation. OTR expression was quantified using WB and immunofluorescence. In addition, explants from CS placentas were cultured in the presence of R-(+)-Methanandamide (Met-AEA), a stable AEA analogue, and AM251, a CB1 receptor antagonist. To assess the impact of Met-AEA on cytotrophoblast and syncytiotrophoblast, BeWo cells were cultured with or without Met-AEA for 48 h. Results: Our findings demonstrated that VD placentas exhibit significantly higher protein expression of OTR compared to CS and PE placentas ($p<0.05$). Furthermore, we observed a noteworthy increase in OTR expression in placentas cultured with Met-AEA, which was inhibited by co-incubation with AM251 ($p<0.05$). Notably, Met-AEA exhibited a significant stimulatory effect on OTR expression in syncytiotrophoblast cells, while no discernible effect was observed on cytotrophoblasts. Conclusions: In summary, our study reveals distinct OTR expression patterns between VD and non labouring placentas (CS and PE), providing insights into the influence of AEA on OTR expression in term placenta.
TOPIC AREA: ENDOCRINOLOGY- METABOLISM - REPRODUCTION.

61. INVOLVEMENT OF GRAPE-DERIVED BIOACTIVE COMPOUNDS ON HIGH-FAT DIET INDUCED MICROBIOTA DYSBIOSIS (R71)

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Introduction: Gastrointestinal tract, and in particular the microbiota, plays a very important role in pathologies associated with excess caloric intake, overweight and obesity. Long-term consumption of a high-fat diet (HFD) can affect the composition of the microbiota. Bioactive compounds can have a major influence on gut microbiota composition, and subsequently, on its impact on overall health. Grape pomace extract (GPE), concentrated in polyphenolic compounds, has shown to attenuate metabolic alterations such as dyslipidemia, hypertension and insulin resistance and prevented adipose tissue inflammation in experimental models of metabolic syndrome induced by high-fat and/or high-fructose diets. Objective: To evaluate how dietary intervention with GPE can modulate metabolic parameters and gut microbiota dysbiosis associated with high-fat diets. Methods: Male C57BL/6 mice (20-25 g) were divided into 4 groups (n = 7-8 each) and fed for 14 weeks as follows: i) Control

group (Ctrl): standard diet; ii) Ctrl group + diet supplemented with GPE: 300 mg/kg body weight (bw)/day iii); iv) HF (high fat) group; control diet containing 60% of total calories from fat and v) HF + GPE 300 mg/kg bw/day. Results: Consumption of a HFD resulted in obesity, dyslipidemia and insulin resistance, shown by a significantly increased in body weight and visceral adipose tissue (AT) gain, total and LDL cholesterol levels, fast glucose and insulin levels and HOMA:IR index, respectively. GPE significantly prevented body weight and visceral AT gain and attenuated the altered metabolic parameters. Among microbiota changes associated with HFD consumption, we observed a significant difference on intestinal microbial community between the HF and Ctrl and Ctrl + GPE groups. In addition, HFD increased the mean abundance of Ruminococcaceae bacteria, whereas GPE supplementation to the HFD attenuated its increase. Conclusion: GPE supplementation attenuates HFD-induced metabolic alterations and may contribute to prevent gut microbiota dysbiosis.

62. MODULATION OF THE HEPATIC CHOLESTEROL PATHWAY IN RABBITS FED DIETS HIGH IN SATURATED AND MONOUNSATURATED FATS (R44)

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Introduction: Hepatic cholesterol (cho) accumulation induced by lipid overload is a major public health problem worldwide, and natural products such as Extra Virgin Olive Oil (EVOO) have proven benefits, but the mechanism remains unclear. Sterol regulatory element-binding protein 2 (SREBP2) leads to intracellular cho metabolism as a transcription factor and is sensitive to dietary fat intake. Objectives: Our objective was to analyze the effects of the addition of EVOO to a high-fat diet (HFD) on the expression of molecules of the hepatic cho metabolism pathway using rabbits as an experimental model of hypercholesterolemia (HC). Methods: New Zealand rabbits were fed a commercial pellet (control), a HFD (14% bovine fat, HC group) or HFD plus EVOO (HFD 7% + EVOO 7%: protected rabbits) for up to 12 months. The expression of SREBP2, HMGCR (3-hydroxy-3-methyl-glutaryl-coenzyme A reductase) and LDLR (low density lipoprotein receptor) was to study by western blot and PCR. Results; the results show that cho increased in HC rabbits but decreased in protected animals. SREBP2 mRNA was not modified by HFD although protein expression decreased in the short term, and raised under a long term HFD. When EVOO was added, in both cases the expression increased significantly. HMGCR expression did not vary significantly with HFD, but increased with the addition of EVOO. LDLR mRNA and protein showed increased with both diets. These results indicate that fat intake deregulates SREBP2 expression, leading to lipid accumulation in rabbit hepatocytes. The addition of EVOO prevented fat diet-induced lipid increase despite rising HMGCR and LDLR expression. The former needs further research as it involves many post-translational regulators; and the LDLR increase is reasonable as the hepatocyte is the main cell involved in the removal of plasma cholesterol through LDLR activity. Conclusion: The improvement in hepatic lipid accumulation is probably related to other mechanisms such as bile production. Finally, all the molecules analyzed here were sensitive to EVOO supplementation, although specific studies are needed to determine the exact mechanism of protection.

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63. THYROID STATUS MODULATES THE FORMATION OF BREAST CANCER METASTASIS (R9)

González G[1], Debernardi MM[1], Menay F[1], Díaz Albuja JA[1], Campos Haedo M[1], Paulazo MA[1], Díaz Flaqué MC[1], Rosemblit C[1], Cayrol F[1], Cremaschi GA[1], Sterle, HA[1].

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Introduction: The association between thyroid status and breast cancer progression has not yet been completely clarified. We have previously described that hyperthyroid mice that have been inoculated with the 4T1 breast cancer cell line showed increased primary tumor growth rate, while hypothyroid mice developed a reduced tumor growth rate but an increased number of lung metastases. Objectives: To evaluate the effect of thyroid status on the mechanisms involved in the metastatic process in breast cancer, particularly on the epithelial-to-mesenchymal transition (EMT), the activity of matrix-metalloproteases (MMPs) and the production of chemokines by the tumor; and the composition of the immune microenvironment in the lungs. Methods: Female Balb/c mice were treated with propylthiouracil (PTU) for 2 weeks to obtain hypothyroid mice, or thyroxine (T4) for 4 weeks to obtain hyperthyroid animals. Mice were then orthotopically inoculated with 4T1 cells and after 21 or 35 days, tumors

and lungs were excised and analyzed. Results: Primary tumors from hypothyroid mice exhibited a higher mRNA expression of Vimentin and a lower expression of E-cadherin than tumors from eu- and hyperthyroid animals ($p<0.05$). In these tumors, no differences in the activity of MMP-2 and MMP-9 were detected by gelatinolytic zymography. Additionally, tumors from hypothyroid mice showed increased mRNA expression levels of CCL-5, CCL-2 and CXCL-16 chemokines, when compared with tumors from eu- and hyperthyroid mice ($p<0.05$). Increased secretion levels of CCL-2 were also detected in tumors and lungs from hypothyroid mice ($p<0.05$). The analysis of the distribution of immune subsets by flow cytometry showed a higher proportion of cytotoxic T cells ($p<0.05$) and B cells ($p<0.05$) in lungs from hyperthyroid mice, but an increased proportion of myeloid-derived suppressor cells CD11+Gr1+ ($p<0.05$) in lungs from hypothyroid mice. Conclusion: Our results suggest that thyroid status modulates the development of breast cancer metastasis through the regulation of the EMT process, the secretion of chemokines by the primary tumor and the distribution of subpopulations of immune cells that infiltrate metastatic sites.
TOPIC AREA: ONCOLOGY-INFLAMMATION

64. EFFECT OF THE INHIBITION OF LEUKOTRIENE A4 HYDROLASE (LTA4H) WITH SC-57461A IN LIVER CANCER PROLIFERATION (R18)

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Introduction: Leukotriene B4 (LTB4) is a lipid mediator generated from arachidonic acid through the sequential action of several enzymes, including LTA4H. LTB4 is overexpressed in different types of cancer and plays an important role in cancer cell proliferation. The use of SC-57461A (a specific inhibitor of LTA4H) has been demonstrated to modulate the immune system in different studies; but its effect on liver cancer has not been reported. Objectives: to evaluate the effect of LTA4H inhibition in liver cancer cells proliferation, both in vivo and in vitro. Methods: In vitro studies: Human cell lines: hepatocellular carcinoma Huh7, endothelial EA.hy926 and hepatic stellate LX2 cells. For dose-response studies cells were treated with different doses of SC-57461A for 72 h or left untreated. Cell viability was determined (MTT assay) and the IC50 was calculated. In vivo studies: A xenograft model for liver cancer was generated in athymic mice by injecting 5x106 Huh7 cells into the flank (n=10). Half of animals were treated with SC-57461A 10 mg/kg, by gavage 2 days/week for 2 weeks and the remaining were left untreated (control group). Tumor dimensions were measured with a calliper and volumes were calculated. Tumors were excised and processed for immunohistochemical studies to detect the marker of proliferation PCNA. Results: In vitro studies: Treatment with SC-57461A showed a dose-dependent decrease in cell viability and the following IC50s were established for each cell line: Huh7 259.45 µM, LX2 316.38 µM and EA.hy926 288.38 µM. In vivo studies: Tumor size was significantly reduced in the group treated with SC-57461A (-68%, $p<0.01$) compared with control group. Also, immunohistochemical analyses revealed a decreased staining for PCNA in the SC-57461A group compared to the control group. Conclusions: SC-57461A reduced proliferation both in hepatocellular carcinoma cells and in critical environmental tumor cells. In addition, in vivo administration of the inhibitor reduced tumor size and proliferation in tumor-bearing mice. These results, despite being preliminary, show that the inhibition of LTA4H has potential as a pharmacological tool to inhibit liver tumor growth.
TOPIC AREA: ONCOLOGY-INFLAMMATION

65. INVOLVEMENT OF INSULIN-LIKE GROWTH FACTOR 2 MRNA-BINDING PROTEIN 1 (IGF2BP1) IN ABC TRANSPORTERS MODULATION IN CACO-2 CELL LINE. (R21)

Gola A.M.[1], Bucci-Muñoz M.[1], Livore V.I.[1], Mottino A.D.[1], Rigalli J.P[2], Ceballos M.P.[1], Ruiz M. L[1].

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Introduction: P-glycoprotein (ABCB1) and multidrug resistance associated protein 2 (ABCC2) are canalicular export pumps that are linked to chemoresistance in a variety of tumors due their ability to eliminate the chemotherapeutic drugs out of the cell. IGF2BP1 is an oncofetal RNA binding protein expressed in tumors which was associated with ABCB1 and chemoresistance in ovarian cancer. However, no relationship has been reported between the expression of IGF2BP1 with chemoresistance in colorectal cancer or with the expression of ABC transporters. Objectives: to evaluate ABCB1, ABCC2 and IGF2BP1 protein expression and the role of IGF2BP1 in ABCB1 and ABCC2 regulation. Methods: Caco-2 cells were incubated at 4, 7, 14 or 21 days. IGF2BP1, ABCB1 and ABCC2 protein levels were quantified by western blotting. IGF2BP1 was inhibited with BTYNB. Viability

assay was performed using BTYNB (0-100 µM). For protein expression studies cells were treated, 7 days after seeding, with 10 µM BTYNB during 48 h and DMSO was used as control. Results: All results are presented as mean±SEM, n=3, *p<0,05 vs 4 days. The protein expression of IGF2BP1 at 7(287±37%*), 14(204±36%*) and 21(245±40%*) days after seeding was increased compared to 4 days(100±1%). ABCB1 protein expression was significantly increased at 7(5888±1229%*), 14(2535±617%*) and 21(2489±791%*) days compared to 4(100±1%) days after seeding. ABCC2 protein expression was significantly different at 7(33±1%*), 14(397±19%*) and 21(498±17%*) days after seeding compared to 4(100±1%) days. Cell viability remain unchanged at all concentrations of BTYNB tested. IGF2BP1 protein expression tend to decrease in presence of 10 µM BTYNB(Control: 100±15%, BTYNB group: 65±4%,p=0,1). ABCB1 protein expression tend to increase (Control: 100±11%, BTYNB group: 127±19%, p=0,2). ABCC2 protein expression showed no difference in presence of BTYNB 10 µM. Conclusion: Only ABCB1 and IGF2BP1 exhibit time-dependent protein expression positive association in Caco-2 cells. Higher inhibitor doses are needed for significant IGF2BP1 inhibition. Further studies are required to explore the role of IGF2BP1 in ABCB1 regulation.

66. EXPRESSION OF IMMUNECHECKPOINT HUMAN LEUKOCYTE ANTIGEN G IN A CHORIOCARCINOMA CELL LINE CULTURED IN HYPOXIC MICROENVIRONMENT (R32)

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Introduction: The immunecheckpoint HLA-G is a non-classical MHC class I molecule that modules negatively the local immune response. This protein has restricted tissue expression in physiological conditions. However, its expression can be ectopically induced under malignant cell transformation in several tumors. The quick growth of tumor cells creates a hypoxic environment where low oxygen levels lead to the accumulation of hypoxia inducible factors (HIF), which modulate gene expression of angiogenesis, cell proliferation and migration further increasing the tumor development. In this context, HIF could positively modulate the HLA-G expression in tumor cells to evade the immune system. Objective: To evaluate the effect of hypoxia on HLA-G expression in a choriocarcinoma cell line called JEG-3. Methods: JEG-3 were incubated with deferrioxamine 200µM (DFX an iron chelator mimicking hypoxia) for 6, 24, 48 and 72 h in DMEM + 10%FBS. HIF and HLA-G expression was measured by western blot (WB), flow cytometry (FC) and RT-qPCR. Results: The effect of DFX was analyzed by WB determining HIF-1α and -2α isoforms at different incubation times. HIF-1α levels increased gradually in a time-dependent manner, but HIF-2α only showed increased values at 6 and 24 h of incubation. Then, HLA-G expression was analyzed. RT-qPCR showed an increment of HLA-G level after 6 and 48 h with DFX (1.8-fold change compared to control cells), however after 24 and 72 h with DFX, the expression levels decreased. By FC, 63% of control JEG-3 were positive for membrane-bound HLA-G, while 64, 75, 78 and 70% of JEG-3 incubated for 6, 24, 48 or 72 h respectively, were positive. Total HLA-G protein levels measured by WB increased gradually in a time-dependent manner. Conclusion: HLA-G protein levels increased during hypoxia culture. As HIF-1α levels also increased gradually, it could be considered that this factor is one of the positive modulators of HLA-G expression. No correlation with HIF-2α levels was observed. Furthermore, RT-qPCR and FC results showed that when HLA-G reaches high levels of mRNA and membrane-bound protein, these start to decline, which could suggest a negative autoregulation of HLA-G.

TOPIC AREA: ONCOLOGY-INFLAMMATION

67. PROTEIN KINASE C ALPHA (PKCA) EXPRESSION AS A POTENTIAL MARKER IN THYROID CANCER (R51)

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Introduction: Thyroid cancer (TC) is the most common endocrine cancer, and its global incidence has been rising in recent decades. While many studies have linked PKCα overexpression to cancer aggressiveness and resistance to therapy, its specific role in TC remains underexplored. Objectives: To assess if PKCα overexpression promotes tumor growth and its association with clinical and tumor factors in TC patients. Methods: In vitro assays were conducted using human TC cell lines to examine PKCα role in tumor proliferation. We assessed protein expression and activation by qPCR, western blot, and Cell Titer Blue assays. To downregulate PKCα expression, siRNA transfection was employed. We further investigated PKCα expression and prognostic significance in TC patients from the TCGA-PanCancer Atlas TC database through bioinformatics tools. R2 Genomics Analysis and Visualization Platform (http://r2.amc.nl) analyses were performed on the GSE126729 dataset; for analyses on the Kaplan-Meier plotter (https://kmplot.com/), cBioPortal (https://cbioportal.org/) and Metascape (https://metascape.org/) platforms, mRNA

sequencing data from the PanCancer Atlas were used. These findings were subsequently validated in TC patients from the British Hospital of Buenos Aires by immunihistochemistry (IHC). Results: PKC expression in human TC cell lines showed high protein and mRNA levels compared to normal cells. PKCα downregulation significantly reduced hormone-induced proliferation. Our results also revealed PKCα's involvement in AKT and Erk phosphorylation. Analyzing PKC family expression using R2 revealed that PKCα expression was highest in TC and anaplastic TC patients. cBioPortal analysis indicated a positive correlation between PKCα and PI3K-AKT pathway. Metascape analysis showed activation of MAPK and PI3K pathways in samples with PKCα overexpression. In the Kaplan-Meier Plotter, higher PKCα expression was associated with poorer overall survival in TC. Additionally, IHC indicated increased PKCα expression in 30% of patients with papillary thyroid microcarcinoma. Conclusion: Our results establish that PKCα overexpression confers an advantage on tumor growth in TC. Therefore, it could act as a prognostic biomarker or therapeutic target in this disease.
TOPIC AREA: ONCOLOGY-INFLAMMATION

68. THE FLAVONOID 2'-NITROFLAVONE REDUCES VIABILITY AND MIGRATION IN HUMAN ENDOTHELIAL CELLS (R52)

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Introduction: Flavonoids are polyphenolic compounds that cause several antineoplastic effects. We previously described that the synthetic flavonoid 2'-nitroflavone (2'NF) inhibits proliferation and migration of human triple-negative breast cancer (TNBC) cells. Objectives: Our aim pointed to find out 2'NF effects on endothelial cells. Methods: We employed EA.hy926 human endothelial cells to perform viability (MTT) and migration (wound-healing) assays. Two experimental approaches were conducted: A) direct treatments with 2'NF (1.25-40 µM) or vehicle on endothelial cells, and B) endothelial cells treated with conditioned media (CM) derived from TNBC cells pre-incubated with 2'NF (1-25 µM) or vehicle. Results: EA.hy926 cell viability was significantly reduced with 2.5 µM 2'NF (77.98±4.06%; n=5; $p<0.05$) in 10% fetal bovine serum (FBS) condition versus vehicle (100%) after 24 h. The 50% inhibitory concentration value for 2'NF rendered 14.5 µM (95% confidence interval: 9.9-21.5 µM) after 96 h for A approach. In B approach, CM from 25 µM 2´NF-treated TNBC cells also decreased endothelial cell viability (84.21±4.68%; n=6; $p<0.05$) in 10% FBS containing medium versus vehicle (100%) after 24 h. To find out 2'NF concentration for migration assays that did not affect cell viability, we performed MTT assays for 24 h in 1% FBS. In approach A, we chose 1.25 µM 2'NF (versus vehicle; n=3; $p>0.05$) while in B, CM derived from 5 µM 2'NF-treated TNBC cells (versus vehicle; n=4; $p>0.05$) were selected. In scratch assays, in the presence of 1% FBS, direct treatment (A approach) with 1.25 µM 2´NF significantly reduced cell migration (vehicle: 100%; 2'NF: 80.78±3.39%; n=4; $p<0.05$) after 24 h. CM from TNBC cells pre-treated with 5 µM 2´NF (B approach) markedly inhibited cell migration (vehicle: 100%; 2'NF: 55.91±7.99%; n=3; $p<0.01$) after 24 h. Conclusion: 2'NF reduces viability and migration of human EA.hy926 endothelial cells. Putative 2'NF anti-angiogenic effects on endothelial cells will be further evaluated.
TOPIC AREA: ONCOLOGY-INFLAMMATION

69. MOLECULAR BASIS OF ABC TRANSPORTERS REGULATION BY INSULIN-LIKE GROWTH FACTOR 2 MRNA-BINDING PROTEIN 1 (IGF2BP1) IN HEPATOCELLULAR CARCINOMA (HCC). (R54)

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Introduction: Previously, we showed that IGF2BP1 knockdown (KD) leads to a decrease in ABCB1 and ABCC3 expression (protein and mRNA). Sorafenib (SFB) treatment induced IGF2BP1, ABCB1 and ABCC3 expression and KD of IGF2BP1 prevented the induction of ABCB1 expression, ABCC3 induction was prevented only at the mRNA level. Objective: To study the molecular mechanism of ABC transporter regulation by IGF2BP1 and its influence on SFB chemotherapy. Methods: KD of IGF2BP1 was done by transfection of a siRNA (IGF2BP1 siRNA), scrambled (C siRNA) was used as control, in HepG2 cells. After 24 h of transfection, cells were treated with the transcription inhibitor Actinomycin D (ActD) 5 ug/ml for 6 h. Also, HepG2 cells were pre-treated with ActD 5 ug/ml for 30 min and incubated with SFB 2µM for 48 h. Control cells received DMSO. IGF2BP1, ABCB1 and ABCC3 mRNA levels were measured by Real Time PCR. Data were presented as mean±SEM, N=3-6, $p<0.05$: * vs. C, # vs CsiRNA, π vs CsiRNA+ActD and ∞ vs SFB+ActD. Statistical analysis was performed using One-Way ANOVA followed by Tukey test. We investigate the impact of combined expression variations of IGF2BP1, ABCC3, and ABCB1 on the survival probability of a

cohort of patients with HCC, we accessed gene-level RNA-seq read counts from The Cancer Genome Atlas Liver HCC dataset (N=368). Results: mRNA levels of ABCB1 and ABCC3 decreased after KD of IGF2BP1 (55±3%#; 55±6%#, respectively) and in presence of ActD mRNA levels of transporters still down regulated respect to CsiRNA+ActD (ABCB1:62±4%π; ABCC3:29±2%π). SFB increased mRNA levels of IGF2BP1, ABCB1 and ABCC3 (160±3*%, 210±4%*,132±8%*; respectively) and ActD was not able to prevent it (100±3%∞,100±2%∞,76±4%∞; respectively). Bioinformatic studies showed that patients with the lowest probability of survival are those with high expression of all three genes and those with high expression of IGF2BP1 and ABCB1, but low expression of ABCC3. Conclusion: ABCC3 and ABCB1 are modulated by IGF2BP1 in a post-transcriptional manner under both constitutive expression and under induction by SFB. Overexpression of IGF2BP1 and ABC transporters is associated with a poor prognosis of survival in HCC.

70. USE OF MULTICELLULAR TUMOR SPHEROIDS AS A MODEL OF HEPATOCELLULAR CARCINOMA TO STUDY THE EFFECTS OF SORAFENIB AND EX-527 ON PROLIFERATION, MIGRATION AND APOPTOSIS (R58)

Palma NF[1,2,3], Chares LG[1], Livore VI[1], Oviedo Bustos L[1,3], Ferretti AC[1,2], Comanzo CG[1,2,3], Vera MC[1,3], Frattini M[2], Claros IA[2], Álvarez ML[1,2,3], Quiroga AD[1,2,3], Ceballos MP[1,3].

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Introduction: Chemoresistance (CR) counteracts the efficiency of sorafenib (SFB), a first-line therapy for hepatocellular carcinoma (HCC). Despite nowadays there exist other treatment options, HCC is a chemorefractory cancer and therapeutic strategies are still challenging. Sirtuins 1 and 2 (SIRT1/2) promote cancer progression and CR. Multicellular tumor spheroids (MCTS) are 3D models that provide more reliable results than standard 2D in vitro cell cultures, since they mimic features of in vivo tumors. Also, it is now accepted that stromal cells in solid tumors contribute to cancer progression and CR. We have shown that a combined treatment of SFB and EX-527 (EX), a SIRT1/2 inhibitor, reduces the viability of MCTS composed of SFB-resistant HCC and stroma cells. Objectives: to study the effects of SFB and EX on proliferation, migration and apoptosis using MCTS with the same composition. Methods: a SFB-resistant HCC cell line was established after incubating Huh7 cells for 6 months with increasing doses of SFB (Huh7-SR). MCTS of Huh7-SR, endothelial (EA.hy926) and hepatic stellate (LX-2) cells, in a ratio 1:0.3:0.3, respectively, were obtained by liquid overlay technique. Then, MCTS were treated for 72h with SFB 4µM(1), EX 40µM(2), SFB 4µM+EX 40 µM(3) or DMSO (control group: C). Proliferation rate and migration capability (in an adherent surface) were calculated as volume 72/0h and area 72/0h, respectively. Caspase-3/7 activity was determined at 72h using a green fluorescent reagent and apoptosis was estimated as fluorescence intensity/volume. Results: treatments reduced the proliferation (1: -16.3%*; 2: -54.7%*; 3: -62.6%*#) and the migration capacity (1: -13.5%*; 2: -13.4%*; 3: -46.2*#) of MCTS. Apoptosis was induced by treatments (1: +114.5*; 2: +127.8%*; 3: +181.6*). *p<0.05 vs. C. #p<0.05 vs. individual treatment. Conclusion: although all treatments were able to reduce proliferation and migration and to induce apoptosis, the combination of SFB and EX was superior to either treatment alone. More importantly, these responses were achieved in an in vitro model that better reflects tumor behavior in a context of SFB resistance and cancer-stroma interactions.

TOPIC AREA: ONCOLOGY-INFLAMMATION

71. GALECTIN-1 AND CD13 PROMOTE LIVER TUMOR-DERIVED SINUSOIDAL ENDOTHELIAL CELL MIGRATION AND TUBE FORMATION (R87)

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Introduction: The β-galactoside–binding protein galectin-1 (GAL1) is overexpressed in liver tumor. CD13 is a glycosylated membrane exopeptidase upregulated in endothelial cells during tumor-related angiogenesis. Previously we described that GAL1 interacts with CD13 in SKHEP1 tumor-derived liver sinusoidal endothelial cells (LSECs) in a glycan-dependent manner. Objectives: To determine GAL1 and CD13 roles in SKHEP1 LSEC migration and tube formation. Methods: Wild type (WT), CRISPR/Cas9-based CD13 knockout (CD13KO) and scrambled (SCR) SKHEP1 LSECs were treated with recombinant GAL1 (rGAL1, 100 µg/ml) or vehicle (V). In some experiments, rGAL1 was preincubated with lactose, a galectin inhibitor. Cell migration was evaluated after 15h by wound-healing assay. For tube formation assay (angiogenesis assay), cells were cultured on a basement membrane matrix (Geltrex) for 8h. Results: Cell migration was increased in WT cells treated with rGAL1 (126±4%, p<0.01, V: 100%). GAL1 pro-migratory effect was inhibited with lactose (95±3%, p<0.05), suggesting the involvement of GAL1 carbohydrate

recognition domain. CD13KO cell migration was reduced (77±6%, p<0.05) vs WT cells (100%). rGAL1 also promoted CD13KO cell migration (132±5%, p<0.05, V: 100%) and lactose inhibited this effect (102±7%, p<0.05 vs untreated cells). SCR cells did not show significant differences in migration respect to WT cells when incubated with rGAL1 (130±8%, V: 100%). The number of closed tubes increased in rGAL1-treated WT cells (318±63%, p<0.05, V: 100%) in a lactose-inhibitable manner. CD13KO cells exhibited a lesser number of tubes (67±2%) than WT cells (100%); this number was not affected after rGAL1 treatment (108±22%, V: 100%). Conclusion: GAL1 promotes SKHEP1 LSEC migration and tubulogenesis in a glycan-dependent manner but only the tube formation process would be dependent on its interaction with CD13. Cooperation between both proteins in liver-tumor associated angiogenesis will be further evaluated.
TOPIC AREA: ONCOLOGY-INFLAMMATION

27/10 Screen 2 • INMUNOLOGY AND NEUROINMUNOENDOCRINOLOGY
15:30 - 17:30 HS

72. HIGH FAT DIET AND CHRONIC STRESS EXPOSURE INDUCE ANXIETY BEHAVIOR AND COGNITIVE PERFORMANCE DECREASE IN MALE MICE. PARTICIPATION OF CORTICOSTERONE AND METABOLISM ALTERATION (R30)

Marcone MP[1], Paulazo MA[1], Burgueño AL[1], Quiroga S[1], Wald MR[1] and Genaro AM[1]
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Introduction: Accumulated evidence suggests that lifestyle -specifically dietary habits and stress exposure- plays a detrimental role in health. Objectives: In this context, our aim was to analyze the interplay of stress and diet, on metabolism, anxiety disorder and cognitive performance. Methods: One-month-old C57Bl/6J mice were fed with a standard diet (SD) or high-fat diet (HFD) for two months and then, exposed or not to chronic stress (CS) for four months. Body weight and food intake were measured weekly. Plasma glucose levels, both basal and 2 h after glucose administration, were determined using test strips. Leptin in plasma and abdominal adipose tissue was measured by ELISA kit and RT-PCR, respectively. Plasma corticosterone levels were measured by HPLC. To evaluate anxiety behavior open field and plus maze tests were done. For cognitive performance spontaneous alterations and spatial object recognition were performed. Results: Body weight and food intake increased significantly in HFD mice, while decreased by CS exposure. These variables were negatively correlated with leptin in plasma and abdominal fat. An increase in blood glucose was observed in HFD decreasing with CS exposure. In addition, corticosterone values showed an increase in HFD mice and a decrease in CS-exposed animals. Both, HFD or CS exposed mice increased anxiety behavior and a decrease in cognitive performance. Conclusion: In metabolism, HFD increased body weight and glucose levels. CS exposure improved these alterations and increased leptin levels. On the other hand, HFD and CS induced deleterious responses on anxiety behavior and cognition, but these effects were not potentiated when the animals were exposed to both treatments. Regarding corticosterone, elevated levels were observed with HFD but not in mice exposed to CS. Considering that there is described an interrelation between HPA axis and leptin, more studies are needed to evaluate it. Additionally, hyper- and hypo-activity in HPA axis have been described in stressful situations, it is interesting to further study this participation in the behavioral and cognitive response in HFD and CS animals.

27/10 Screen 2 • ENDOCRINOLOGY, METABOLISM AND REPRODUCTION SESSION II
15:30 - 17:30 HS

73. PRENATAL STRESS ALTERS LIPID AND ENERGY METABOLISM IN MALE BALBC MICE (R3)

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Introduction: Prenatal stress exposure and high-fat diet intake independently contribute to the development of metabolic diseases such as obesity, diabetes, and metabolic syndrome. Previously, we reported that males exposed to prenatal stress showed a decrease in Sirt1 mRNA expression in visceral adipose tissue. After high-fat diet feeding, we observed a rise in glucose and insulin levels and an increase in visceral adipose tissue gene expression of adipokines. Considering the liver is the main organ responsible for regulating lipid metabolism; dysfunction of this organ can lead to developing metabolic disorders due to the storage of lipids. This study aims to analyze the impact of prenatal stress and high-fat diet intake on liver mRNA expression in male and female BALB/c mice. Objective: We studied liver mRNA expression of genes that regulate lipid and energy

metabolism and are related to metabolic diseases such as obesity and hepatic steatosis. Methods: We exposed pregnant mice to movement restriction stress for two hours daily between gestational days 14 and 21. Offspring consumed a standard or a high-fat diet for 24 weeks, starting at four weeks of age. We studied liver mRNA expression of Pgc1α, Pparα, and Sirt1 and associated genes by qPCR. Results: In males exposed to prenatal stress, we found 1) increased body weight, triglyceride levels, and total cholesterol, 2) increased inflammation (mRNA levels of Socs3), and 3) decreased mRNA expression of genes related to metabolic diseases (Pgc1α, Pparα, and Sirt1) and lipid metabolism (Mttp and ApoB). Similar disturbances were observed in non-prenatally stressed males fed a high-fat diet and in all females under a high-fat diet, regardless of prenatal treatment. Conclusion: Our results suggest that prenatally stressed males were more susceptible to an increased risk of hepatic fat accumulation, leading to steatosis, without a synergistic effect of high-fat diet intake.
TOPIC AREA: ENDOCRINOLOGY- METABOLISM - REPRODUCTION.

74. SIMVASTATIN AFFECTS BODY WEIGHT AND VISCERAL FAT IN NORMOCHOLESTEROLEMIC RATS (R36)

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Introduction: Simvastatin(SMV) is often prescribed to treat hypercholesterolemia. It acts on the rate-limiting step in cholesterol biosynthesis (the conversion of HMG-CoA to mevalonate). Experimental findings have suggested a role for mevalonate-derived isoprenoids in regulating adipose tissue function. Previously, we demonstrated the beneficial effects of SMV on liver and metabolism in a diet-induced hypercholesterolemia model. As SMV has been shown to have pleiotropic effects beyond cholesterol lowering, the aim of this study was to analyze the effect of statins on body weight, liver and visceral fat in normocholesterolemic rats. Methods: 30 adults female Wistar rats(200g) were randomly assigned to control(C) or SMV(orally,5mg/day) groups. Animals were fed standard chow ad libitum and food consumption was measured. After 3, 4 and 5 weeks, 5 rats/group were euthanized, blood was drawn for: serum lipids determination(Cholesterol=chol, Triglycerides=TG;mg/dL) and transaminase activities [AST,ALT(UI/l)].The liver and visceral adipose tissue(VAT) were removed and weighed. Weight-gain velocity(WG), hepato-somatic index(HSI%) and VAT % were calculated (organ mass(g)/body mass(g)%).Hepatic samples were processed with H&E staining for NAFLD score. Results (mean±SD,two-wayANOVA):After 5 weeks, SMV showed changes in the TG values (SMV37,84±7,16 < C67,64±10,91 mg/dL; $p<0,001$) but no differences in the Chol and HIS% values. SMV particularly reduced AST ($p<0,001$). A lower WG was observed 3 weeks after SMV administration and was maintained over time (SMV13,11±3,49 < C33,31±2,89; $p<0,001$), as the visceral depots of adipose tissue decreased at 5 weeks (total VAT SMV1,60±0,06 < C2,64±0,61 %; $p<0,001$). However, there were no differences in dietary intake. Conclusion: This study suggests that the reduced weight gain was due to a reduction in fat mass rather than changes in dietary intake. SMV showed an anti-adipogenic effect that could be attributed to increased energy expenditure. Understanding the pleiotropic effects of statins may improve their efficacy in the treatment and prevention of CVD and as a potential therapeutic strategy for obesity, diabetes and metabolic syndrome. Keywords: simvastatin, body weight, visceral fat, liver, pleiotropic effects
TOPIC AREA: ENDOCRINOLOGY- METABOLISM - REPRODUCTION.

75. HYPOTHYROIDISM AND HEART FUNCTION: CARDIOVASCULAR PROFILES IN RATS WITH CONGENITAL AND POSTNATAL ONSET (75)

Eugenia Pietronave[1,2], Sofía Magalí Epíscopo[1,2], Kiara Peloso[1,2], María Belén Ces[1,2], Juan Manuel Gaetani[1,2], Gabriela Noceti[1,2], Noelia Arreche[1,2], Mónica Navarro[1,2], Celeste Ferrari[1,2], Andrea Fellet[1,2]
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Introduction: Thyroid hormones play an important role in cardiovascular function. Objectives: The aim of this study was to evaluate if congenital and postnatal hypothyroidism affect cardiovascular function in rats similarly. Methods: Sprague-Dawley cubs were divided into Group A (cubs from mother with free access to 0.02% methimazole (MMZ) water during gestation and lactation) and Group B (hypothyroid rats induced by free access to 0.02% MMZ water during 28 days). Body mass, tibia length and tail were measured weekly. Systolic arterial pressure (PAS, mmHg) was evaluated (tail cuff method). Echocardiographic measurements were left ventricle internal diameter, posterior and anterior wall thickness, ejection fraction and heart rate (HR,

bpm). Results are mean values ± SEM. Statistical procedures were performed using the SPSS statistical software, statistical significance was set at P < 0.05 versus B group. Results: A showed decreased growth parameters than B until third week. After, they increased in A compared to B. A had a higher growth rate than B. A had a higher PAS and HR (122 ± 3*; 331 ± 12*) than B (107 ± 1; 206 ± 12). Echocardiographic measurements were similar in both. Conclusion: Cardiac function is disrupted similarly regardless of time of onset. Foetal programming of hypothyroidism would maintain hemodynamic parameters within normal range. Time of onset may differentially modulate growth related factors. Although these results are preliminary, they allow us to present an initial model of congenital hypothyroidism.
TOPIC AREA: ENDOCRINOLOGY- METABOLISM - REPRODUCTION.

27/10 Screen 2 • CARDIOVACULAR PHYSIOLOGY ANF HYPERTENSION SESSION II
15:30 - 17:30 HS

76. ELECTROPHYSIOLOGICAL EFFECTS OF G PROTEIN-COUPLED ESTROGEN RECEPTOR (GPER) ACTIVATION IN HYPERTROPHIC CARDIOMYOCYTES (R4)

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Introduction: The G protein-coupled estrogen receptor (GPER) has been described as an important mediator of cardioprotective effects in various pathologies. However, changes in electrophysiological properties induced by activation of this receptor have not been extensively studied. In our group, it has been determined that the activation of GPER by its synthetic agonist G1 mediates a cardioprotective response against cardiac hypertrophy; however, ionic current studies have not been studied.
Objective: To study ionic currents involved in the cardiac action potential of hypertrophic myocytes and their modulation by GPER signaling.
Methods: 3-month-old male C57Bl/6 mice were subjected to cardiac hypertrophy by transverse aortic constriction (TAC mice) using titanium clip. Control mice were subjected to the same procedure without TAC (SHAM mice). Echocardiographic parameters were measured before and 28 days after surgery. Mice were sacrificed and ventricular myocytes were isolated using the Langendorff technique. Electrophysiological recordings were performed using the Patch Clamp technique. Results: TAC mice presented a significant increase in left ventricular mass index. Increased of action potential duration (APD) was observed in TAC myocytes, characteristic of the hypertrophy model. In addition, a decrease in resting membrane potential (PMR) was observed in these mice. L-type calcium current (ICaL), potassium inwardly rectifier current (IK1) and potassium outward transient current (Ito) were determined. A significant decrease in ICaL and IK1 was detected in TAC myocytes (pA/pF - ICaL: SHAM: -5.29±0.47, n=17. TAC: -3.36±0.21, n=13. IK1: SHAM: -12.16±0.95, n=16; TAC: -9.06±0.86, n=12.currents). No significant changes were observed in Ito. Importantly, G1 significantly decrease ICaL and increase IK1 and Ito currents in TAC myocytes. Finally, G1 significantly decrease ADP90 and increase PMR in TAC myocytes. Conclusion: The selective activation of GPER by G1 modifies the activity of calcium and potassium channels and the configuration of the action potential, partially reversing the alterations caused by TAC, possibly providing a cardioprotective mechanism.
TOPIC AREA: CARDIOVASCULAR PHYSIOLOGY-HYPERTENSION

77. PERINATAL PROGRAMMING WITH HYPERTONIC SODIUM: EFFECTS ON SODIUM INTAKE AND HYPERTENSION INDUCED BY DOCA-SAL MODEL (R43)

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Introduction: Our previous results indicate that voluntary maternal intake of hypertonic NaCl during gestation/lactation affects the offspring sodium/water intake, brain mRNA expression of angiotensin receptor 1a(Agtr1a), vasopressin, basal number of renal glomeruli, renal gene expression of the TRPV1 channel and Agtr1a and causes a sustained increase of blood pressure after a sodium overload. Objective: Our aim was evaluating the effects of perinatal imprinting induced by voluntary consumption of hypertonic NaCl on: -the pattern of hypertension onset in adult offspring, using the desoxycorticosterone acetate and Salt (DOCA-Salt) hypertensive model; - the 1% NaCl intake; - the body size and brain mRNA expression of Agtr1a, and serotonin

receptor 2c(Htr2c) in the sufornical organ and paraventricular nuclei. Methods: The perinatal manipulation period embraced dams (Wistar rats) from 1 week before conception until offspring at postnatal day 28 when the animals received access to NaCl (0.45M), food, and water (MP-NaCl group) or food and water (MP-Ctrol group). On 50 postnatal day, male offspring were subjected to DOCA treatment for 4 weeks(25 mg/kg twice a week) and water was replaced with a 1% NaCl solution. The systolic blood pressure was evaluated in conscious rats using the non-invasive tail cuff method, intake volume of 1% NaCl, and brain Agtr1a and Ht2c receptors mRNA expression were measured. Results: The DOCA-salt hypertensive treatment to MP-NaCl and MP-Ctrol animals did not differentially modify blood pressure between them and it did not affect the brain expression of Agtr1a and Ht2c receptors, but salt consumption significantly increased in PM-NaCl groups [F (1,10) = 11.907; p=0.006]. Conclusion: These results, in addition to previous data, indicate that the availability of a rich source of NaCl solution during the perinatal period induces long-term changes in osmoregulatory mechanisms and circuits, altering the functioning of the renal and brain angiotensin and vasopressin systems, which together modulate behavioral, endocrine, and renal responses to achieve hydroelectrolytic and cardiovascular homeostasis after different challenges (including DOCA-SAL).

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TOPIC AREA: CARDIOVASCULAR PHYSIOLOGY-HYPERTENSION

78. DYNAMIC EXPRESSION AND EFFECTS OF GALECTIN 1 AND GALECTIN 3 ON M2-MACROPHAGES AND INFLAMMATION AFTER MYOCARDIAL INFARCTION IN MICE (R61)

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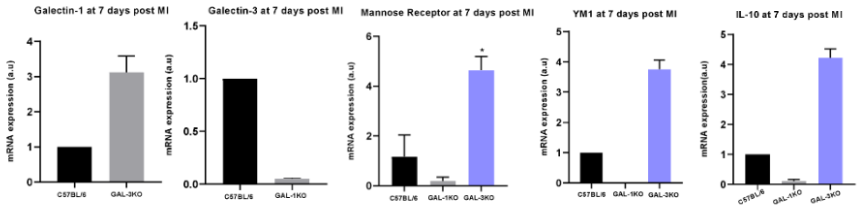
Background: In previous works, we found that lack of Galectin 1 and 3 (Gal-1 and 3) reduces the inflammation and macrophages infiltration after myocardial Infarction (MI). However, if the lack of Gal-1 and 3 are associated with the compensatory increase of Gal-3 and 1 respectively is unknown. Objectives: To investigate the dynamical expression of Gal-1 and Gal-3 and if the balance of both galectins regulates the expression of alternative activation markers of macrophages (M2) and cytokines after MI.

Methods: Male C57BL/6J, Gal-3 knockout (KO) and Gal-1 KO mice were subjected to permanent coronary ligature or sham. After 1 week, animals were euthanized and the hearts were harvested, snap frozen or fixed in formaldehyde. At 1 week post-MI M2 macrophages markers and cytokines expression were quantified by rt-PCR. Results: X±SEM. Cardiac mass index (CMI) was increased in Gal-3 KO with MI as compared with C57BL/6 mice (7,5±0,2 vs 6,1±0,5, p=0,03), CMI was similar between Gal1 KO mice and control. In the infarct zone, the expression levels of Gal-1 were increased 358% in the infarct zone of Gal-3 KO mice whilst the expression levels of Gal-3 in Gal-1KO mice was 6 %. Mannose receptor (5±0,4 a.u), YM1 (2±1 a.u) and IL-10 (4±1 a.u) were increased in GAL-3 KO mice compared with control.



Conclusion: these preliminary results shows that the lack of Gal-3 tended to increase the expression of Gal-1 but not inversely. In addition, the lack of Gal-3 was associated with the increase of M2 macrophages and anti-inflammatory cytokine expression while the lack of Gal-1 leads to opposite effects on those parameters. New studies to investigate the interactive role of both Gal in the temporal evolution of MI are require.

79. THE ω3 SUPPLEMENTATION INHIBITS THE DEVELOPMENT OF THE SLOW FORCE RESPONSE IN OVARIECTOMIZED RATS

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Introduction: Myocardial stretch induces a rapid increase in force, followed by the slow force response (SFR). The production of stretch-induced reactive oxygen species (ROS) is crucial for its development and the activation of cardiac Na+/H+ exchanger

(NHE1). Many studies describe that the consumption of polyunsaturated fatty acids of the omega-3 (ω3) series has cardioprotective effects. We recently demonstrated that ovariectomized (OVX) rats have increased expression and NHE1activity. Objectives: Characterize the SFR development in OVX rats supplemented with ω3. Methods: Two-month-old female rats were randomized into two groups: Sham (S) and bilaterally ovariectomized (OVX). One month after surgery, a group of OVX received 200 mg/kg/day ω3 orally, for 3 months (OVX ω3). Echocardiographic parameters and systolic blood pressure (SBP) were determined in the all protocol. After 3 months, the development of SFR in isolated papillary muscles, the ROS production and NHE1 expression in left ventricular slices were evaluated. Results: Ovariectomy produced uterine atrophy and body weight increase without affecting SBP. The ω3 did not modify these parameters. Left ventricle mass index (LVMI) was higher in OVX (mg/mm: 20.21 ± 1.0, n= 13, p<0.05 vs S), and ω3 was able to decrease it (17.7 ± 0.6, n=15, p<0.05 vs OVX). The development of SFR was similar in S and OVX rats (115.7 ± 1.8%, n=8; 116.8 ± 1.7%, n= 8, respectively, ns), but was abolished in OVXω3 (101.3 ± 0.9%, n= 10, p<0.05 vs S). The OVX showed greater basal ROS production (IF/µg: 187.7 ± 21.4 n=9, p<0.05 vs S) and the dietary supplement with ω3 prevented it (134.3 ± 12.8% n =10, p<0.05 vs OVX). We observed an increase in ROS production after stretch in S and OVX (% vs non stretched muscle: 38.9 ± 21.7 n=4; 30.4 ± 23.5, respectively, ns), but was absent in OVXω3 (% vs non stretched muscle -7.8 ± 10.27 n= 4). The NHE1 expression was higher in OVX (165.3 ± 17.1% n=3, p<0.05 vs S), ω3 was able to decrease it but not significant. Conclusion: The results show that ω3 supplementation in OVX rats canceled the development of SFR, an effect that is probably a consequence of the decrease in ROS production in response to stretch.

80. CHARACTERIZATION OF A MECHANO-IMMUNO-INFLAMMATORY SIGNALING PATHWAY IN RAT HEART PAPILLARY MUSCLES (R83)

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Introduction: Sustained mechanical stress leads to adverse cardiac remodeling. It has been shown that mechanical stress triggers an inflammatory response that can damage the heart. However, if axial mechanical stretch induces inflammation has not been fully explored, and the mechanosensors that lead to inflammation in this context have not been examined. Objectives: To determine if mechanical stretch triggers inflammation in papillary muscles and evaluate the mechanosensors involved, in an attempt to describe a novel mechano-immuno-transduction pathway. Methods: Papillary muscles were dissected from left ventricles of male Wistar rats and mounted in a tissue chamber in HEPES buffer at 30ºC. Lipopolysaccharide (1µgr/ml) was added to the medium to induce NLRP3 priming. After determining minimum length, one muscle was stretched during the following 2 hours to reach 98% of maximum force development length (STR), while the other remained unstretched (CTR). To evaluate microtubule involvement, the same protocol was performed but in presence of the microtubule inhibitor Colchicine (10 µM) (CTR+COL or STR+COL). RNA was extracted from muscle samples and cDNA was generated by reverse transcriptase reaction. Q-PCR was used to determine expression level of proinflammatory genes TNF-α, IL-18 and NLRP3. Statistical analysis was performed by T Test. Data are expressed as means ± SEM. Differences were considered significant at $p \leq 0.05$. Results: A significant increase in TNF-α, IL-18 or NLRP3 gene expression was detected in stretched muscles. This increase was prevented by Colchicine treatment. TNF-α (%ratio of TNF-α /GAPDH mRNA: CTR: 100 ± 11,5(N:6); STR: 333,5 ± 89,5(N:7); CTR+COL: 100 ± 21,7 (N:7); STR+COL: 223,9 ± 65,2 (N:8)). IL18 (%ratio of IL18 /GAPDH mRNA: CTR: 100 ± 18,7(N:7); STR: 330,6 ± 90,5(N:7); CTR+COL: 100 ± 16 (N:8); STR+COL: 197,1 ± 60,4 (N:8)). NLRP3 (%ratio of NLRP3 /GAPDH mRNA: CTR: 100 ± 20.4(N:6); STR: 235,5 ± 48,3(N:7); CTR+COL: 100 ± 15.8 (N:7); STR+COL: 103,5 ± 18,63 (N:7)). Conclusion: Mechanical stretch induces inflammation and the microtubular network is involved in transducing this mechanical stress into the activation of a proinflammatory response.
TOPIC AREA: CARDIOVASCULAR PHYSIOLOGY-HYPERTENSION

81. CARDIAC MITOCHONDRIAL MODIFICATIONS INDUCED BY TRAINING IN SHR (R89)

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Introduction: Essential hypertension produces mitochondrial dysfunction in the myocardium, along with other cell metabolic changes. Long-term exercise promotes cardioprotection, at least due to redox rebalancing. Specific mitochondria effects of exercise are yet elusive. Objectives: To determine whether aerobic training influence mitochondrial O-GlcNAcylation, membrane potential (ΔΨm), mitochondrial membrane lipid composition, pH, and ATP production in the myocardium of spontaneously hypertensive rats (SHR). Methods: Adult male SHR were randomized into sedentary (S) or trained by 8-week (5day/week) swimming routine (T) groups. Western blots were carried out with left ventricles. HP-TLC and functional experiments were carried out with isolated mitochondria. Results are shown as mean ± SEM (n) and considered statistically different when

p<0.05, otherwise p-value is stated. Normality was assessed and t-tests were carried out. Results: T rats had diminished O-GlcNAcylation levels in isolated mitochondria (%S: 73.8±6.5 (10)). OGA was not changed by training (%S: 95.4±14.6 (5)), whereas OGT trended to decrease (%S: 71.9±5.7 (4), p=0.16). No differences were found in cardiolipin, phosphoethanolamine, phosphatidylserine, and phosphatidylcholine between groups. T rats had an increased mitochondrial matrix pH (pH, S: 7.12±0.03 (5), T: 7.28±0.05 (5)) and a greater acidification velocity (pH/sec, S: 0.013±0.006 (5), T: 0.052±0.01 (5)), the later effect was canceled when exposed to the Na+/H+ exchanger inhibitor HOE648 even though the expression of this exchanger was similar in T and S. Training enhanced ΔΨm (mV, S: -157.6±9.2 (5), T: -183.6±2.9 (5)), ATP content (%S, T: 131.1±9.1 (11)) and production (lum/sec, S: 0.073±0.01 (8), T: 0.123±0.02 (8)). Conclusion: Training enhanced mitochondrial function by improving ΔΨm and mitochondrial pH, both with beneficial impact on ATP production, which was indeed increased. No changes were detected regarding mitochondrial lipid classes. Lesser O-GlcNAcylation found in mitochondrial proteins might explain in part the better mitochondrial phenotype. However, further data is needed to completely elucidate the underlying mechanisms.
TOPIC AREA: CARDIOVASCULAR PHYSIOLOGY-HYPERTENSION

AWARDS

SAFIS AWARD

HOW BENEFICIAL IS ZINC SUPPLEMENTATION IN RATS WITH METABOLIC SYNDROME?

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Introduction: Previously, we demonstrated an association between zinc deficiency during growth and the development of cardiometabolic alterations in adult male Wistar rats. Objective: To evaluate the effects of postnatal zinc supplementation on skeletal muscle, systolic blood pressure (SBP) and intermediate metabolism in male Wistar rats fed a high-fat and fructose diet during post-weaning growth. Methods: Male Wistar rats received from weaning, 21 days of life, up to 81 days of life: control diet (C, Zinc: 30 ppm), high fat diet (CHF; fat calories: 60%, Zinc: 30 ppm) and fructose at 10% in drinking water or a high-fat diet supplemented with zinc (ZHF; fat calories: 60%, Zinc: 190 ppm) and fructose at 10% in drinking water. On day 81, SBP, plasma lipid profile, oral glucose tolerance test, morphological changes in skeletal muscle and skeletal muscle vessels were determined. Statistics: Values are mean±SEM. ANOVA I Factor. Bonferroni: ***p<0.001 **p<0.01 y *p<0.05 Vs C; $$$p<0.001, $$p<0.01 and $p<0.05 Vs CHF. N=7-9 per group. Results: When comparing CHF with C group, it was observed that CHF showed an increase in body weight (C: 462±12; CHF: 523±11*; ZHF: 477±11$$ g), in SBP (CC:133±2;CHF: 159±2*; ZHF:144±1$$); in the area under the oral glucose tolerance curve (C: 25868±752;CHF: 30012±1469*; ZHF: 25650±589$$); and in triglycerides levels (C: 115±4; CHF: 156±6**, ZHF: 154±9 mg/dL). CHF group showed a decrease in skeletal muscle cells area (C: 1857±99;CHF: 1433±92*;ZHF: 1821±90$ µm2), and an increase in media layer area/lumen area ratio (C: 1.6±0.3;CHF: 2.9±0.4**;ZHF: 1.8±0.1$) and in the perivascular collagen area/lumen area ratio (C: 3.6±0.4;CHF: 5.4±0.5**;ZHF: 2.3±0.3$$$) compared to C. The ZHF group showed lower body weight, area under the oral glucose tolerance curve, SBP and morphological changes in skeletal muscle and skeletal muscle vessels than CHF. Conclusion: Zinc supplementation could have a beneficial impact on the adult life of these animals, preventing and/or reducing cardiovascular and metabolic damage associated with metabolic syndrome.

TOPIC AREA: ENDOCRINOLOGY- METABOLISM - REPRODUCTION.

NADPH OXIDASE-GENERATED REACTIVE OXYGEN SPECIES ARE INVOLVED IN ESTRADIOL 17ß-D-GLUCURONIDE-INDUCED CHOLESTASIS.

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Introduction: Estradiol 17β-D-glucuronide (E17G) induces cholestasis by impairing the activity of canalicular transporters such as Mrp2. We have recently presented evidence that E17G induces a rapid increase in intracellular reactive oxygen species (ROS) levels, which mediate the alteration of Mrp2 function and subcellular localization. We also reported that NADPH oxidase (NOX) seems to be the main source of these ROS, and evidenced that NOX shares the MEK-ERK1/2 and p38MAPK signaling pathways activated by E17G, downstream these kinases. Aim: to corroborate the participation of ROS in a more physiological model and to gain more direct evidence of the involvement of NOX in the impairment of Mrp2 localization and activity. Methods: In isolated and perfused rat livers (IPL) we evaluated the effects of the antioxidant compound, N-acetylcysteine (NAC, 1mM) on the alteration of bile flow and the biliary excretion of the Mrp2 substrate dinitrophenyl-glutathione induced by a single intraportal injection of E17G (3 µmol/liver) or its solvent (DMSO/10% ASB). In sandwich-cultured rat hepatocytes (SCRH) we performed a knockdown of the regulatory cytosolic subunit of NOX, p47phox, with specific siRNA, evaluating its expression by RTqPCR. In transfected SCRH treated with E17G, we evaluated the activity of Mrp2 by assessing the initial transport rate (ITR) of glutathione-methylfluorescein by epifluorescence microscopy, and the subcellular distribution of Mrp2 by immunofluorescence and confocal microscopy analysis. Results: In IPL, E17G rapidly decreased bile flow, which then started a slow recovery. NAC prevented the initial drop in bile flow and accelerated its recovery. siRNA transfection significantly decreased p47phox mRNA

expression. Confocal images of E17G-treated transfected SCRH showed a significant prevention of the alteration in Mrp2 localization; consequently, this maneuver also prevented the functional alteration of Mrp2. Conclusion: E17G generates a rapid increase of ROS via NOX, which are partially responsible for the internalization of the canalicular transporter Mrp2, the consequent canalicular secretory failure and the cholestasis induced by this estrogen.
TOPIC AREA: GASTROENTEROLOGY

ROLE OF AGOUTI-RELATED PROTEIN-EXPRESSING NEURONS IN REWARD-RELATED BEHAVIORS DURING CALORIC RESTRICTION

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Introduction: Animals under calorie restriction (CR) display enhanced reward-related behaviors towards palatable stimuli, and the molecular basis underlying such adaptations remain uncertain. Agouti-related protein (AgRP)-expressing neurons, located in the arcuate nucleus (ARH), are able to sense circulating factors. AgRP neurons project to many brain centres, including the paraventricular hypothalamus (PVN), the lateral hypothalamic area (LHA), the paraventricular thalamus (PVT) and the bed nucleus of the stria terminalis (BNST). AgRP neurons are activated under energy deficit conditions, such as CR, and the connection between AgRP neurons and reward-related behaviours is established. Objective: We studied if AgRP neurons orchestrate the enhancement of reward-related behaviours observed in mice subjected to CR. Methods: We used an experimental protocol in which male mice were fed with 40% of their daily ad libitum food intake for 5 days. Additionally, they were daily exposed to a solution of the non-caloric sweetener saccharin for 4 hours before each meal. Results: We found that WT mice under CR showed an increase of saccharine intake and an induction of the neuronal marker activation c-Fos expression in several brain regions, including the ARH-AgRP neurons, LHA, PVN, PVT and BNST. Furthermore, using an animal model in which the ARH was ablated, we found that the ARH integrity was required for CR-induced enhancement of saccharine intake and for the induction of c-Fos in most ARH-targets. By utilizing loxP-Cre technology in mice, we selectively expressed inhibitory DREADDs (Designer Receptors Exclusively Activated by Designer Drugs) in the AgRP neurons, and we observed that CR-induced enhancement of saccharine intake was reduced when AgRP neurons were selectively inhibited. Moreover, employing the same technology but using excitatory DREADDs selectively in AgRP neurons, we found that the pharmacogenetic activation of AgRP neurons alone was sufficient to induce saccharine intake in ad libitum fed mice. Conclusion: AgRP neurons' activation is required for the enhancement of saccharine intake in CR, and that AgRP neurons' activation is sufficient to induce saccharine intake in ad libitum fed mice.
TOPIC AREA: ENDOCRINOLOGY- METABOLISM - REPRODUCTION

THE CANNABINOID RECEPTOR CB1 IN NORMAL AND PATHOLOGICAL PLACENTA. IMPLICATIONS IN THE PATHOPHYSIOLOGY OF PREECLAMPSIA.

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Introduction: The placenta serves essential roles during gestation, including endocrine functions, nutrient exchange, and immune modulation. In human gestation, maternal blood directly interacts with chorionic villi, primarily through the syncytiotrophoblast (STB). Proper syncytialization of the trophoblast is crucial for a successful pregnancy. However, pathological conditions like preeclampsia are associated with alterations in this process. Preeclampsia is a common pregnancy disorder characterized by hypertension and proteinuria. Its multifactorial origin is believed to be related to abnormal placentation, particularly inadequate remodeling of uterine spiral arteries by extravillous cytotrophoblasts, leading to irregular uteroplacental blood flow and intermittent hypoxia-reoxygenation (HR), causing oxidative stress. The endocannabinoid system (ECS), a cellular signaling network comprising endocannabinoids, cannabinoid receptors (CB1, CB2), and related enzymes, has been found to be altered in preeclamptic placentas. Objectives: Evaluate HR's effect on CB1 receptor expression in normal human placentas.

Investigate HR's impact on CB1 expression and syncytialization in a villous cytotrophoblast model. Materials and Methods: Chorionic villi explants were obtained from normal and preeclamptic term placentas. The BeWo choriocarcinoma cell line was used to study villous cytotrophoblast and syncytialization. Villi explants from normal placentas and BeWo cells were subjected to HR. Results: Preeclamptic placentas exhibited significantly increased CB1 and CB2 expression compared to normal placentas. HR increased CB1 expression in chorionic villi explants. In BeWo cells, HR elevated CB1 and CB2 levels. HR and Met-AEA treatment reduced syncytialization markers in BeWo cells, which was prevented by CB1 blockade with SR141716 antagonist.
Conclusions: HR mimics CB1 changes observed in preeclampsia, supporting its role in this disease. These changes are replicated in an isolated trophoblast model. Additionally, CB1 stimulation reduced syncytialization markers, suggesting increased CB1 may contribute to placental function alterations.
TOPIC AREA: ENDOCRINOLOGY- METABOLISM - REPRODUCTION

CAMILIÓN DE HURTADO AWARD

ACUTE CHANGES IN [GLUCOSE]e INDUCE ARRHITHMOGENIC EVENTS PARTIALY DEPENDENT ON SGLT1 ACTIVITY IN MICE CARDIOMYOCYTES

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Introduction: In the late decade, the inhibitors of Na+/glucose cotransporter (SGLT) 2 have taken relevance not just as a hypoglycemic drugs in Diabetes Mellitus Type 2, but also as a drug for cardiovascular diseases (hearth failure). The direct effect on the heart is controversial since the unique isoform present in this organ is SGLT-1. Has been reported that the acute changes in [glucose]e (ΔGE), under insulin deprivation, alter the intracellular Ca2+ handling, inducing arrhythmogenic events (AE) Ca2+-Calmodulin kinase II (CaMKII) dependent, which activates the electrogenic Na+-Ca2+exchanger (NCX) in the forward mode. This pathway did not consider that the entering Na+, which would generate ectopic beats, could be given by SGLT1. In this context, the AE inducing by ΔGE has not been studied. Objetives: Study the role of SGLT-1 in the arrythmogenic events induced by changes in [glucose]e. Methods: We used isolated myocytes, of males C57 mice, loaded with Fura-2-AM, in which we measured intracellular Ca2+i by epifluorescence (intracellular Ca2+ transient [CaiT] amplitude and dynamics), and developed pressure in ex vivo Langendorff perfused whole heart. Both preparations were exposed to 5.5Mm (low glucose, LG) or 11mM (normal glucose, NG) glucose solutions until stabilization of contractibility and transient Ca2+ (CaiT), to then be abrutpty perfused with 11mM and 25mM (high glucose, HG) extracellular glucose, respectively. Osmotics controls were performed with choline chloride or sucrose. Phlorozin 10µM was used as SGLT1 inhibitor. Cytochalasin B 1µM was used as GLUT1 and GLUT4 inhibitor. We also performed Western Blot of cardiac homogenates that were previously perfused with NG or HG solutions and with or without 10 µM Dorsomorphin (an AMPK inhibitor).We did electrocardiograms (ECG) in the in vivo animals under intraperitoneal high glucose stimulation. Results: Arrhythmogenic events induced by changes in [glucose]e: The ΔGE from LG to NG and from NG to HG, majorly induced alteration in Ca2+i handling, such as an increase or a decrease of the CaiT. Independently on the effects on CaiT after the ΔGE, we observed AE in 96% from LG-NG and 76% from NG-HG. In isolated whole hearts, we observed the same pattern of AE as we observed in isolated myocytes with the ΔGE. Osmotic controls: To ensure that these effects are not doe to changes in osmolarity, we used as osmotics control choline chloride and sucrose and no differences were seen with the control group. Effects SGLT-1 inhibition: When we use phlorozin, the emergence of AE is significantly prolonged in isolated myocytes. In whole heart preparation, the washout of phlorozin unmasked the ΔGE-induced AE. Effects GLUT-1 and GLUT-4 inhibition: When using cytochalasin B, no prolongation of latency time was seen. However, the glucose change induced an increase in pGLUT-4, a fact that was counteracted by the addition of dorsomorphin. Effect of acute hyperglycemia on ECG in vivo: ECG traces showed that i.p. glucose administration induced ventricular extrasystoles (VES) in all glucose-treated mice when challenged with digoxin (20 mg/Kg) and adrenaline (1mg/ml/Kg), meanwhile, some non-treated mice showed no VES, o fewer arrhythmic events. Conclusion: The acute in [glucose]e would induce AE dependent on SGLT1 activity and independent of osmolarity. iSGLT increases the latency time of AE generation. Moreover, acute hyperglycemia induces arrhythmias in in vivo animals.

PATHOPHYSIOLOGICAL ROLE OF INOSITOL 1, 4, 5-TRIPHOSPHATE RECEPTOR (IP3R) BINDING PROTEIN RELEASED WITH IP3 (IRBIT) IN THE MYOCARDIUM

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Introduction: IP3R binding protein released with IP3 (IRBIT) was originally identified as a competitive inhibitor of the receptor. Cardiac hypertrophy has been associated with increased generation of IP3 and overexpression of IP3R was found in both human and animal models of heart failure. Given the involvement of IP3R in cardiac hypertrophy, it is plausible that IRBIT may also play a role in this pathological process. Aims: Although IRBIT heart expression has been reported, its function in cardiac tissue remains unclear. Thus, we aimed to study the cardiac outcomes of up and down-regulating IRBIT to establish its pathophysiological role. Materials and methods: We conducted IRBIT overexpression experiments in neonatal and 3-month-old male mice using AAV9-IRBIT-mCherry (5x1011 and 3x1011 vp, respectively), with AAV9-mCherry/saline as controls. For IRBIT downregulation, 3-month-old male mice received AAV9-shIRBIT (4x10^11 vp) or saline. Echocardiography analysis was performed. Cardiac hypertrophy and fibrosis-related molecular markers were measured. IRBIT expression was evaluated in human cardiomyopathy heart samples. Shapiro-Wilk normality test and Student's t-test or two-way ANOVA were used. Results: IRBIT overexpressed mice showed an increase in left ventricular mass index (LVMI: Saline: 3.55±0.23, n=11; AAV9-IRBIT*: 5.61±0.7, n=8; $p<0.05$ vs. Saline) and a decrease in cardiac systolic function. Moreover, an augmented heart weight to body weight ratio was found. Cardiac hypertrophy markers (Nppa, MYH7) were significantly increased in IRBIT-upregulated mice. Conversely, IRBIT downregulation did not alter cardiac hypertrophy parameters and fibrosis molecular markers. We evaluated IRBIT expression in human dilated and ischemic cardiomyopathy hearts samples and we discovered IRBIT was differentially expressed: it was reduced in dilated cardiomyopathy patients and increased in ischemic cardiomyopathy patients. Conclusion: Data presented here support IRBIT as a novel cardiac protein involved in cardiac hypertrophy development. Further research is needed to fully elucidate the precise mechanisms through which IRBIT influences heart function, as well as its potential as a therapeutic target in cardiovascular diseases.