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LECTURES

. Immune cell regulation of the induction of metaplasia in the stomach.

James R. Goldenring. MD, PhD Epithelial Biology Center, Vanderbilt University School of Medicine and the Nashville VA Medical Center, Nashville, TN, USA.

Intestinal type gastric cancer evolves in a field of metaplastic mucosal changes thought to be triggered by H. pylori-induced oxyntic atrophy. Intestinal metaplasia and spasmolytic polypeptide-expressing metaplasia (SPEM) are considered neoplastic precursors of gastric adenocarcinoma. Both metaplasias are marked by altered gene expression profiles in comparison to the normal stomach mucosa. SPEM evolves from transdifferentiation of chief cells into mucous metaplasia, and increasing evidence suggests that SPEM gives rise to intestinal metaplasia. A number of important studies have now revealed that the process of cancer genesis in the stomach represents a continuum of mucosal changes. Recent investigations in mouse models have demonstrated that SPEM lineages are critical components of the repair response following acute gastric mucosal injury. These results demonstrate that metaplasia is a physiological response to injury. Protein-secreting chief cells transdifferentiate into SPEM following loss of parietal cells in the corpus mucosa. The process of transdifferentiation follows a orderly pathway with down-regulation of the transcription factor Mist1, up-regulation of CD44v9 and xCT to cope with ROS due to ER stress, autophagy of zymogen granules, unwinding of DNA to alter the transcriptome and finally upregulation of mucous granule transcripts. Our studies have also found that immune cell infiltrates, particularly M2 macrophages, are critical for the conversion of a benign reparative metaplasia into a more proliferative intestinalizing metaplasia as found in association with chronic H. pylori infection. Additionally, ILC2 cells through secretion of IL13 are involved in initiation and maintenance of the metaplasia. Thus, immune cell influences are critical drivers of the progression of metaplasia towards neoplasia. Together these studies indicate that induction of metaplasia in the stomach is a highly coordinated event induced by severe damage in the stomach and represents the initial process responsible for development of pre-cancer.

. Rewiring adult brain circuits by neurogenesis and experience.

Alejandro Schinder. Laboratorio de plasticidad neuronal del Instituto Leloir. CONICET.

The adult hippocampus, a region of the brain involved in learning and memory, has the capacity to generate thousands of new neurons each day in all mammals, including humans. New neurons derive from neural stem cells and develop during several weeks, growing in size and complexity, and establishing synaptic connections to communicate with the preexisting network. While they integrate, new neurons contribute to information processing. Adult neurogenesis is tightly regulated by physiological and pathological conditions. For instance, it decreases with aging but it increases with voluntary exercise (running) or after epileptic seizures. However, seizures also produce marked alterations in migration and connectivity of adult-born neurons that further contribute to dysfunction. My laboratory is interested in understanding how adult neurogenesis remodels hippocampal circuits to optimize function. We are also dissecting cellular and molecular mechanisms guiding neuronal development and integration. In my talk I will discuss some of these mechanisms, their modulation by experience, and the alterations occurring in the aging brain.

SYMPOSIUM CARDIOVASCULAR PHYSIOPATHOLOGY: FROM MOLECULES TO TREATMENT

. Reprogramming heart rhythm with biological pacemakers.

Eugenio Cingolani. Cedars-Sinai Heart Institute, Los Angeles, California, USA.

Abnormally slow heart rhythms affect many people in the USA, and those numbers are steadily increasing as the population ages. While electronic pacemakers are the mainstay of therapy for these conditions, complications from device implantation, such as infections and pacing-induced cardiomyopathy may occur. As an alternative to electronic devices, we propose to study select biological pacemaker candidates in a pre-clinical model of complete heart block, and to develop the most promising candidate as a potential therapeutic agent
for first-in-human application.

Chronic right ventricular (RV) pacing can cause RV pacing-induced cardiomyopathy (RPVIC). Approximately 20% of patients paced from the RV apex develop RVPIC, with a dramatic depression of systolic function. Symptomatic heart failure is not infrequent, and long-term outcomes are poor. Clearly, alternatives to RV pacing are desirable, but there are no validated preclinical models of RVPIC to help understand mechanisms and to guide therapy. Here we've developed a non tachycardia pacing model of RVPIC using a physiologic heart rate in a porcine model of complete heart block. Gene-based biological pacemakers (BioP) were first described more than a decade ago; somatic gene transfer of various constructs (a dominant-negative mutant of the inward rectifier channel [Kir2.1AAA], wild-type HCN channels, and a transcription factor [Tbx18]) have all been shown to create BioP activity. However, until recently, in vivo preclinical applications have been mostly limited to highly-invasive open-chest models. We have developed a clinically-realistic minimally-invasive delivery technique and used it to create BioP in a porcine model of complete heart block. Here, we propose to use this approach to compare two "finalist" therapeutic candidates with fundamentally different mechanisms of action. The first one is a wild-type ion channel (HCN2) that artificially induces automaticity in ventricular cardiomyocytes by functional re-engineering. The goal is not to create a faithful replica of a pacemaker cell, but rather to manipulate a single component of the membrane channel repertoire so as to induce spontaneous firing in an excitable but normally-quiescent cell. The active principle of the second therapeutic candidate, Tbx18, reprograms ventricular cardiomyocytes into sinoatrial node (SAN)-like pacemaker cells (induced SAN [iSAN] cells). No

one determinant of excitability is selectively over-expressed: the entire gene expression program is altered, with resultant changes in fundamental cell physiology and morphology. This proposal utilizes the above mentioned percutaneous delivery method to reduce to refine and validate, in a large-animal model of bradycardia, the approaches required for translation to the clinic. We will characterize and compare the pacing efficacy and safety of HCN2 and Tbx18-derived BioP, testing the hypothesis that iSAN cells will provide superior chronotropic support as compared to HCN2. We will go on to perform long-term efficacy, toxicology and biodistribution studies with the more promising therapeutic candidate. Once designating the most promising therapeutic candidate, we will then test the utility of BioP in the setting of RVPIC. We hypothesize that restoring antegrade conduction by his-bundle pacing with a BioP can attenuate or reverse the adverse ventricular remodeling associated with right ventricular pacing. This research proposal is designed to lay the pre-clinical groundwork for testing of an optimized BioP in a pre-clinical model of RVPIC.

. Mesenchymal stem cell-derived exosomes for cardiac regeneration.

Gustavo Yannareli. Instituto de Medicina Traslacional, Trasplante y Bioingeniería (IMETTYB), Universidad Favaloro, Buenos Aires.

Mesenchymal stem/stromal cells (MSCs) are employed in numerous cell therapy clinical trials for a broad range of pathologies, including ischemic myocardium. Paracrine action of MSCs, rather than direct regeneration by differentiation into cardiomyocytes, explains the hemodynamic improvement as engraftment in the heart is not required. MSCs have potent anti-inflammatory and immunosuppressive properties. We found that MSC therapy mediates a switch from infiltration of pro-inflammatory (M1) to anti-inflammatory (M2) macrophages at the infarct site. In this context, MSC-secreted extracellular vesicles (EVs) are increasingly recognized as key paracrine mediators responsible for the biological and therapeutic function of MSCs. Special attention has been paid to the very small EVs (50 to 150 nm in diameter) that are referred to as exosomes. Exosomes contain proteins, mRNA, microRNA and other regulatory molecules that can be transferred into recipient cells to modulate their function. Thus, exosomes constitute a potential off-the-shelf and cell-free alternative to classical MSC-based therapies, providing a superior safety profile. Moreover, specific cargoes can be loaded to create what is currently known as designer exosomes. In the last years, we developed a novel and clinically scalable chromatographic procedure for the purification of anti-inflammatory exosomes from MSC culture media. In addition, we recently set-up an *in vitro* macrophage assay that makes it possible to rank different preparations of exosomes by their anti-inflammatory activity. Their ranking predicts their efficacy in suppressing lipopolysaccharide (LPS)-induced systemic inflammation in mice. Our data infer that EVs ability to modulate M1/M2 macrophage phenotype represents one of the mechanisms mediating cardiac regeneration after MSC therapy.

. Sarcoplasmic Reticulum-Mitochondria interaction in prediabetic heart.

Julieta Palomeque. MD, PhD. Centro de Investigaciones Cardiovasculares (CIC), La Plata. CONICET.

We have previously shown that myocytes from prediabetic hearts present Ca2+ mishandling manifested as sarcoplasmic reticulum (SR) Ca2+ leak and spontaneous Ca2+ release events, associated with an arrhythmic pattern at the whole animal level. Moreover, we have detected in the prediabetic mice hearts Ca2+-calmodulin kinase II (CaMKII) hyperactivity, which led in part, mitochondria injury, ultrastructure disarrangements, and apoptosis. The SR-mitochondria interplay is pivotal in pathophysiological situations (included metabolic diseases) and mitochondria structure and metabolisms are essential to the fate of cells. Therefore, the previous findings justify a deeper understanding of the interaction between these two organelles which are essential for a correct excitation-contraction coupling, rhythmicity and survival of the cell and are altered by a prediabetic condition.

We hypothesize that in prediabetic hearts, SR-Ca2+ leak is due to increased ryanodine receptors 2 (RyR2) activity via CaMKII-dependent pathway, which in turn produces mitochondrial Ca2+ overload and mitochondrial metabolic changes, which play a role in the enhanced SR-mitochondria proximity and ultrastructure alterations in prediabetic hearts.

We measured spontaneous Ca2+ release events (SCaRE), 3H-Ryanodine ([3H]Ry) binding assay, mitochondrial Ca2+ retention capacity (CRC), ATP and H2O2 production, and O2 uptake, morphology and fission/fusion proteins expression in a prediabetic model induced by fructose-rich diet (FRD) in WT and AC3I mice, which express a CaMKII-inhibitor at heart level.

Confocal images showed significantly increased SCaRE in WT FRD vs WT CD myocytes, which were prevented in AC3I mice. [3H]Ry binding assay revealed higher Bmax in WT FRD than WT CD and AC3I FRD. In isolated mitochondria, CRC, ATP production rate and O2 uptake were decreased, while H2O2 production rate was increased. Transmission Electron Microscopy (TEM) photographs confirmed decreased distance between SR and mitochondria in WT FRD specimens vs WT CD. Moreover, TEM photographs showed decreased mitochondria area and diameter in WT FRD respect to WT CD, whereas Feret diameter and roundness were higher in WT FRD than WT CD. A clear disarrangement in WT-FRD tissue was evident. We found a decreased pDRP-1/DRP (pro-fission protein in the dephosphorylated state) and no differences in Mfn-2 or OPA-1 (pro-fusion proteins) in WT FRD.

We conclude that CaMKII is involved in the increase of RyR2 activity, SR Ca2+ leak and mitochondria morphology changes of FRD. Moreover, the increased in SR Ca2+ leak and SR-mitochondria proximity would favor the decreased CRC, favoring mitochondrial Ca2+ overload and decrease mitochondrial bioenergetics: increased mitochondrial H2O2 production and decreased O2 consumption and ATP production. Finally, the fission process would be present over fusion.

. Galectins and heart inflammation

Germán González. Instituto de Bioquimica y Medicina Molecular (IBIMOL), Buenos Aires, Argentina.

Galectins (Gal) are β-galactoside-specific binding lectins widely expressed in the immune system. They play a critical role in the regulation of innate and adaptive immunity, inflammation, wound healing and carcinogenesis. Currently, the family of Gal consist of 15 different types, each with a specific function. During the last few years a growing body of evidences either from clinical and experimental research strongly suggests that Gal plays an important role in the regulation of essential physiological mechanism of cardiomyocyte as well as cardiovascular inflammation. Therefore, the role of Gal in the development and evolution of cardiovascular disease has progressively gained attention. Under pathological conditions, these lectins are very important players in the evolution of cardiovascular disease to heart failure. Undoubtedly, Gal-3, a chimera type galectin, is the most widely studied in cardiovascular disease and cardiac failure. Galectin 3 is widely expressed in the immune system, fibroblasts and cardiomyocytes. Over the past few years, this lectin merged as a strong prognostic factor in patients with coronary artery disease and heart failure since it is markedly involved in cardiovascular pathophysiology as a proinflammatory and profibrotic molecule. In rodents, the synthesis of Gal-3 is highly increased over the pressure overload and it is a key player in target organ damage in hypertension. Moreover, previous studies from our and other groups suggest that Galectin3 is a key regulatory factor in the temporal evolution of macrophage infiltration and their phenotypic polarization, tissue repair, remodeling and function after myocardial infarction in mice. Along the presentation, it will summarize the main concepts on different aspects of Galectins as key regulator of inflammation and fibrosis associated to cardiac disease.

SYMPOSIUM METABOLIC SIGNALING. IMPACT ON CELLULAR AND TISSUE PHYSIOPHATOLOGY

. Cyclooxygenase-2 (COX-2)-derived prostaglandins in the regulation of liver cell metabolism: Role in the prevention of insulin-resistance, fibrosis and ischemia/reperfusion injury.

Daniel Francés. Instituto de Fisiología Experimental (CONICET-UNR).

Prostanoids (prostaglandins, prostacyclines and thromboxanes) are short-lived lipid messengers that derive from arachidonic acid. Cyclooxygenase (COX) is the enzyme that catalyzes the limiting step in prostanoid biosynthesis. A series of tissue-specific isomerases and oxidoreductases generates the different products: prostaglandins (PGE_2, PGD_2, PGF_2), prostacyclines (PGI_2) and thromboxanes (TXA_2). PGE_2 is the main product present in liver. Prostaglandins are implicated in homeostatic processes like platelet aggregation, maintainance of gastric mucose, reproduction etc; in addition, these compounds play an important role in the onset of inflammation, mitogenic responses and cancer. Two COX isoenzymes have been identified. COX-1 is ubiquitous and constitutively expressed in a wide variety of tissues and is responsible for the low and continuous prostaglandin synthesis required in tissue homeostasis. COX-2 is undetectable in most tissues; however, a variety of extracellular and intracellular stimuli, such as inflammation and other cellular stresses, can rapidly induce COX-2 in a variety of cell types. COX-2 is also related with cellular growth and carcinogenesis. In the liver, COX-2 is not expressed in the adult hepatocyte and is not induced by pro-inflammatory stimuli as in other tissues. However, COX-2 is expressed in non-parenchymal cells, in the fetal hepatocyte and in some hepatoma cell lines.

Our group has focused in the relationship between COX-2 expression and liver pathology: hepatic insulin-resistance, fatty liver disease (NAFLD), fibrosis (NASH), hepatocellular carcinoma (HCC) and ischemia/reperfusion injury. Using different animal experimental models and human biopsies, we have deepend the study of the molecular mechanisms implicated in the above mentioned processes. By means of a transgenic murine model constitutively expressing human COX-2 in the hepatocyte, we have shown that in acute liver processes that undergo apoptosis, PGs exert a protective action due to their anti-apoptotic/anti-necrotic effect and stimulate hepatocyte proliferation. Moreover, we tested the role of constitutive expression of COX-2 in chronic liver injury. In this regard, our studies show that the expression of COX-2 in hepatocytes protects the liver against the damage induced by hyperglycemia, insulin resistance and obesity. PGs increase insulin sensitivity and induce thermogenesis and fatty acid oxidation, thus suggesting an improvement in mitochondrial function and oxidative phosphorylation.

MicroRNAs (miRNAs) are noncoding RNAs that function as key post-transcriptional regulators of gene expression. Although it is known that COX-2 expression is regulated by miRNAs, there are little data regarding COX-2 involvement in miRNA regulation. We evaluated the role of COX-2 in the regulation of a specific set of miRNAs implicated in insulin signaling in liver cells. Our results suggest that COX-2 represses the expression of several miRNAs in hepatocytes, thus promoting protection against insulin resistance by increasing its signaling. Later, when we corroborated that constitutive COX-2 expression in hepatocytes ameliorates NASH progression and liver fibrosis development by reducing inflammation, oxidative stress and apoptosis and by modulating activation of hepatic stellate cells (HSCs), respectively, we suggested a possible protective role for COX-2 induction in NASH/NAFLD progression. Again, miRNAs emerged as key regulatory molecules in this context. Our results provide evidence that COX-2 represses miRNAs in HSC that promotes protection against fibrosis by decreasing the levels of pro-fibrogenic markers. Moreover, we have recently demonstrated several beneficial and protective effects of hepatocyte COX-2 induction and its dependent PGs during ischemic pre-conditioning as a key hepatic mechanism triggered against ischemia/reperfusion-derived liver damage.

These results shed new insights into a possible physiological protective mechanism of COX-2 induction in the progression of several hepatic pathological processes. This hypothesis is sustained in the fact that using COX-2 transgenic mice we confirmed that continuously derived hepatic PGE_2 production plays a protective role against the development of hepatic insulin-resistance, NASH and hepatic fibrosis progression, and ischemia/reperfusion liver injury. Thus, our data suggest that the use of stable PG analogs or mimicking COX-2 signaling in the liver could represent valuable therapeutic options to ameliorate the progression

of the above mentioned diseases, and/or might provide evidence-based support for further therapeutic interventions after liver injury.

. Epigenetic consequences of cholesterol loss in the aging brain.

Mauricio G. Martín. Instituto Ferreyra-INIMEC-CONICET-UNC, Córdoba.

Aging is characterized by a progressive decline in cognitive capacities. Several recent reports indicate that epigenetic mechanisms are affected in the aged brain and contribute to the aged brain phenotype. It is, however, unclear whether or not and how typical features of old neurons alter the epigenetic regulation of learning and memory genes. We have analyzed the effect of changes in lipid composition in the brain, a constitutive feature of aging, on the epigenetic regulation of gene expression. We found that neuronal cholesterol loss impairs the proper function of neuronal receptors and downstream signaling pathways leading to the formation of a repressive chromatin structure in old neurons. In addition, altered levels of sphingolipid metabolizing enzymes contribute to gene repression by increasing histone deacetylase activity. Oral administration of Voriconazole, an inhibitor of the cholesterol catabolic enzyme cholesterol-24-hydroxylase in mammalian brain, rescued hippocampal age-associated cholesterol loss and improved cognitive abilities of old mice. These results contribute to unveil some of the mechanisms involved in the cognitive decline of the old.

. Regulation of multiple pathways by a unique kinase in sperm capacitation.

Darío Krapf. Laboratorio de Cascadas de Señalización Celular.IBR (CONICET-UNR).

Protein kinase A (PKA) is a broad-spectrum Ser/Thr kinase involved in the regulation of several cellular activities. Thus, its precise activation relies on being localized at specific subcellular places known as discrete PKA signalosomes. A-Kinase anchoring proteins (AKAPs) form scaffolding assemblies that play a pivotal role in PKA regulation by restricting its activity to specific microdomains. Because one of the first signaling events observed during mammalian sperm capacitation is PKA activation, understanding how PKA activity is restricted in space and time is crucial to decipher the critical steps of sperm capacitation. Our work describes that anchoring of PKA to AKAP is not only necessary but also actively regulated during sperm capacitation. However, we show that once capacitated, the release of PKA from AKAP promotes a sudden Ca^{2+} influx through the sperm-specific Ca^{2+} channel CatSper, starting a tail-to-head Ca^{2+} propagation that triggers the acrosome reaction. Three-dimensional super-resolution imaging confirmed a redistribution of PKA within the flagellar structure throughout the capacitation process, which depends on anchoring to AKAP. These results represent a new signaling event that involves CatSper Ca^{2+} channels in the acrosome reaction, sensitive to PKA stimulation upon release from AKAP.

SYMPOSIUM MEMBRANE TRANSPORTERS: INVOLVEMENT IN PHYSIOLOGICAL AND PATHOLOGICAL CONDITIONS

. Role of aquaporins in the development of the human placenta. implications in the pathophysiology of preeclampsia.

Alicia E. Damiano. Laboratorio de Biología de la Reproducción, Instituto de Fisiología y Biofísica Bernardo Houssay (IFIBIO)- CONICET-Facultad de Medicina, Universidad de Buenos Aires. Buenos Aires, Argentina. Cátedra de Biología Celular y Molecular, Departamento de Ciencias Biológicas, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires.

Preeclampsia is a multisystem syndrome unique to human pregnancy characterized by hypertension and proteinuria after 20 weeks of gestation in a previously normotensive woman. This gestational disorder affects 7–10% of pregnancies worldwide. In Latin-American countries, preeclampsia is the leading cause of maternal and fetal mortality and is estimated that 26% of maternal deaths are related to this syndrome.

Despite the importance of preeclampsia and several decades of extensive research, its etiopathogenesis remains unclear. Accumulated evidence suggests that an abnormal placentation and an altered expression of a variety of trophoblast transporters are associated to preeclampsia. In this regard, we have previously reported an abnormal expression of AQP3 and AQP9 in these placentas.

Described evidence suggests that placental AQPs are not only simple transporting proteins and that may participate in relevant processes required for a normal placental development, such as cell migration and apoptosis.

Recently we reported that inhibition of AQP3 significantly reduced the migration of extravillous trophoblast (EVT) cells. This might lead to a shallow trophoblast invasion and poor remodeling of the maternal spiral arteries resulting in fluctuations of oxygen tension, a potent stimulus for oxidative damage and trophoblast apoptosis. In this context, the increase of oxygen and nitrogen reactive species could nitrate AQP9, producing the accumulation of a non-functional protein affecting the survival of the villous trophoblast (VT). This may trigger the exacerbated release of necrotic and aponecrotic VT fragments into maternal circulation producing the systemic endothelial dysfunction underlying the maternal syndrome.

Therefore, our hypothesis is that the alteration in the expression of placental AQPs observed at the end of gestation may take place during the trophoblast stem cell differentiation, disturbing both EVT and VT cells development, or during the VT differentiation and turnover. In both situations, VT is affected and at last the maternal vascular system is activated resulting in the development of the clinical symptoms of preeclampsia.

. Relevance of canalicular transporter endocytosis in hepatocellular cholestasis.

Fernando A. Crocenzi. Instituto de Fisiología Experimental (CONICET-UNR).

Bile formation is an osmotic process driven by the gradient generated by osmotically active compounds between sinusoidal blood and the bile canaliculi. Creation and maintenance of this gradient depend on the activity of canalicular hepatocellular

transporters belonging to the ABC superfamily, such as BSEP and MRP2, which actively transport bile salts and organic anions such as glutathione, respectively, into bile. Activity of these transporters depends on their proper localization at the apical domain of hepatocytes, and acute changes at this level take place in both physiological and pathological conditions, by modulating their recycling between the canalicular membrane and intracellular subapical endosomal compartments. Thus, an increase in the number of transporters inserted in the canalicular membrane domain, and consequently in transporter secretory function, can occur in physiological conditions under demand (e.g., by intracellular increase of cAMP or bile salts). On the other hand, these transporters can suffer endocytic internalization and intracellular retention in cholestatic conditions; this leads to a decreased secretion of osmotically active biliary solutes and impairment of canalicular bile flow. Eventually, long lasting intracellular retention leads to increased degradation of the endocytosed transporters. Endocytic retrieval of canalicular transporters has been described in several experimental models of hepatocellular cholestasis, for example, that induced by monohydroxylated bile salts, hyperosmolarity, estrogens and endotoxin. More importantly, this phenomenon has been reported in several human cholestatic pathologies, such as primary sclerosing cholangitis, obstructive cholestasis, and primary biliary cholangitis. The mechanisms underlying the endocytic retrieval of canalicular transporters in hepatocellular cholestasis are not fully understood at present. Our group has focused on the study of the molecular mechanisms governing this process in the acute cholestasis induced by the endogenous metabolite of estradiol, estradiol 17β-D-glucuronide (E17G), a main responsible of intrahepatic cholestasis of pregnancy. According to the evidence gathered so far, E17G activates signaling proteins in three complementary pathways; two pathways initiate with the estrogen receptor GPR30, and the remaining one initiates with the activation of PKCα. These signaling pathways lead to *i*) a shift of the transporters from "rafts" to "non-rafts" membrane microdomains followed by an increased microtubule-independent and clathrin-dependent endocytic retrieval of canalicular transporters, and *ii*) a delayed microtubule-dependent exocytic reinsertion of the canalicular transporter-containing vesicles occurring during the recovery phase of cholestasis. The understanding of this crucial pathomechanism at a molecular level opens new possibilities for treatment of cholestatic hepatopathies.

. Modulation of luminal ATP levels by extracellular vesicles along the nephron: potential role in intrarenal communication and electrolyte reabsorption.

Juan Pablo Rigalli. Department of Physiology, Radboud Institute for Molecular Life Sciences, Radboud University Medical Center, Nijmegen, the Netherlands.

Purinergic signaling results from binding of extracellular ATP and its hydrolysis products to P1 and P2 receptors. In the kidney, the epithelial sodium channel (ENaC) mediates sodium reabsorption in the collecting duct. While the acute regulation of ENaC activity by ATP is well acknowledged, long-term effects have been less investigated. Extracellular vesicles (EVs) are membrane-enclosed nanosized particles released from a large variety of cell types. All vesicles carry bioactive molecules such as proteins, nucleic acids and lipids. In the kidney, EVs can be transported along the nephron and interact with cells downstream. Although evidence obtained in extrarenal tissues indicates modulation of extracellular ATP levels by EVs, it is not known if a similar process may take place in the kidney and how this could impact ATP sensitive processes.

The aim of this study was to investigate the effect of EVs from proximal (HK2) and collecting duct (HCD) cells on extracellular ATP levels and the impact on the expression of αENaC. Also, the effect of fluid-shear stress (i.e. mimicking pro-urine flow) on EV release was addressed using culture in microfluidics chambers and nanotracking-analysis.

EVs were isolated from HK2 and HCD cells by ultracentrifugation and characterized by electron microscopy, nanotracking-analysis and immunoblotting. Extracellular ATP levels were measured by chemiluminescence. First, ATP hydrolyzing activity was determined in a non-cellular system, where only apical EVs from HCD cells decreased ATP levels. Following, HK2 cells were incubated with HK2 EVs, and HCD cells were incubated with HK2 and HCD EVs. EV-uptake dependence was evaluated using the inhibitor dynasore. Addition of HK2 EVs to HCD cells increased extracellular ATP (+25%*) in an uptake-dependent process. Downregulation of the ectonucleotidase ENTPD1 (-22%*) by the EVs may explain this observation. Expression of the ATP releasing channel *PANX1* was not affected by EVs. Conversely, HCD EVs decreased extracellular ATP in HCD cells (-40%*), being the effect uptake-independent and probably attributed to ATP hydrolysis by HCD vesicles. Differential proteomic analysis of HK2 and HCD EVs pointed the protein 14-3-3 as a potential candidate to mediate this effect. In addition, expression of *SCNN1A* (i.e. encoding the α subunit of ENaC) was determined by RT-qPCR after incubation of HCD cells with both types of EVs. While HK2 EVs decreased *SCNN1A* expression (-26%*), *SCNN1A* expression was up-regulated by HCD EVs (+46%*). γ-S-ATP and apyrase mimicked the effects of HK2 and HCD vesicles on *SCNN1A*, respectively, supporting an association between extracellular ATP and αENaC expression. Finally, flow-mediated release of EVs by HCD cells was observed, while EV release by HK2 cells remained flow-unsensitive. Altogether, these observations suggest a differential effect of proximal and collecting duct EVs on αENaC via modulating purinergic signalling, where the autocrine effect of HCD EVs might be sensitive to changes in pro-urinary flow. (*$p<0.05$, $n=4$).

SYMPOSIUM POLARITY PROTEINS. COMMON ACTORS OF MULTIPLE CELLULAR FUNCTIONS

. A key function for Microtubule-Associated-Protein 6 in activity-dependent stabilization of actin filaments in dendritic spines.

Mariano Bisbal. Instituto de Investigación Médica Mercedes y Martin Ferreyra. CONICET.

Synaptic modifications that underlie plasticity, learning and memory critically depend on the remodelling of actin filaments within dendritic spines, the neuronal membrane protrusions that support the post-synaptic side of most excitatory synapses in the adult brain. Emerging evidence indicates that microtubule-associated proteins (MAPs) are implicated in synaptic

function; in particular, mice deficient for MAP6 exhibit striking deficits in plasticity and cognition. How MAP6 connects to plasticity mechanisms is unclear. Here, we address the possible role of this protein in dendritic spines. We find that in MAP6-deficient cortical and hippocampal neurons, maintenance of mature spines is impaired, and can be restored by expressing a stretch of the MAP6 sequence called Mc modules. Mc modules directly bind actin filaments and mediate activity-dependent stabilization of F-actin in dendritic spines, a key event of synaptic plasticity. *In vitro*, Mc modules enhance actin filament nucleation and promote the formation of stable, highly ordered filament bundles. Activity-induced phosphorylation of MAP6 likely controls its transfer to the spine cytoskeleton. These results provide a molecular explanation for the role of MAP6 in cognition, enlightening the connection between cytoskeletal dysfunction, synaptic impairment, and neuropsychiatric illnesses.

. Cell polarity proteins as common targets for viral pathogenesis.

Daniela Gardiol. Instituto de Biología Molecular y Celular de Rosario (IBR)-CONICET, Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario.

Cellular polarity is maintained by the interconnected activity of polarity complexes whose components have a well-defined subcellular localization and, in addition, are key regulators of the intercellular junctions. Proteins derived from different viruses can interfere with the functions of polarity regulators, mainly the PDZ domain-containing proteins. These interferences result in significant biological alterations, important for virus entry, dissemination and pathogenesis.

In our group, we have focused on the oncogenic mechanisms linked to polarity disruption during Human Papillomavirus (HPV) infections that are associated to the development of cervical cancer. We have characterized some of these cellular targets, such as the human Discs large oncosuppressor (DLG1) and the tight junction Partitioning defective 3 protein (PAR3). By diverse methodological tools, such as traditional, histotypical and organotypical raft cultures, we have evaluated the changes in the expression of these cell proteins mediated by HPV. Remarkably, clear alterations in the levels and the distribution along the epithelium, as well as in the subcellular localization, were observed for these proteins, particularly for DLG1. As these findings may entail the deregulation of cellular processes involved in tumour suppression, the data obtained have encouraged us to search for malignant progression biomarkers.

We also extended our studies to other tumor viruses, such as the human T-cell lymphotropic virus type 1 (HTLV-1). By fluorescence microscopy we evaluated the aberrant accumulation of polarity regulators in vesicle-like structures, in the presence of the HTLV-1 Tax oncoprotein, with potential significance in lymphocyte deregulation and in the development of lymphoid pathologies.

Moreover, disruption of cell polarity and alterations of intercellular contacts are not restricted to tumor viruses. As a result, we are currently interested in the analysis of these processes during infections by regional arboviruses, like the Zika virus, which has been associated with serious neurological and developmental pathologies.

The knowledge about conserved mechanisms of viral pathogenesis could help to the understanding of basic viral biological processes and to the identification of special targets for novel diagnostic or therapeutic tools.

. Golgi apparatus plays a pivotal role in the trafficking of the Linker for Activation of T cells (Lat) to the immune synapse (IS) and in T-cell activation.

Andrés Zucchetti. Institut Curie, PSL Research University, INSERM, U932 Crosstalk between T cells and Dendritic cells team, 26 rue d'Ulm, 75248 Paris Cedex 05, France.

The IS is a site of intense vesicular trafficking. The adapter molecule LAT orchestrates the formation of signalosomes upon T cell receptor (TCR) stimulation. LAT is present in different intracellular pools and is dynamically recruited to the IS during T cell activation. However, the mechanisms permitting the spatial organization of this vesicular transport are unknown.

We show herein that, T-cell activation induces the retrograde transport of the endocytic pool of LAT through the Golgi–trans-Golgi network (TGN), where it is repolarized to the immune synapse. Using in vitro and in vivo models, we have demonstrated that this retrograde transport of LAT depends on the small Syntaxin-16–and Rab6, two regulators of the endosome-to-Golgi/TGN retrograde transport. These results establish that the canonical retrograde traffic of LAT from the plasma membrane to the Golgi-TGN is required for polarized delivery of LAT to the IS and T cell activation.

These results suggested that the Golgi apparatus plays a pivotal role for the transport of a signaling molecule, which plays a key role in T cell activation. In line with this, we have also shown here that the Golgin GMAP210, known to capture vesicles and to organize membrane traffic at the Golgi apparatus, is involved in the delivery of vesicles containing LAT to the IS. Specifically, we found out that GMAP210 silencing hampered: LAT recruitment to the IS, formation of signaling complexes and production of cytokines upon TCR activation. We have also decrypted the mechanisms involved by showing that GMAP210 binds vesicles containing LAT. A deep structure/function analysis showed that the tethering activity of GMAP210 is key to the polarized delivery of LAT-vesicles to the IS. All together, these results pointed towards the Golgi as a central organelle for TCR-signaling organization, IS formation and lastly T-cell activation.

. Regulation of the immune synapse by polarized membrane trafficking: impact on b cell responses.

María-Isabel Yuseff. Department of Cellular and Molecular Biology. Faculty of Sciences. Pontificia Universidad Católica de Chile. Santiago, Chile. Supported by Fondecyt Project #1180900.

Engagement of the B cell receptor (BCR) with surface-tethered antigens leads to the formation of an immune synapse (IS), which coordinates the uptake and processing of immobilized antigens by B cells. A pivotal step in this process is the rapid polarization of the centrosome, which guides the recruitment and local secretion of lysosomes at the synaptic membrane to facilitate antigen extraction. Conserved polarity proteins, such as Cdc42 and Par3, coordinate centrosome repositioning with lysosome recruitment at the IS, however the molecular machinery involved in the docking and fusion of lysosomes remains to

be defined. A semi-quantitative proteomic analysis of centrosome-rich fractions obtained from activated versus non-activated B cells revealed that the exocyst complex and regulatory subunits of the 19S proteasome become differentially associated with the centrosome of B cells. We have begun to elucidate the associated molecular mechanisms by which the exocyst and the proteasome regulate the immune synapse by characterizing their subcellular localization, assembly, or activation during B cell stimulation. Our results show that engagement of the B cell receptor enhances microtubule stability, thereby releasing the centrosome-associated exocyst subunit, Exo70, which becomes redistributed to the immune synapse. This process is coupled to the recruitment and activation of the GTPase, GEF-H1, which is required for assembly of the exocyst complex, used to promote tethering and fusion of lysosomes at the immune synapse. On the other hand, our results show that resting B cells contain an active proteasome pool at the centrosome, which is required for efficient actin clearance at this level. Upon activation, the proteasome is transported to the peripheral domain of the IS, where it controls local actin cytoskeleton remodeling and cell signaling. Silencing components of the exocyst complex or interfering with proteasome activity severely impairs the ability of B cells to extract and present immobilized antigens. Altogether, we propose that the centrosome of B cells acts as a hub where specialized cellular machinery is focused and used by B cells to regulate morphological and functional parameters of the immune synapse.

POSTER SESSSIONS

Cardiovascular Physiology

C5. Mechanisms that define sarcoplasmic reticulum Ca release restitution in cardiac myocytes.

Alejandra Cely-Ortiz*, Juan I. Felice*, Carlos A. Valverde, Marilén Federico, Luciana Sapia, Julieta Palomeque, Elena Lascano, Jorge Negroni, Alicia Mattiazzi (* Equal contribution). Centro de Investigaciones Cardiovasculares UNLP/CONICET La Plata.

During EC-coupling, Ca influx induces the release of Ca from the sarcoplasmic reticulum (SR). The release mechanism needs time between stimuli to produce a second full Ca release (Ca release restitution, CRR). Dissection of the mechanisms determining CRR is important since its alteration is associated with arrhythmias. However these mechanisms are unclear with controversial results, mainly regarding the role of SR Ca uptake on CRR.

Aims. To dissect the mechanisms that define CRR. Methods. Experiments were performed in hearts and myocytes isolated from wild type (WT) mice, mice with increased SR Ca uptake (due to phospholamban ablation, PLNKO), mice with increased SR Ca channel (RyR2) activity (due to constitutive pseudo-phosphorilation of RyR2-Ser2814 CaMKII site, S2814D), and double-mutant mice (SDKO) obtained by crossbreeding S2814D and PLNKO. Intracellular Ca was measured using fluorescent probes. CRR curves were obtained by a two pulse protocol and the time constant (Tau) was used to evaluate CRR, assuming an exponential relationship between the Ca released and the time between stimuli. Hearts from each strain were frozen for Western blot analysis (WB). A previously validated human cardiac myocyte model that represented each experimental condition was used to perform simulations of the CRR protocol.

Results. WB analysis showed 33% decrease in RyR2 expression in PLNKO and SDKO hearts. Analysis of CRR curves indicated that CRR depends on SR Ca load (Increasing SR Ca by increasing extracellular Ca from 2 to 4 mM in WT mice, decreased Tau by 52%, $p < 0.05$), and on RyR2 activity (Tau value was not statistically different in S2814D vs WT mice, in spite of the fact that SR Ca content was 20% lower). The rate of SR Ca reuptake had no influence on Tau. The simulations obtained with the model reproduced the experimental results. However, when these simulations were carried out in PLNKO and SDKO representing only the increase in Ca uptake (i.e. without any decrease in RyR2 expression), the results of the model showed that the rate of SR Ca uptake is largely responsible of CRR.

Conclusion. CRR depends on SR Ca content, RyR2 sensitivity/activity and SR Ca uptake rate.

C6. Stabilization of Hypoxia-Inducible Factor-1 alpha enhances the tubulogenic capacity of genetically modified Muse cells.

Castillo Martha G., Giménez Carlos S., Nuñez Cristian N. Crottogini Alberto J., Olea Fernanda D, Cuniberti Luis A. Instituto de Medicina Traslacional, Trasplante y Bioingeniería (IMETTYB-Universidad Favaloro-CONICET).

Angiogenesis is the formation of new blood vessels from existing vasculature and is an integral part of both normal and pathological processes. Muse cells are a subpopulation of Mesenchymal stem cells (ASC) that has been reported to repair acute myocardial infarction (AMI) in animal models.

The objective of our study was to improve genetically human Muse cells transduced with the double-mutated HIF-1a gene to assess their angiogenic ability in an in-vitro tube formation assay.

Methods.

ASC and Muse cells were isolated from human lipoaspirates. In a third group, Muse cells were modified with a baculovirus that encodes a mutant form of the HIF-1a transcription factor that does not degrade under normoxia conditions (dmHIF-1a-Muse). Changes in the expression of the angiogenic genes HIF-1a, VEGF and FGF were studied by RT-qPCR. Additionally, in vitro tube formation assay was performed and the rings formed per unit area were quantified.

Results. HIF-1a expression increased 5.9 fold in Muse and 2555 fold in Muse-HIF compared to its expression in ASCs. VEGF and FGF expression showed similar behavior; VEGF 3.3 and 7.9 and FGF 3.5 and 5.9 fold increases, respectively.

In the tubulogenesis test, significant differences were observed in the number of rings per area (mm2) between dmHIF-1a-Muse group (31.42 ± 12.47) compared to the Muse group (21.75 ± 12.50, $p<0.05$) and ASC group (21.33 ± 5.63, $p<0.01$, One way ANOVA Bonferroni). However

non-significant difference was observed between Muse and ASC groups.
Conclusion.
Muse cells modified with dmHIF-1a increased their angiogenic capacity at the level of expression of molecules involved in the proliferation of vascular endothelial cells, as well as in the formation of three-dimensional vessels in functional assays.

C7. Subcellular mechanisms underlying the low cardiotoxicity of istaroxime.

María Florencia Racioppi, Juan Ignacio Burgos, Malena Morell, Martín Vila Petroff y Luis Alberto Gonano. Centro de investigaciones cardiovasculares Horacio E Cingolani.

Pharmacological inhibition of sodium/potassium ATPase (NKA) with digitalis improves cardiac contractility but also promote diastolic Ca2+ waves. We have demonstrated that acute exposure to high doses of digitalis induces CaMKII-dependent phosphorylation of RyR2 and Ca2+ related arrhythmias. In parallel, we showed that low "therapeutic" doses of digitalis activate CaMKII-dependent apoptosis in ventricular cardiomyocytes. Istaroxime has a dual effect which combines NKA inhibition with sarcoplasmic reticulum (SR)-Ca2+ uptake acceleration due to SERCA-PLB dissociation. This peculiar combination makes istaroxime an inotropic-lusitropic agent with lower arrhytmogenic potential compared to digitalis compounds. However, the impact of istaroxime on cardiomyocyte viability and apoptosis has never been addressed. Our hypothesis is that istaroxime can reach a significant inotropic response with lower induction of CaMKII-dependent cardiomyocyte apoptosis and lower occurrence of arrhythmogenic Ca2+ release during diastole.
To investigate this, mouse ventricular cardiomyocytes were paced at 1 Hz and superfused with Hepes-buffer at in the presence of ouabain 2 µM or istaroxime 10 µM, detecting that both promoted a similar increase in cell shortening. We also measured SR Ca2+ waves in Fluo-4 loaded cells to confirm if, after 1 hour of incubation, istaroxime is less arrhytmogenic than ouabain. On average, 2 µM ouabain-treated cells presented a significantly higher Ca2+ waves frequency compared to 10 µM istaroxime-treated cells (0,135±0,020 and 0,061±0,013 waves/min respectively. n= 23 per group). Cardiomyocytes were cultured for 24 hours at 37ºC and we observed a significant reduction in cell viability accompanied by an increase in caspase-3 activity in ouabain-treated cells. Interestingly, 10 µM and even 20 µM istaroxime did not significantly affect cell viability and caspase-3 activity compared to control without drugs (n=5 cultures per group). We conclude that istaroxime is less cardiotoxic than ouabain, suggesting its potential benefit for the treatment of chronic heart failure.

C8. The specific inhibition of the cardiac electrogenic sodium/bicarbonate cotransporter (NBCe1) leads to cardiac hypertrophy.

Di Mattia, Romina A.; Jaquenod - De Giusti Carolina; Blanco, Paula G.; Portiansky, Enrique L.; Aiello, Ernesto A.; Orlowski, Alejandro. Centro de Investigaciones Cardiovasculares Dr. "Horacio E. Cingolani"

Introduction: The Na+/HCO3- cotransporter (NBC) is one of the main alkalinizing transporters on cardiomyocytes. There are two isoforms of NBC: the electrogenic NBCe1 and the electroneutral NBCn1. Although both isoforms enters Na+ into the cell, NBCe1 contributes with half of Na+ per HCO3-, therefore it has a major efficiency. We have previously found in cardiac hypertrophy (CH) models that there was a reduction of NBCe1 activity together with an increased NBCn1 activity. However, we were unable to demonstrate the exact cause-consequence involvement of these NBC isoforms in the development of CH.
Experimental design: We developed an interference RNA cloned in an adeno-associated vector (siNBCe1) to study the effect of the specific inhibition of NBCe1 in CH. We delivered the virus through a lateral tail vein injection in male 3 months old Wistar rats and then performed a series of studies to assess CH, using an AAV9-siCtrl as control. Both vectors contained GFP.
Results: After 30 days of injection, euthanasia was performed. We first made sure that cardiomyocytes were green and that we had a significance reduction on NBCe1 expression. In addition, we compared the left ventricle mass index (LVMI) obtained by echocardiography. We found an increase of LVMI in hearts injected with the siNBCe1 (siCtrl:0.99±0.09;siNBCe1*:1.55±0.16;*p<0.05 vs siControl). This result was consistent with the analysis of cross-sectional area of the cardiomyocytes (in µm2;siCtrl:247.5±13.7;siNBCe1*:297.6±15.9;*p<0.05 vs siCtrl). No differences were found in blood pressure or collagen abundance. Additionally, some preliminary results indicate a compensatory increase in NBCn1 and Na+/H+ exchanger (NHE) expression.
Conclusion: Overall, these results suggest that the CH is developed, at least in part, by the decrease in NBCe1 expression. We propose that this reduction triggers a compensatory response involving the increase of the expression and activity of the remaining alkalinizing transporters. This mechanism would in turn induce the enhancement of intracellular Na+ levels, leading to Ca+2 overload trough Na+/Ca+2 exchanger acting in its reverse mode. Such increase of Ca+2 could lead to CH.

C9. Generation and transduction optimization of a baculoviral vector encoding the antimitotic gene meis1 as a preliminary in vitro model to evaluate possible cell cycle regulators.

López AE1, Bauzá MDR1, Simonin JA2, Halek JM1, Crottogini A1, Cuniberti L1, Belaich MN2, Olea FD1, Locatelli P1 Instituto de Medicina Traslacional, Trasplante y Bioingeniería (IMETTYB-Universidad Favaloro-CONICET). 2- Universidad Nacional de Quilmes.

Introduction: Ischemic heart disease, including acute myocardial infarction, is the main cause of death worldwide. Considering the poor results of clinical trials on cardiac regeneration, there is a growing interest in developing gene therapies aimed at inducing the adult cardiomyocyte mitosis, an approach that would warrant efficient electromechanical coupling of new cells to the myocardial sincitium.
Among the strategies to deliver genes to the heart is the use of viral vectors. Genetically engineered baculovirus, an originally insect-infecting virus, has been shown to efficiently infect mammalian cells while conferring a good safety profile. Our aim was to design a set of baculoviral vectors to overexpress the cell cycle inhibitor meis1 and 2 shRNAs targeting this gene, and evaluate the effects on

the proliferation rate in the rat myoblast cell line H9c2. Material and methods: Plasmids containing the genetic constructions were commercially obtained and employed to generate the baculoviral particles (Bv) through Bac-to-bac system: BacMeis1-GFP; BacshRNA A-mCherry and BacshRNA B-BFP. H9c2 cells were transduced at different multiplicities of infection (MOI) with a Bv encoding GFP (BacGFP).Transduction efficiency (TE) was measured by flow cytometry. Then the peak of expression of meis1 was assessed at 24, 36, 48 and 72 hours by RT-qPCR. The effect of meis1 overexpression on cell proliferation was evaluated by MTS assay at 5 days post-transduction (PT).
Results: TE was 26,41 %, 49,72 % and 53,6 % for MOI 100, 300 and 900, respectively. The peak expression of meis1 was observed between 36 and 48 hours PT reaching a 6-fold increase vs. meis1 basal levels. MTS proliferation assay revealed a decrease in cell division rate at 5 days PT (area under the curve: 3±0.099 BacMeis1-GFP vs. 3.2±0.031 BacGFP, $p<0.05$).
Conclusion: The transduction of H9c2 cells with a baculovirus encoding meis1 was optimized, the peak expression of the transgene was detected and it induced a reduction in proliferation rate, confirming the role of meis1 as a cell cycle inhibitor in rat and providing an in vitro system to assess the effects of the shRNAs.

C10. Selective activation of the G-protein-coupled estrogen receptor (GPER) decreases cardiac contractility through inhibition of the L-type calcium channel (ICa) via Nitric Oxide Synthase

Leandro A. Diaz Zegarra, María S. Espejo, Alejandro M. Ibañez, Verónica C. De Giusti, Ernesto A. Aiello. Centro de Investigaciones Cardiovasculares "Horacio E. Cingolani", Facultad de Ciencias Médicas, UNLP-CONICET.

GPER was described as an orphan membrane receptor associated with protein G. Years later, estradiol was proposed as a ligand. The activation of GPER by its synthetic agonist G1 has cardioprotective effects. However, the mechanisms involved in these effects have not been fully clarified. The objective is to evaluate the role of GPER on baseline cardiac contractility. Rat heart ventricular myocytes loaded with the fluorescent calcium indicator FURA-2 were used. Sarcomeric shortening (SS) and transient changes in intracellular calcium concentration (Ca2+i) were measured by video camera and epifluorescence, respectively. Calcium influx through the L-type channel (ICa) was evaluated with the patch-clamp technique. * indicates $p<0.05$ vs control. G1 (1 μM) caused a decrease in contractility (% of effect (n=15): -34.0±6.0*, respect to Control (Ctrl)), accompanied by a reduction in Ca2+I (% of effect (n=11): -27.6±8.6, respect to Ctrl). Both effects were prevented with the antagonist of GPER, G36 (1 μM) (% of effect in SS (n=14): G36+G1: -0.91±2.48 respect to Ctrl; % of effect in UA (n=9): G36+G1: -10.72±2.11 respect to Ctrl). G1 induced a significant decrease in ICa (pA/pF 0 mV, Ctrl: 4.44±0.71; G1: 3.44±0.47*; n=14), consistent with the negative inotropic effect observed above. Also, the effect was prevented with G36 (pA/pF 0 mV: G36: 5.23±0.48; G36+G1: 4.97±0.43, n=6). Finally, thinking that a possible pathway involves Nitric Oxide Synthase (NOS), the experiments were performed in the presence of the NOS inhibitor, L-NAME (10 μM). No differences were found in baseline contractility respect to L-NAME + G1 (% of effect (n=9): L-NAME+G1: 4.73±2.56, respect to Ctrl), in Ca2+i (% of effect (n=10): L-NAME+G1: -0.11±2.60, respect to Ctrl) and ICa (pA/pF 0 mV, L-NAME: 2.92±0.25, L-NAME+G1: 2.71±0.25, n=12, $p>0.05$). We conclude that the selective activation of cardiac GPER induces a decrease in contractility as a consequence of the inhibition of ICa, through activated NOS, however other intermediaries are not ruled out.

C11. CaMKII determines arrhytmogenic events induced by acute exposure to high glucose

Sofía López, Marilen Federico, Guillermina Nuozzi, Matilde Said, Alicia Mattiazzi, Julieta Palomeque. Centro de Investigaciones Cardiovasculares (CIC) UNLP, CONICET.

Glycaemia is continuously fine-tuned according to our food intake and consumption. However this regulation could be disrupted under certain metabolic conditions as impaired glucose tolerance, metabolic syndrome and diabetes mellitus; or even in stress context, i.e. surgery, cardiac infarct. Given that pre-diabetic and diabetic hearts present calcium (Ca2+) mishandling due, in part, by Ca2+-Calmodulin Kinase II (CaMKII)hyperactivation, we hypothesized that an acute increase in glycaemia causes Ca2+ handling abnormalities capable to trigger arrhythmias in a CaMKII dependent pathway. We used isolated mice cardiomyocytes loaded with Fura 2-AM to evaluate intracellular Ca2+ handling by epifluorescence, Langendorff perfused hearts to measure developed pressure (DP) and monophasic action potential (MAP) and hearts homogenates to perform [3H]Ryanodine binding assay to evaluate ryanodine receptor 2 (RyR2) activity.
The change from normal glucose (NG) buffer (11 mM, 325.61mOsm) to high glucose (HG) buffer (25 mM, 339.26 mOsm) significantly increased Ca2+ transient (CaiT) amplitude (60.4±20.5%) and arrhythmogenic events (71.429%). A similar increment in osmolality induced by sucrose or choline chloride did not produced any effect either in CaiT or rhythmicity. The effects of HG were prevented by treatment with AIP (2.5μM), a specific CaMKII inhibitor and in S2814A mice myocytes (which have the S2814 residue of RyR2 mutated to A, unphosphorytable by CaMKII). In Langendorff perfused hearts, HG perfusion increased DP (30.0±3%) and produced ectopic beats with respect to NG perfusion. [3H]Ry binding assays showed a significantly increase in RyR2 sensitivity (1.05±0.27 vs 2.30±0.38μM) and maximal activity (Bmax: 48.09±2.35, vs 31.58±1.98) under HG vs. NG conditions.
We conclude that acute administration of HG induces changes in Ca2+ handling and arrhythmic events that are dependent on CaMKII activation, probably through phosphorylation of RyR2.

C12. SR- leak, mitochondria injury and SR-mitochondria miscommunication culminate in apoptosis in prediabetic hearts

Federico Marilen(1), López Sofía(1), Zavala Maite(1), Vico Tamara(3), Portiansky Enrique(4), Alvarez Silvia(3), Villa Abrille Celeste(1), Mattiazzi Alicia(1), Palomeque Julieta(1,5) Centro de Investigaciones Cardiovasculares, UNLP, CONICET.

Prediabetic hearts present Ca2+ mishandling, Ca2+-calmodulin kinase II (CaMKII) hyperactivity, mitochondria injury, ultrastructure disarrangements and apoptosis. The SR-mitochondria interplay is pivotal in patho-physiological

situations (included metabolic diseases) and mitochondria structure and metabolisms is essential to fate of cells. We hypothesize that in prediabetic hearts, SR-Ca2+ leak is due to increased ryanodine receptors 2 (RyR2) activity via CaMKII-dependent pathway, which in turn produces mitochondrial Ca2+ overload, mitochondrial metabolic changes, which play a role in the enhanced SR-mitochondria proximity of prediabetic hearts and ultrastructure alterations.

We measured spontaneous Ca2+ release events (SCaRE), 3H-Ryanodine ([3H]Ry) binding assay, mitochondrial Ca2+ retention capacity (CRC), ATP and H2O2 production, and O2 uptake, morphology and fission/fusion proteins expression in a prediabetic model induced by fructose rich diet (FRD) in WT and AC3I mice, which express a CaMKII-inhibitor at heart level.

Confocal images showed significantly increased SCaRE in WT FRD vs WT CD myocytes, which were prevented in AC3I mice. [3H]Ry binding assay revealed higher Bmax in WT FRD than WT CD and AC3I FRD. In isolated mitochondria, CRC, ATP production rate and O2 uptake were decreased, while H2O2 production rate was increased. Transmission Electron Microscopy photographs showed decreased mitochondria area and diameter in WT FRD respect to WT CD, whereas Feret diameter and roundness was higher in WT FRD than WT CD. A clear disarrangement in WT-FRD tissue was evident. A decreased pDRP-1 was found and no differences were found in Mfn-2 or OPA-1 in WT FRD.

We conclude that CaMKII is involved in the increase of RyR2 activity, SR Ca2+ leak and mitochondria morphology changes of FRD. Moreover, the increased in SR Ca2+ leak and SR-mitochondria proximity would favor the decreased CRC, favoring mitochondrial Ca2+ overload and decrease mitochondrial bioenergetics: increased mitochondrial H2O2 production and decreased O2 consumption and ATP production. Finally, fission process are present without fusion.

C13. Role of soluble adenylate cyclase (sAC) in basal of normotrophic and hypertrophic hearts.

Noelia Rossetti, María S. Espejo, Alejandro M. Ibañez, Ernesto A. Aiello, Verónica C. De Giusti. Centro de Investigaciones Cardiovasculares. UNLP. CONICET.

In the heart, cyclic AMP (cAMP), stimulating the protein kinase A (PKA) is associated to the excitation-contraction coupling regulation. Historically, the only known source of cAMP were the transmembrane adenylyl cyclases (tmACs). However, recently a cytosolic adenylyl cyclase (sAC) was described, which represents an alternative source of localized cAMP. Besides sAC was described to be present in the heart, its pathophysiological function is unknown. In addition, it is known that the cardiac Na+/HCO3- cotransporter (NBC) promotes the cellular co-influx of HCO3- and Na+. Since sAC activity is regulated by HCO3-, our purpose was to investigate the potential functional relationship between NBC and sAC in the cardiomyocyte. Male Wistar rats were used as a normotrophic model, whereas spontaneously hypertensive rats (SHR) or mice with thoracic aortic constriction (TAC) were used as hypertrophic model. Rat and mouse ventricular myocytes were isolated and loaded with Fura-2 in order to measure Ca2+ transient (Ca2+i) by epifluorescence. Sarcomere or cell shortening were measured as index of contractility. * indicates $p<0.05$ vs control (pared t-test). The NBC blocker S0859 (10 μM), the selective inhibitor of sAC, KH7 (1 μM) and the PKA inhibitor, H89 (0.1 μM) induced a negative inotropic effect (NIE) in normotrophic (Wistar: control 19±3.23 vs S0859 14.5±2.64* (9); control 13.3±1.7 vs KH7 10.2±1.6* (9); control 9.43±0.94 vs H89 6.65±0.77* (12)) and hypertrophic hearts (TAC: control 10.37±0.87 vs S0859 6.96±0.63* (14); control 9.87±0.16 vs KH7 7.43±0.18* (10); control 6.84±0.93 vs H89 4.19±0.55* (7); SHR: control 9.66±0.84 VS S0859 7±0.70* (16); control 12.58±1.32 vs KH7 10.74±1.07 (9); control 10.54±1.40 vs H89 8.57±1.54* (14)). These contractile behavior was associated to a significant decrease in Ca2+i. No effect of these drugs was observed in the absence of HCO3-. These results demonstrated that the complex NBC-sAC-PKA plays a relevant role in Ca2+ handling and basal contractility, both in normotrophic and hypertrophy hearts.

C14. Transverse Aortic Constriction (TAC): Technique Minimally Invasive for induction of left ventricular hypertrophy in mice.

Noelia S. Rossetti, Leandro Diaz Zagarra, Omar Velez Rueda, Ernesto A. Aiello, Verónica C De Giusti. Centro de investigaciones cardiovasculares. UNLP. CONICET.

Transverse aortic constriction (TAC) in mice is one of the most commonly used surgical techniques for experimental investigation of pressure overload-induced left ventricular hypertrophy induced by overload.

In the majority of the reported investigations, this procedure is performed with intubation and ventilation of the animal which renders it demanding and time-consuming and adds to the surgical burden to the animal.

In our laboratory we have developed a minimally invasive technique without intubation and ventilation of mice, significantly reducing surgical time and complications.

Surgical Technique: C57 Muss Musculus male mice (11 weeks old, 28-30 g, n = 40).

Position: Place the animal supine on a heating pad. Skin asepsis is performed with 70% alcohol.

Anesthesia: Inject intra - peritoneally a single dose of a mixture of ketamine (51.4 mg/kg) and xylazine (3.3 mg/kg) diluted in saline solution (0.9% NaCl).

Incision: we perform a longitudinal cervicotomy of more than 10 mm with an 11-blade knife. Dissection of pretracheal muscles, mediastinal fat and thymus using curved soft-tip microsurgical forceps to visualize the aortic arch. Clips are placed in the aortic arch between the left common carotid artery and the left subclavian artery, achieving an occlusion of 66% of the lumen of the artery.

Skin closure: 5/0 continuous polypropylene monofilament suture. We perform the simulated procedure identical to the constriction operation but without placing the clip.

Results: cardiac hypertrophy demonstrated by ultrasound is already present on day 21 after surgery, compared to simulated surgery.

The echocardiogram was performed at 3 weeks showing a significant increase in the left ventricular mass.

in conclusion, our cost-effective minimally invasive technique in mice has a very low operative and postoperative mortality close to 4% and is highly efficient in inducing LVH. Simplify the operating procedure and

reduce the strain on the animal. It can be easily done following the critical steps described in our technique.

C15. Tbx20 gene overexpression increases cell proliferation and the levels of pro mitotic genes in post-natal rat cardiomyocytes.

Bauzá María del Rosario(1), López Ayelén Emilce(1), Simonin Jorge Alejandro(1), Halek Julia María(1), Belaich Mariano Nicolás(2), Cuniberti Luis(1), Crottogini Alberto José(1), Locatelli Paola(1), Olea Fernanda Daniela (1). (1) Instituto de Medicina Traslacional, Trasplante y Bioingeniería-Universidad Favaloro-CONICET (2) Universidad Nacional de Quilmes.

Introduction: myocardiogenic therapies have been proposed to induce cardiac regeneration after myocardial infarction. One of possible approaches would be to stimulate the adult cardiomyocyte to re-enter the cell cycle and divide into daughter cells. To achieve this, positive and negative cell cycle regulator molecules could be down regulated. The induced overexpression of TBX20 transcription factor in transgenic mice increased cell proliferation.

Objective: To evaluate the cell proliferation and gene expression of the cell cycle stimulator genes (Cyclin D1 and MEF2C) and repressor gene (p21) in post-natal rat cardiomyocyte culture.

Methods: Baculoviral vectors encoding Tbx20 (BvTbx20) and GFP (BvGFP) genes were generated. Post-natal cardiomyocytes (CM) were cultured and transduced at 3 days of culture with BvGFP at different multiplicities of infection (MOI: 25, 50, 100, 200 and 500). Transduction efficiency was measured by flow cytometry.

Cell proliferation (MTS assay) and gene expression of Cyclin D1, MEF2C and p21 (RT-qPCR) were measured in CM transduced with BvTbx20 at different days.

Results: Transduction efficiency was optimal at MOI 100 (41.72%). Percentage of cell proliferation was higher in transduced CM (Tbx20CM: 109.6±4.5) respect to non transduced CM (ntCM: 100±3.2, $p<0.05$) at 5 days of culture. Consistently, both the expression of Cyclin D1 (Tbx20CM:5.2±0.2; vs ntCM: 4.7±0.2 mRNA relative level, $p<0.05$), and MEF2C expression (Tbx20CM:1.1±0.6; vs ntCM: 0.5±0.3, $p<0.05$) were higher at 5 days of culture. Furthermore at 8 days of culture, the cyclin D1 expression was increased in Tbx20CM (16.4±5.8) vs ntCM (9.9 ±5.6, $p<0.05$) and p21 expression was decreased in CM-BvTbx20 (1±0.3) vs ntCM (1.8±0.4, $p<0.01$).

Conclusion: Tbx20 overexpression in post-natal cardiomyocytes cell culture increased cell proliferation and gene expression of pro-mitotic genes (Ciclin D1 and MEF2C) and decreased anti-mitotic gene (p21). These results suggest that Tbx20 is a positive cell cycle regulator and may be potentially useful as a therapeutic factor to induce myocardial regeneration.

C16. Acute myocardial glucocorticoid receptor activation decreases NHE1 activity.

María E. Amarillo, Camila Barbieri, Juan P. Prieto Díaz, Néstor G. Pérez and Romina G. Díaz. Centro de Investigaciones Cardiovasculares “Dr. Horacio E. Cingolani”, Facultad de Ciencias Médicas, Universidad Nacional de La Plata.

Background/Aims: Glucocorticoid receptor (GR) is highly expressed on cardiac tissue, but its specific activity remains rather unknown. GR activation following an ischemic insult ameliorates cardiac function through a mechanism that involves calcineurin pathway inactivation. Since cardiac NHE1 activation has been linked to an increased calcineurin activity, we sought to determine if acute GR activation could modulate NHE1 activity.

Methods: Experiments were performed in isolated rat papillary muscles. NHE1 activity was assessed by Na+-dependent pHi recovery after acute transient acidosis (ATA). AKT and NHE1 phosphorylation, NHE1 expression and dimerization were determined by western blot. Hydrocortisone (HC) was used to trigger GR activation. Experimental groups: Non-acidotic control, ATA, ATA+HC 1nM, and ATA+HC 10nM.

Results: HC promoted a dose-dependent decrease in NHE1 activity after ATA (JH+: 1.22±0.19 ATA, n=5; vs. 0.68±0.11 HC1, n=4; vs. 0.56±0.15 HC10, n=4, $p<0.05$). NHE1 expression was not affected by the acute HC treatments. Since it was reported that corticoid stimulation promotes membrane changes, and NHE1 dimerization/activity is affected by changes in membrane composition, we next analyzed dimerization status of NHE1 by non-reducing western blot. No differences between dimeric and monomeric NHE1 were detected among groups. Acute GR-activated signaling pathway has been linked to activation of AKT in zebrafish. Since AKT-mediated NHE1 phosphorylation decreases exchanger activity, AKT phosphorylation (as index of protein activation) in homogenates, as well as phosphorylation of AKT consensus sequence (AKT-cs) in immunoprecipitated NHE1 samples following HC stimulation were explored. An increase in AKT phosphorylation was detected under HC stimulation, and a HC dose-dependent increase in NHE1 AKT-cs was also found (%AKTcs-phosphorylated NHE: 155±23 HC10 vs. 100±5 non-stimulated control $p<0.05$).

Conclusion: Current results show for the first time that acute GR activation decreases NHE1 activity in the myocardium. Our findings preliminary suggest that AKT phosphorylation/activation would be part of this inhibitory signaling pathway

C17. Hyperactivity of the NHE1 Na+/H+ exchanger in a mouse model of type 2 diabetic cardiomyopathy.

Fernanda E. Carrizo Velásquez1, Rayen De Fazio1, Paula N. Arrías2, Carolina Jaquenod De Giusti1. 1 Centro de Investigaciones Cardiovasculares, Facultad de Ciencias Médicas, UNLP-CONICET.

Heart failure is the leading cause of death among diabetic people. Cellular and molecular entities leading to diabetic cardiomyopathy (DCM) are poorly understood. The role of Na+/H+ exchanger 1 (NHE1) and Ca2+ load was studied in obese type 2 diabetic mice (ob-/-) and control heterozygous littermates (ob+/-).

Echocardiography showed elevated systolic interventricular septum thickness and systolic posterior wall thickness in ob-/- mice at 16 weeks. ob-/- mice showed increased left ventricular weight/tibia length ratio and increased cardiomyocyte cross-sectional area as compared to controls, indicating cardiac hypertrophy. Immunoblot analysis did not show altered NHE1 expression but augmented NHE1 phosphorylation in ob-/- hearts. NHE1-dependent rate of intracellular pH (pHi) normalization after acid loading of isolated cardiomyocytes was higher in ob-/- mice vs. ob+/-. Enhanced myocardial NHE1 activity could promote Na+ and subsequently cytoplasmic Ca2+ loading, leading to

changes in mitochondrial Ca2+ content. We detected altered Ca2+ handlings in cardiac mitochondria of ob-/- mice. ob-/- mice presented reduced swelling and Ca2+ retention capacity. In addition, these mitochondria presented reduced membrane potential. All these could be initially explained by increased cardiac mitochondria Ca2+ load. However, we detected increased NHE1 expression on mitochondrial membranes, NHE1 hyperactivity could affect mitochondria function by altering Na+ content and affecting NCX activity. In fact, NHE1 inhibition resulted in reversion of this phenotype, indicating that the observed alterations could be due to increased NHE1 activity in ob-/- mice. These findings suggest that exacerbated NHE1 activity contributes to mitochondrial dysfunction, a primary recognized pathogenic response in diabetic cardiomyopathic hearts.

C18. Functional studies of six uncharacterized mutations in low-density lipoprotein receptor gene.

Gomez Andrea, Giunta Gustavo, Helman Lorena, Toscanini Ulises, Cuniberti Luis. Laboratorio de Lípidos, Aterosclerosis y Biología Vascular-IMETTyB-Universidad Favaloro, Fundación Favaloro-CONICET, Buenos Aires, Argentina.

Introduction: Familial Hypercholesterolemia (FH) is a primary hyperlipemia. It is an autosomal dominant genetic disorder of lipoproteins metabolism mainly caused by mutations in the lipoprotein receptor gene (LDLR).

Aim: We aimed to investigate the functional impact on the low density lipoprotein receptor (LDLR) activity of six uncharacterised variants located in the coding region of the LDLR gene, namely c.428G>T, c.640T>C, c.1708C>T, c.1736A>T, c.1981C>G and c.2114C>G (NM_000527.4) and to attempt to define their clinical status.

Material and Methods: Functional studies were carried out using site-directed mutagenesis techniques and expression of LDLR protein in vitro. Results were correlated with clinical data and in silico analyses in order to assess the physiopathological role of these variants.

Results: This work provides functional information about 6 uncharacterized mutations in LDLR. The six variants studied here appeared to affect the LDLR function in vitro to different degrees, ranging from receptors with normal to slightly reduced activity to receptors exhibiting less than 10% of the wild-type activity.

Conclusion: According to these studies and The American College of Medical Genetics and Genomics (ACMG) Standards and Guidelines, two variants could be classified as “Likely Benign” (p.(Ala705Gly) and p.(Leu570Phe)), three variants as “Pathogenic” (p.(Asp579Val), p.(Cys143Phe) and p.(Trp214Arg)) and one variant as “Likely Pathogenic” (p.(Pro661Ala)).

C19. Lipoprotein (a) contributes to non-monogenic Familial Hypercholesterolemia status?

Gomez Andrea, Giunta Gustavo, Helman Lorena, Toscanini Ulises, Cuniberti Luis. Laboratorio de Lípidos, Aterosclerosis y Biología Vascular-IMETTyB-Universidad Favaloro, Fundación Favaloro-CONICET, Buenos Aires, Argentina.

Introduction: Among traditionally coronary risk factors, high levels of Lipoprotein (a) (Lp(a)) represent a disorder associated with major risk of coronary artery disease in patients with clinical diagnosis of familial hypercholesterolemia (FH) and in general population. FH is an autosomal dominant genetic disorder of lipoprotein metabolism, mainly associated with the presence of mutation in three conventional genes (low-density lipoprotein receptor (LDLR), apolipoprotein B (APOB) and proprotein convertase subtilisin/kexin type 9 (PCSK9)), characterized by elevated plasma concentration of low-density lipoprotein cholesterol (c-LDL), presence of tendon xanthomas, and it is associated with early cardiovascular disease.

Aim: We aimed to analyze the measures of Lp (a) in a cohort of patients with clinical diagnosis of Familial hypercholesterolemia whom had been tested for monogenic disorder.

Material and Methods: We have evaluated 38 patients with clinical diagnosis of familial hypercholesterolemia. Mutations screening were done using Sanger sequencing in the coding region and adjacent intronic areas of LDLR and PCSK9 genes and exon 26 of APOB gene. Lp(a) levels were determinated by a turbidimetric method using inmunoglobuling G anti-human Lp(a) (Quantia Lp(a) 7K00-01) in an Architect autoanalyzer C16000 (Abbott Diagnostics) and elevated Lp(a) was defined as levels > 50 mg/dL.

Results: The thirty nine point five percent of the analyzed patients did not have pathogenic mutations in coding region and adjacent intronic areas of LDLR, PCSK9 neither in exon 26 of APOB gene. On the other hand, they exhibited higher levels of Lp(a) (at least twofold, p=0,02) in comparison with the group of patients with a monogenic disorder probed.

Conclusions: We concluded that when the monogenic disorder is not present in patients with clinical diagnosis of FH, high levels of serum Lp(a) could be indicative of polygenic or oligogenic cause. Screening for LPA gene variants, such as the kringle IV type 2 (KIV-2) copy number variants would be interesting to elucidate in future analyses.

C20. Modulation of cardiac function by the chronic supplementation with n-3 polyunsaturated fatty acids.

Maite R. Zavala, Lucrecia Longarzo, María J. Almada, Omar Vélez Rueda, Sabina M. Maté, María C. Villa-Abrille. Centro de Investigaciones Cardiovasculares Dr. Horacio E. Cingolani (CIC)-Instituto de Investigaciones Bioquímicas de La Plata “Profesor Doctor Rodolfo R. brenner” (INIBIOLP)

Several studies have shown that supplementation with polyunsaturated n-3 fatty acids (PUFAs) leads to modifications on the cardiac physiology. Dietary fatty acids have been found to affect lipid raft composition, affecting cell function. The Na+/H+ exchanger (NHE1), an integral membrane protein, is involved in the maintenance of the intracellular pH (pHi). Its activity is regulated by allosteric site sensitivity for H+, phosphorylation and by the union of ATP, lipids, growth factors in its cytoplasmic tail. The objective of the present work was evaluate the effect of two major PUFAs, docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) on the modulation of NHE1 activity and cardiac function in normo (Wistar, W) and spontaneously hypertensive rats (SHR).

The animals received orally 200 mg/kg body mass/day of EPA and DHA. After 3 months of treatment we measured: systolic blood pressure (SP), echocardiographic parameters (left ventricular mass index LVMI, fractional shortening FS%, and ejection fraction EF %). The rats were

sacrificed and obtain ventricular cardiomyocytes for measured the NHE1 activity.
The SHR rats showed a significant increase in the SP compared with the Wistar rats. However, although the treatment with PUFAs did not affect the SP in SHR, we observed a modestly, but not significant, reduction in the cardiac hypertrophic index (LVMI).
The activity of NHE1 measured as the velocity of pHi recovery (dpHi/dt) after intracellular acidification, was significantly higher in SHR compared with the Wistar rats. The dietary PUFAs did not affect the activity of the exchanger in Wistar rats. On the other hand in SHR, we observed a modest, although not significant, decrease in the NHE1 activity, showing a velocity recovery similar to Wistar rats.
Together, these preliminary results allow us to suggest that the PUFAs supplement in hypertensive rats could improve the cardiac function through the decrease in the NHE1 activity and the cardiac hypertrophy, despite similar values of SP (afterload)

C21. Quercetin electrophysiological protective effects in a proarrhythmic dysmetabolic rat model.
Villanueva F, Prado NJ, Vazquez Prieto MA, Miatello RM, Ponce Zumino AZ, Diez ER. Facultad de Ciencias Médicas, Universidad Nacional de Cuyo, Mendoza, Argentina.
BACKGROUND: Severe arrhythmias during reperfusion increase in patients with metabolic syndrome. Quercetin is a bioactive flavonol found in high concentrations in grapes and has potential cardioprotective actions, but little is known about its electrophysiological properties.
OBJECTIVE: To evaluate electrophysiological effects of chronic quercetin administration in rats with increased arrhythmogenesis during reperfusion due to a high fat and fructose diet (HFFD).
METHODS: Male Wistar rats were fed for six weeks with control diet (C), HFFD (20 % fat and 20% fructose w/w), HFFD supplemented with quercetin in a dose of 20 mg/Kg/day (HFFDQ) or quercetin alone (Q). Isolated hearts were perfused with a Krebs-Henseleit solution and after stabilization, submitted to 10 min of regional ischemia followed by10 min of reperfusion. Epicardial action potential and arrhythmic events were analyzed.
RESULTS: HFFD and HFFDQ developed metabolic syndrome, but action potential at 90% of repolarization lengthening only increased in HFFD (79±6 vs 54±7 ms, $P<0.01$ by ANOVA). HFFD increased ventricular fibrillation incidence (HFFD 8 of 9;C 2 of 9 $P=0.0152$ by Fisher Exact test). Both groups treated with quercetin had low arrhythmic incidence (HFFDQ 2 of 10 and Q 1 of 9; $P<0.0055$ and $P<0.0034$, respectively when compared with HFFD). The severity of arrhythmias persisted high during reperfusion only in HFFD. Other less severe arrhythmias like salvos and extra beats occurred in similar proportion in the three other groups.
CONCLUSION: The above results confirm that a HFFD facilitates the occurrence of severe arrhythmias during reperfusion. Quercetin reduces arrhythmias and electrophysiological remodeling despite metabolic syndrome. Since quercetin is one of the most abundant dietary flavonoids, our results reinforce its nutraceutical potential.
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Endocrinology

EN1. Osmotic stress and human amnion aquaporins.
Di Paola, Mauricio1,2; Sierra, Matías Nicolas1; Doroshnko, Brian1; Fernandez, Nazarena1; Erlejman, Alejandra3; Damiano, Alicia E1,2. 1Cátedra de Biología Celular y Molecular, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires. 2 Laboratorio de Biología de la Reproducción, Instituto de Fisiología y Biofísica Bernardo Houssay (IFIBIO)- CONICET- Facultad de Medicina, Universidad de Buenos Aires. Buenos Aires, Argentina. 3 Departamento de Química Biológica, Facultad de Ciencias Exactas, Universidad de Buenos Aires.
INTRODUCTION: Amniotic fluid (AF) is essential for normal fetal growth. Aquaporins (AQPs) may have a key role in the AF volume homeostasis. Therefore, altered expression of these proteins might be associated with pathologies as oligo and polyhydramnios. However, the etiology of these syndromes remains unknown.
Recently, we demonstrated that AQPs facilitate water transport across human amnion, being the AQP1 the most important contributor.
OBJETIVE: To study the effect of an osmotic stress on the expression and regulation of AQPs in amniotic cell line WISH.
METHODS: WISH cell line were cultured in hypo (150 mOsm) and hyperosmolar (400 mOsm) conditions. AQP1, AQP3 and AQP9 expressions were examined. Tetraethylammonium (TEA) and $CuSO_4$ were used to block AQP1 and AQP3, respectively. Cell viability and integrity were studied by the MTT and LDH assays. Cell swelling was studied with phase contrast microscope and Image-J software. Nf-kB and I-kB expression were assessed in nuclear and cytosolic fractions.
RESULTS: AQP1 and AQP9 significantly decreased (n=6; $p<0.05$) in WISH cells cultured in hyperosmolarity while AQP3 significantly increased (n=7; $p<0.05$). On the other hand, hypoosmolarity significantly increased AQP1 and AQP9 (n=6; $p<0.001$ and $p<0.05$ respectively) and decreased AQP3 (n=6; $p<0.001$). Under hypoosmolar conditions, AQP1 blocking affected negatively cell viability (n= 9; $p<0.01$) but it was not modified under hyperosmolar condition. AQP3 inhibition reduced cell viability in both conditions (n=9; $p<0.01$). Cell swelling studies showed that hypoosmolar treatment increased cell volume and AQP1 inhibition prevented it. However, AQP3 blocking was not enough to avoid cell swelling (n=7; $p<0.05$).
In hyperosmolar condition, Nf-kB p-65 nuclear expression significantly increased (n=3, $p<0.01$), while I-kB significantly decreased compared to control (n=3; $p<0.01$). No changes were observed when cells were cultured in hyoposmolar condition.
CONCLUSION: Our findings suggest that AQP1 and AQP3 may have a dual role in the function and cell survival of human amnion. AQP1 may be required for water transport

across the amnion, while AQP3 may be involved in the cell survival.

EN3. Effects of hyperthyroidism on the cellular antioxidant system and the expression of DNMT-1 in peripheral blood mononuclear cells of patients with Graves' disease.

Costilla Melisa; Saban Melina; Klecha Alicia; Di Cugneo Mariana; Curria Marina; Cremaschi Graciela; Barreiro Arcos María Laura. Instituto de Investigaciones Biomédicas (BIOMED)-CONICET, Buenos Aires; Hospital Británico de Buenos Aires-Servicio de Endocrinología, Buenos Aires.

Introduction: Graves' disease is the most frequent cause of hyperthyroidism. It is characterized by the presence of autoantibodies anti-TSH receptor, which causes overproduction of T3 and T4. Although its etiology has not been fully clarified, it is due to the complex interaction between genetic and environmental factors. Objectives: Analyze the effect of oxidative stress induced by the hyperthyroid state, on the cellular antioxidant system and the expression of DNMT-1, an enzyme that could regulate the transcription of genes involved in autoimmune processes. Methodology: Peripheral blood mononuclear cells (PBMC) of euthyroid subjects, patients with Graves' disease recently diagnosed or treated with methimazole were purified by Ficoll-Hypaque. Serum levels of T3, T4 and autoantibodies were quantified by immunoassays. Reactive oxygen species (ROS) and apoptosis were evaluated by cytometry. Nuclear morphology was analyzed by microscopy. Catalase activity was determined by spectrophotometry and glutathione concentration was quantified by HPLC/MS. The genomic and protein expression of catalase and DNMT-1 was determined by PCR and western blot. Results: PBMC of patients with Graves' disease had increased levels of ROS that were reverted to control by preincubation with N-acetyl cysteine (NAC, 2 mM). ROS levels in patients treated with methimazole were similar to the euthyroid subjects. Only the PBMC of patients with Graves' disease had a significant increase in the expression and activity of catalase and decreased levels of GSH. They also showed a higher genomic and protein expression of DNMT-1, which decreased in the presence of NAC. PBMC of patients treated with methimazole expressed levels of DNMT-1 similar to controls. Cellular apoptosis was similar in the three study groups. Conclusions: The oxidative stressexpression and activity of catalase and decreasing the content of GSH. ROS detoxification protects cells from apoptosis. On the other hand, ROS induces the expression of DNMT-1, enzyme responsible for DNA methylation, silencing immunoregulatory genes and contributing to the pathogenesis of Graves' disease.

Cell Physiology

FC1. The loss of functionality of aquaporin 9 adversely affects the survival of trophoblast cells.

Medina Yollyseth, Di Paola Mauricio, Anud Carolina, Brian Dorosshnko, Reppetti Julieta, Damiano Alicia E. IFIBIO HOUSSAY UBA-CONICET.

Introduction: Trophoblast apoptosis is a physiological process during placentation and increases progressively throughout gestation. It is notably exaggerated in pregnancy complications such as preeclampsia. We have previously reported that aquaporin 9 (AQP9) protein expression increased in preeclamptic placentas buy it was not functional. Recently, we demonstrated that the exacerbated oxidative and nitrative stress may induce the nitration of AQP9 resulting in a non-functional protein. In addition to water, AQP9 can permeate small uncharged solutes such as lactate. Lactate can be used as an energy substrate to prevent cell death.
Objective: Our aim was to explore the role of AQP9 in the survival of trophoblast cells.
Methodology: This study was approved by the ethics committee of the Hospital Nacional Dr. Prof. A. Posadas. Explants from normal term placentas were cultured in low glucose conditions with or without L-lactate, and in presence and absence of AQP9 inhibitors (0.3 mM $HgCl_2$, a general blocker of AQPs, and 0.5mM Phloretin, to block AQP9). Bax/Bcl-2 protein expression ratio by Western blot, cell viability by MTT assay and cytotoxicity by LDH release were assessed.
Results: In explants cultured in low glucose medium, MTT decreased and Bax/Bcl-2 ratio increased compared to controls, showing that cells were dying ($n=5$, $p<0.02$). In both conditions, the blocking of AQP9 did not modify these results. However, when explants were culture in low glucose medium supplemented with L-lactate, MTT and Bax/Bcl-2 ratio were similar to controls, suggesting L-lactate could substitute glucose. In this case, the blocking of AQP9 resulted in an increased of cell dying ($n=4$, $p<0.05$). In addition, LDH released did not change in any situation.
Conclusions: Our results suggest that AQP9 may have a key role in lactate transport in human placenta. Thus, the blocking of lactate transport mediated by AQP9 negatively affects the survival of trophoblast cells. Therefore, the expression of non-functional AQP9 in preeclamptic placentas may play a direct role in the pathogenesis of preeclampsia.

FC2. Effects of α-hemolysin on cell volume and intracellular sodium and potassium of human erythrocytes.

Lauri Natalia a, b, Cane Lucia c, Mouro-Chanteloup Isabelle d, e, Leal Denis María Florencia a, Alvarez Cora a, Lefevre L. Sophie d, e, Ostuni Mariano A. d, e, Herlax Vanesa c, Alleva Karina a, f, Schwarzbaum Pablo J. a, b. a Universidad de Buenos Aires, Consejo Nacional de Investigaciones Científicas y Técnicas, Instituto de Química y Fisico-Química Biológicas (IQUIFIB) "Prof. Alejandro C. Paladini", Facultad de Farmacia y Bioquímica, Junín 956 Buenos Aires, Argentina. b Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Departamento de Química Biológica, Cátedra de Química Biológica Superior, Junín 956, Buenos Aires, Argentina. c Universidad Nacional de La Plata, Consejo Nacional de Investigaciones Científicas y Técnicas, Instituto de Investigaciones Bioquímicas de La Plata (INIBIOLP) "Prof. Dr. Rodolfo R. Brenner", Facultad de Ciencias Médicas, Av. 60 y Av. 120, La Plata, Argentina. d Université de Paris, Integrated Biology of Red Blood Cell UMR_S1134, INSERM F-75015, Paris, France. e Institut National de la Transfusion Sanguine, Laboratoire d'Excellence GR-Ex, F-75015 Paris, France. f Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Departamento

de Fisicomatemática, Cátedra de Física, Junin 956, Buenos Aires, Argentina.

The hemolytic toxin α-hemolysin (HlyA) is a virulence factor produced by several strains of Escherichia coli. It is involved in urinary tract infections, peritonitis, meningitis and septicemia. HlyA causes lysis of mammalian cells, including human erythrocytes (RBCs).

In this work, we studied the extent to which changes in cell volume kinetics and the concentration of intracellular diffusible ions affect cell volume regulation of RBCs exposed to HlyA. RBCs from healthy donors and from aquaporin 1 KOs (AQP1KO) individuals were used.

Cells at hematocrit 10% were treated with 0.1 ng/µl HlyA or vehicle at 37 °C for 1, 5 and 10 min. Cell volume was measured using the coulter counter principle, while cell volume changes at short periods of time were assessed by light scattering using a stopped flow rapid mixing equipment. For stopped flow experiments exponential curves were fitted to data and best fit exponential coefficients (k) were used to compare treatments. Intracellular concentrations of sodium and potassium were evaluated by flame photometry of lysed RBCs. HlyA induced changes in volume and shape of RBCs, which shrunk during the first min followed by continuous swelling, reaching 10% over control values after 10 min. Under a similar treatment, volume changes correlated with a 5-8 fold increase of intracellular Na+ and 5-7 fold decrease of intracellular K+ concentrations.

In wild type RBCs, cell volume kinetics was affected by toxin treatment. Values of k were 8.5 ± 0.5 seg-1 for untreated cells vs 6.5 ± 0.4, 4.1 ±0.4 and 3.4 ±0.3 seg-1 for cells treated with the toxin for 1, 5 and 10 min, respectively. In the absence of toxin, k values were 5 fold lower for AQP1KO cells, as compared to wild type cells. Nevertheless, the relative decrease of k values upon HlyA exposure was similar for both types of cells.

HlyA dependent decrease of volume, followed by swelling, correlated with acute and simultaneous Na+ uptake and K+ efflux. Values of the kinetic constant k decrease with time of exposure to the toxin, irrespective of the presence of aquaporin 1 in the cells.

FC3. Bioassay standardization to assess exosomes anti-inflammatory activity in vitro.

Ricardo Malvicini, Diego Santa-Cruz, Natalia Pacienza, Gustavo Yannarelli. Instituto de Medicina Traslacional, Transplante y bioingeniería-(IMeTTyB- CONICET)

Exosomes (Exo) are small sized-extracellular vesicles (40-150 nm), released by almost all kind of cells, and play a major role in cell-to-cell communication. In the last years, they have drawn attention due to its potential application in clinical diagnostics and therapeutics. However, determining their biological activity and potency has proven difficult, owing to the lack of biological assays. Here, we standardized an in vitro assay to assess the anti-inflammatory potential of mesenchymal stem cells (MSCs)-derived exosomes based on their ability to prevent the acquisition of the M1 phenotype in LPS-stimulated RAW264.7 macrophages. M1 phenotype was characterized by the induction of IL-1β, IL-6 and iNOS as determined using qRT-PCR. Nitric oxide (NO) released by iNOS turns into NO2-, that can be easily quantitated in the culture media by Griess reaction. Moreover, phenol red present in culture media did not interfere in the spectrophotometric detection of NO2-. Thus, we first tested different assay conditions in 96-well-plates, including two seeding densities (2x104 and 4x104 cells), four LPS doses (1, 10, 100 and 1000 ng/ml) and two time-points (16 and 24 h), in order to determine the best set-up to accurately measure NO2- production as a marker of M1 macrophage polarization. We found that seeding 2x104 cells/well and stimulating with 10 ng/ml LPS for 16 h allowed us to inhibit the inflammatory response by 60% using Dexamethasone (1 ug/ml). Using these established conditions, we were able to test different exosomes preparations in sextuplicate (5 µg protein/well) and to rank then by their anti-inflammatory activity. Finally, conditioned media containing NO2- can be processed immediately or stored at -20°C, as we found that NO2- is stable in culture media. In summary, we standardized a quick, cheap and reproducible in vitro macrophage assay that allows evaluating and estimating the anti-inflammatory activity of MSCs-derived exosomes. The assay is convenient for comparing multiple samples and, therefore, should be useful in developing protocols to improve the purification and characterization of anti-inflammatory exosomes.

FC4. Aquaporin-4 facilitates cell proliferation in retinal Müller cells: Implications in Neuromyelitis Optica.

Vanina Netti, Alan White, Gisela Di Giusto, Juan Fernández, Paula Ford, Miriam Echeverría, Claudia Capurro. Laboratorio de Biomembranas, IFIBIO-Houssay (UBA-CONICET)

Müller cells are involved in controlling extracellular homeostasis in the retina, regulating cell swelling by a regulatory volume decrease (RVD) mechanism that depends on the efflux of solutes and water through Aquaporin-4 (AQP4). Müller cells are also important for retinal integrity, as they respond to injury by re-entering the cell cycle for tissue repair. Since AQP4 was reported to modulate cell volume during cell cycle progression and facilitate proliferation in astrocytes, the aim of this study was to evaluate, using the novel inhibitor TGN-020, if AQP4 was involved in human Müller cells' proliferation in physiological conditions. Considering that AQP4 is the target of autoantibody IgG-NMO present in the sera of patients with Neuromyelitis Optica (NMO), we also evaluated if cell proliferation was altered in the presence of IgG-NMO. MIO-M1 human Müller cells were exposed to 100 nM TNG-020 or vehicle or to 1/50 dilution of IgG-NMO positive or control sera. Cell volume (videomicroscopy) and cell proliferation (cell count, cell cycle analysis by flow cytometry and BrdU incorporation by immunofluorescence) were measured. AQP4 inhibition with TGN-020 reduced osmotic water permeability (Pf, µm/s) from 20.3±1.2 to 12.2±0.4 (n=5, p<0.001) and %RVD 15min from 54±4 to 17±3 (n=5, p<0.001). MIO-M1 cell proliferation was decreased by TGN-020 (doubling time in hours, control vs. TGN-020: 31±1 vs. 40±3, n=4, p<0.05) without affecting cell viability. TGN-020 also increased the % of cells in G1/G0 phase, decreased the S phase of cell cycle and reduced BrdU incorporation by 20%. IgG-NMO positive sera decreased AQP4 plasma membrane expression in MIO-M1 cells, reducing Pf from 22.4±1.5 to 15.9±0.6 µm/s (n=6, p<0.001) and %RVD 15min from 66±5 to 48±4 (n=6, p<0.005), as well as cell proliferation (doubling time in hours, control vs. IgG-NMO: 59±5 vs. 86±4, n=3, p<0.05) in comparison to control sera. We

propose that inhibition or removal of AQP4 from the plasma membrane reduces AQP4-mediated water permeability altering cell proliferation. This is of particular importance in NMO, as the decreased ability of Müller cells to proliferate may affect retinal tissue repair.

FC5. Regulation of oxidative stress on the activity of Nrf-2 factor and the expression of antioxidant enzymes in lymphoid cells of a murine model of hyperthyroidism.

Costilla Melisa; Macri Delbono Rodrigo; Klecha Alicia; Cremaschi Graciela; Barreiro Arcos María Laura. Instituto de Investigaciones Biomédicas (BIOMED)-CONICET, Buenos Aires.

Introduction: Hyperthyroidism is an endocrine disorder characterized by excessive secretion of T3 and T4 and low levels of TSH. Thyroid hormones exert pleiotropic actions on numerous tissues and induce an overall increase in metabolism, with an increase in energy demand and oxygen consumption. Objectives: Study the effect of oxidative stress on the activation of the nuclear factor Nrf-2 and the transcription of antioxidant enzymes in lymph node and spleen cells of euthyroid and hyperthyroid mice. Methodology: Hyperthyroidism was induced in Balb/c mice by treatment with 12 mg/l of T4 in drinking water for 30 days. Reactive oxygen species (ROS) were evaluated using the fluorescent probe DCFH-DA and flow cytometry. The expression of catalase (CAT), glutathione peroxidase-1 (GPx-1) and superoxide dismutase (SOD) was determined by PCR and western blot. Nrf-2 phosphorylation and its translocation to the nucleus were evaluated by western blot and confocal microscopy, respectively. Protein kinase C (PKC) activity was evaluated by measuring the incorporation of 32P into histone H1. PKC isoenzyme expression and the extracellular signal-regulated kinase (ERK) phosphorylation was evaluated by western blot. Results: We found a significant increase in the genomic and protein expression of CAT and GPx-1 in lymph node and spleen cells of hyperthyroid mice that was correlated with the increase in the ROS production. Lymphoid cells of euthyroid mice treated in vitro with H2O2 (250 μM) increased the expression of antioxidant enzymes. The hyperthyroidism increased the phosphorylation levels of Nrf-2 and ERK and the kinase activity of the classic isoenzymes of PKC (α, β and γ). Nrf-2 phosphorylation was decreased by preincubation of lymphoid cells with staurosporine (PKC inhibitor; 5 nM) or PD 98059 (ERK inhibitor; 20 μM). Conclusions: Hyperthyroidism increases ROS and kinase activity of PKC and ERK. The activation of these transduction signals leads to phosphorylation and translocation of Nfr-2 to the nucleus where it induces the transcription of antioxidant enzymes. These results indicate the modulation of hyperthyroidism of the cellular antioxidant system.

FC6. Aquaporin-2 and Na+/H+ exchanger isoform 1 modulate the efficiency of renal cell migration.

Di Giusto Gisela, Pizzoni Alejandro, Rivarola Valeria, Beltramone Natalia, White Alan, Ford Paula, Capurro Claudia. Laboratorio de Biomembranas, IFIBIO-HOUSSAY, FMED, UBA-CONICET.

Aquaporin-2 (AQP2), in addition to its canonical role as a water channel, promotes renal cell migration by the modulation of integrin β1 trafficking and the turnover of focal adhesions. This novel role described for AQP2 opens the possibility to further investigate if AQP2 also works in concert with other components of the cell migration machinery. Na+/H+ exchanger isoform 1 (NHE1) is a well-known protein involved in the regulation of cell migration, which is proposed to act at the leading-edge membrane to direct migration. NHE1 activity is highly modulated by Ca2+ and we recently showed a physical interaction between AQP2 and the Ca2+ channel TRPV4. Then, the aim of our work was to investigate the possible crosstalk between AQP2, its mechanosensitive partner TRPV4, and NHE1 to regulate cell migration. We used two renal cell models: one not expressing AQPs and another one expressing AQP2. We performed wound healing and cell tracking assays to evaluate cell migration; immunofluorescence assays to evaluate lamellipodia volume, focal adhesions, and assembly of F-actin; and fluorescence videomicroscopy to measure lamellipodia pHi and NHE activity. Our results confirm that AQP2 promotes renal cell migration during wound closure, AQP2-expressing cells follow a less tortuous route compared with AQP2-null cells. Lamellipodia of AQP2-expressing cells exhibit significantly smaller volumes and size of focal adhesions and more alkaline pHi due to increased NHE1 activity than AQP2-null cells. The blockage of AQP2 or TRPV4 significantly reduced lamellipodia NHE1 activity. Also, the blockage of NHE1 significantly reduced the rate of cell migration, the number of lamellipodia and the assembly of F-actin only in AQP2-expressing cells. Altogether these results let us propose that during lamellipodia protrusion the presence of AQP2 activates its partner TRPV4, leading to Ca2+ entry and to the consequent activation of NHE1. It is likely that the interplay between AQP2, TRPV4, and NHE1 defines the pH dependent-actin polymerization, providing mechanical stability to delineate lamellipodia structure and consequently the speed and directionality of cells, promoting the migration.

FC7. Release of ATP by TRPV4 activation is dependent upon the expression of AQP2 in renal cells.

Pizzoni Alejandro, Bazzi Zaher, Di Giusto Gisela, Rivarola Valeria, Capurro Claudia, Schwarzbaum Pablo J., Ford Paula. Laboratorio de Biomembranas, Depto. de Fisiología y Biofísica, Facultad de Medicina, IFIBIO HOUSSAY

The involvement of purinergic signalling in kidney physiology and pathophysiology is rapidly gaining recognition. Purinergic signalling influences water and electrolyte transport in all segments of the renal tubule. In several tissues, there is increasing evidence that ATP release is dependent upon activation of the transient receptor potential cation channel (TRPV4). Because we have recently found that TRPV4 physical and functional interacts with the water channel AQP2 in cortical collecting ducts cells (CCD) (1), the aim of this work was to examine the possibility that TRPV4/AQP2 interaction influences ATP release in these cells. We used two rat CCD cell lines expressing AQP2 (AQP2-RCCD1) or not (WT-RCCD1). Extracellular ATP (ATPe) measurements were carried out with cells laid on coverslips that were mounted in the assay chamber of a custom-built luminometer. Cells were stimulated with the specific TRPV4 activator GSK1016790A (GSK, 10 nM) and ATPe was measured using firefly luciferase. We found that GSK stimulate ATP release only in AQP2- expressing cells (MaxAQP2 = 222.9 ± 32 nM (n=10)). ATP release stimulated by GSK in AQP2-RCCD1 cells was inhibited by the TRPV4 specific antagonist HC-

067047 (1 µM) and by extracellular calcium removal. In order to identify if ATP release occurs via a conductive or an exocytic route, before stimulating cells with GSK, we incubated the cells with carbenoxolone (100 µM, to block pannexin 1 and connexin hemi-channels) or brefeldin A (5 µg/ml, an intracellular vesicular transport inhibitor). We found a similar reduction of ATP release with both inhibitors. Interestingly, when we tested both inhibitors together, an additive reduction was observed, suggesting that both mechanisms function independently. In addition, blocking purinoceptors with PPADS (20 µM) strongly reduced ATP release. In conclusion, these findings suggest that in CCD cells AQP2 is critical for the release of ATP induced by TRPV4 activation. Moreover, ATPe, in turn, acts in an autocrine and/or paracrine manner to stimulate PPADS-sensitive purinergic receptors leading to ATPe-induced ATP release.

1. Pizzoni et al, J Cell Biochem 2018 119(5): 4120-4133

FC8. Estradiol 17ß-d-glucuronide-induced impairment of MRP2 activity in rat hepatocytes involves NADPH oxidase-mediated oxidative stress.

Gimena Salas, Anabela C. Medeot, Virginia S. Schuck, Romina B. Andermatten, Gisel S. Miszczuk, Nadia Ciriaci, Ismael R. Barosso, Enrique J. Sánchez Pozzi, Marcelo G. Roma, Cecilia L. Basiglio, Fernando A. Crocenzi. IFISE (Instituto de Fisiología Experimental), CONICET.

Estradiol 17ß-D-glucuronide (E17G) is an endogenous metabolite of estradiol that is considered a main responsible of intrahepatic cholestasis of pregnancy. E17G induces acute cholestasis in animal models via activation of several kinase-mediated signaling pathways leading to an increased endocytosis and intracellular retention of canalicular transporters and the consequent canalicular secretory failure. Oxidative stress has been shown to induce internalization of canalicular transporters and cholestasis. Here, we investigated the possible involvement of oxidative stress in E17G-induced impairment of canalicular Mrp2 function. Transport function of Mrp2 was analyzed by the measurement of canalicular vacuolar accumulation (cVA) of glutathione-S-methylfluorescein (GS-MF), a fluorescent glutathione-conjugated metabolite of chloromethylfluorescein diacetate (CMFDA) in rat hepatocyte couplets (RHC), and also by measuring the initial transport rate (ITR) of GS-MF in sandwich-cultured rat hepatocytes (SRH). The role of reactive oxygen species (ROS) in E17G-induced Mrp2 function impairment was evaluated by preincubating hepatocytes for 15 to 30 min with the antioxidant compounds vitamin C (VitC), mannitol (Man), N,N'-diphenyl 1,4-phenylenediamine (DPPD), or with apocynin (Apo), a specific inhibitor of the ROS-producing enzyme NADPH oxidase (NOX), prior to a 20-min exposure to E17G. cVA of GS-MF, expressed as percent of control, was significantly reduced by E17G (45±2, $p<0.05$ vs control, n=7); this effect was significantly prevented by preincubation with the antioxidant compounds (VitC: 61±5, Man: 71±7, DPPD: 69±6, $p<0.05$ vs E17G and control, n=4) and by inhibition of NOX with Apo (72±5, $p<0.05$ vs E17G and control, n=3). Neither the antioxidants nor Apo alone affected the cVA of GS-MF. Equivalent results were obtained when ITR of GS-MF was assessed in SRH after these treatments. These results support a role of ROS in the impairment of Mrp2 transport function induced by E17G in hepatocytes, probably by participating in the internalization of this transporter induced by this cholestatic compound. An increased activity of NOX induced by E17G seems to represent the source of ROS.

FC9. Role of ATP homeostasis in the microparticles produced by erythrocytes infected with Plasmodium falciparum.

Cora Lilia Alvarez, María Victoria Espelt, Mariano Anibal Ostuni, Jean-Philippe Semblat, Claudine Lapouméroulie, Claude Hattab, Silvia Repetto, Débora González, Matías Barbieri Van Haaster, Pablo Julio Schwarzbaum. Instituto de Química y Fisicoquímica Biológicas Prof. A. C. Paladini. Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Buenos Aires.

Malaria is a worldwide disease caused by the infection of red blood cells (RBCs) with Plasmodium falciparum. Previous studies have demonstrated that infected RBCs produce microparticles (MPs) of 0.1-1 µm by plasma membrane blebbing. RBCs release ATP when challenged by various physio- and pathological stimuli. The resulting extracellular ATP (ATPe) can control vasodilatation. Interestingly, malaria patients show microcirculatory impairments which seems to be associated with altered ATPe homeostasis. ATPe regulation depends on the balance between ATPase activity and ATP release. This balance can be altered by MPs, which display their own capacity to regulate ATPe.

The aim of this study was to determine whether MPs can contribute to ATPe homeostasis of P. falciparum infected RBCs.

MPs were isolated from culture supernatant by centrifugation from both infected and control RBCs. Luciferin-luciferase-based luminometry was used to measure the ATP content and the rate of ATPe hydrolysis of MPs.

Transmission electron microscopy of MPs showed rounded membrane vesicles (diameter: 131 ± 35 nm). The ATP content of MPs, determined after permeabilization with digitonin, was higher in MPs obtained from infected RBCs culture than from control RBCs culture (2603 ± 1007 and 1659 ± 678 nM ATP/mg of protein, respectively). MPs were exposed to various ATP concentrations (450-1800 nM) and the time course of ATPe decay was determined. Initial rate values of ATPe presented a linear function with [ATPe]s, from which KATP accounted for the slope. KATP was 3 fold higher in MPs obtained from infected RBCs than from control RBCs culture (0.99 ± 0.05 and 0.32 ± 0.03 nM ATP/(mg min), respectively).

Our results show that infection triggers the formation of MPs. These MPs exhibited ATPe hydrolysis in the nM range. Previous results, in which infected RBCs exhibited higher ectoATPase activity than non-infected RBCs, correlated with higher ATPe hydrolysis observed in MPs obtained from infected RBCs culture than in MPs obtained from control RBCs culture.

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FC10. Microfluidics to study deformability of human erythrocytes exposed to alpha hemolysin.

M.F. Leal Denis1-2, M. A. Lizarralde Iragorri3-5, W. El Namer3-5, M.A. Ostuni3-5, P.J. Schwarzbaum1,6. 1- IQUIFIB. CONICET-UBA. 2- Cátedra de Química Analítica, Dpto. de Química Analítica y

Fisicoquímica, Facultad de Farmacia y Bioquímica. UBA. 3- Biologie Intégrée du Globule Rouge UMR_S1134, Inserm, Univ. Paris Diderot, Sorbonne Paris Cité, Univ. de la Réunion, Univ. des Antilles. 4- Laboratoire d'Excellence GR-Ex, Paris, France. 5- Institut National de la Transfusion Sanguine, F-75015, Paris, France. 6- Cátedra de Química Biológica Superior, Dpto. de Química Biológica, Facultad de Farmacia y Bioquímica. UBA.

The hemolytic toxin alpha-hemolysin (HlyA) is a virulence factor produced by several strains of Escherichia coli. It is involved in urinary tract infections, peritonitis, meningitis and septicemia. HlyA of E. coli irreversibly binds to human erythrocytes (RBCs) initiating a series of biochemical and morphological events leading to hemolysis.
We studied the capacity of HlyA-treated RBCs to deform under mechanical stress, by passing the cells though a microfluidic device (biochip) that combines a network of microchannels of 5 um high and a serial of restrictions of 5 µm separated by 20 and 30 µm-wide areas, designed to mimicked the mechanical stress exerted on RBCs in the microcirculation.

RBCs (20% hematocrit) were treated with 0.1-0.2 ng/uL of HlyA (HlyA-RBCs), with 1-2 ng/uL of the un-acylated protoxin ProHlyA (Pro-RBCs) or vehicle (c-RBCs) and loaded on the biochip. The biochip was connected to a pump (350 mBar) and mounted on an inverted microscope coupled with a high speed camera. Samples were perfused through the biochip and frame sequences ware taken throughout the experiment. Hemolysis due to mechanical stress was evaluated by measuring free hemoglobin and deformability was estimated through the deformation index (DI).

Results showed no significant differences in the hemolytic response among different treatments and the control (0.3 –1.0 %). On the other hand, exposure to 0.2 and 2 ng/uL ProHlyA reduced 16 % the DI, while exposure to HlyA 0.1 and 0.2 ng/uL reduced deformability 30 % and 35 %, respectively.

HlyA-treated RBCs were equally resistant to lysis than c-RBCs and ProHlyA-RBCs in the experimental conditions. However, both Pro- and HlyA-treated RBCs presented a lower DI, indicating loss of cell elasticity. In Pro-RBCs the DI was independent of the assayed concentration since no further decreased was observed with higher concentrations of ProHlyA. On the other hand, exposure to increased concentrations of HlyA leaded to a slight but significant decrease on the DI.

It can be concluded that in the assayed conditions, all treated-RBCs showed sufficient flexibility to allow them to bend in order to flow through narrow capillaries without hemolysing.

FC11. Characterization of AKAP350 participation in actin reorganization at the immune synapse in natural killer cells.

Alejandro Pariani, Evangelina Almada, Florencia Hidalgo, Leandra Marín, Cristián Favre, Cecilia Larocca. Instituto de Fisiolología Experimental, CONICET, Universidad Nacional de Rosario.

The immune synapse (IS) refers to a specialized cell-cell junction established between an immune cell and its target cell (TC). When a natural killer cell (NK) is activated by a susceptible TC, the actin cytoskeleton reorganizes at the IS, facilitating integrin and receptor clustering. Subsequently, the centrosome polarizes to the IS, enabling the directional secretion of cytotoxic components, which triggers the lysis of the TC. AKAP350 is an A-kinase anchoring protein essential in the regulation of microtubule dynamics. AKAP350 interacts with the microtubule plus end binding protein 1 (EB1), which participates in the crosstalk between microtubule and actin cytoskeleton. In non-lytic T cell IS, AKAP350 participates in LFA-1 clustering, whereas EB1 is involved in T cell receptor activation. Our previous results indicate that AKAP350 participates in NK cytotoxic response, by regulating actin accumulation and LFA-1 clustering at the IS. The aim of our work was to demonstrate if AKAP350 participation occurs exclusively downstream LFA-1 activation and to evaluate EB1 as a possible mediator. YTS NK cells with decreased expression of AKAP350 (AKAP350KD) were activated for 30 min using specific ligands for LFA-1 or CD28. Actin accumulation and LFA-1 clustering were analyzed by confocal microscopy. Actin accumulation at the IS was impaired in AKAP350KD cells activated via LFA-1 (-37%*) or CD28 (-73%*). Concomitantly, AKAP350KD cells showed decreased LFA-1 localization at the IS in cells activated via either LFA-1 (-40%*) or CD28 (-60 %*). In order to analyze AKAP350 role in EB1 recruitment to microtubules at the IS, YTS AKAP350KD cells were incubated with KT86 cells (TC) for 30 min. EB1 localization was analyzed by confocal microscopy. YTS AKAP350KD showed decreased EB1 localization at the IS edge (-45%*) and at IS microtubules (-55%*). The latter results were confirmed by western blot analysis of microtubule fractions of YTS cells activated via LFA-1. Overall, our results suggest that AKAP350 participates in actin accumulation and receptor clustering downstream both LFA-1 and CD28activations and position EB1 as a putative mediator of this effect. *p<0.05.

FC12. Chalcone derivative synthesis and cytotoxicity in hepatocellular carcinoma cells.

Fabiola Kamecki, Mariel Marder, M. Fernanda Troncoso, M. Victoria Espelt. Instituto de Química y Fisicoquímica Biológicas. CONICET

Hepatocellular carcinoma (HCC) is a lethal tumor that often occurs in patients with chronic liver disease and cirrhosis. It has poor prognosis due to its high recurrence rate and resistance of chemotherapy. Lately several clinical investigations occurred in order to improve clinical outcome of HCC patients.

Chalcones (1,3-diaryl-2-propen-1-ones) belong to the flavonoid family. They are composed by two aromatic rings joined by three carbon α,β-unsaturated carbonyl system. Natural and synthetic chalcone derivatives have shown promising biological activities as antioxidant, anti-inflammatory, anticancer, among others. So the aim of this work was to synthesize new chalcone derivatives and analyze their cytotoxicity in HCC cell lines.

For chalcone synthesis, we used the reaction of each acetophenone and the corresponding benzaldehyde (aldol condensation) in the presence of aqueous NaOH solution. The synthesized chalcone derivatives were characterized using 1H nuclear magnetic resonance (1H-NMR), 13C-NMR and mass spectrometry (MS). HepG2 and Huh-7 cells viability was assessed using MTT assay.
We synthesized the following chalcones: 3-chloro-2´-hydroxy-4´-methoxychalcone, 2´-hydroxy-4´-methoxy-4-chlorochalcone, 2´-hydroxy-3-nitrochalcone, 2´-hydroxy-6´-methoxychalcone; 2´-hydroxy-5´-methoxychalcone, 2´-

hydroxy-4´-methoxychalcone, 2´-hydroxychalcone, 2´-hydroxy-3-chlorochalcone. After 48 h incubation with 50 µM of each chalcone, cell viability was reduced between 35-60 % (versus vehicle 100%) in both cell types. Cells were also incubated during 48 h with 50 µM quercetin, a natural flavonoid with antigrowth activities against several types of cancers, that has no effect on cell viability in HepG2 or in Huh-7 cells. We also determined the 50% inhibitory concentration (IC50) for chalcone, the nucleous base of all derivative compounds, which was 27.32 (20.14 to 37.07)µM for HepG2, and 23.81 (14.96 to 37.89)µM for Huh-7 after 72 h incubation.

Our findings demonstrate that these new sythesized chalcones show cytotoxic effects on HCC cells. Future studies will focus on the relationship between the type of substituents on the chalcone core and the mechanism involved in HCC cell death.

FC13. Tauroursodesoxicolate prevents activation of pro-cholestatic signalling pathways in estradiol 17ß-d-glucuronide -induced cholestasis independently of protein phosphatases and the integrin receptor.

Anabela C. Medeot, Paula M. Maidagan, Andrea C. Boaglio, Maria V. Razori, Romina B. Andermatten, Nadia Ciriaci, Fernando A. Crocenzi, Marcelo G. Roma. Instituto de Fisiología Experimental – IFISE. CONICET.

Estradiol 17ß-D-glucuronide (E217G), a main etiologic agent of pregnancy-induced cholestasis, induces bile flow drop by endocytosis and intracellular retention of the main canalicular transporters involved in bile secretion, Mrp2 and Bsep, via activation of the pro-cholestatic signaling pathways cPKC and PI3K, respectively. The most effective medication for pregnancy-induced cholestasis is ursodeoxycholic acid (UDCA), but its action mechanisms are still unknown. We tested here whether the main active metabolite of UDCA, tauroursodeoxycholate (TUDC), prevents the secretory failure induced by E217G by inhibiting the activation of cPKC and PI3K/Akt signaling pathways, and whether protein phosphatases (PPs) or integrin receptor are involved in this mechanism. Activation of cPKC and Akt was quantified by assessing the plasma membrane amount and the phosphorylated amount of these proteins, respectively, in isolated rat hepatocytes by Western Blot. Pretreatment with TUDC (100 µM), followed by exposure to E217G (100 µM), prevented activation of cPKC (-34±4%) and Akt (-37±2%); $p<0.05$ vs. E217G. Mrp2 and Bsep transport activity was assessed in rat hepatocyte couplets (RHC) by quantifying the % of RHC displaying canalicular vacuolar accumulation (CVA) of fluorescent substrates GSH-S-methylflourescein (GS-MF) and cholyl-lysilfluorecein (CLF), respectively. E217G decreased CVA of CLF by 41±3% ($p<0.05$ vs. control), while TUDC partially prevented this alteration by 79±7% ($p<0.05$ vs. E217G). E217G-induced impairment in CVA of the Mrp2 substrate GS-MF was similarly prevented by TUDC. The pretreatments with the PP1/PP2A inhibitors, tautomycin (1 nM) and okadaic acid (5 nM), the PP2B inhibitor tacrolimus (1 µM), or the integrin receptor inhibitor RGD (20 and 30 µM) did not prevent the CVA improvement afforded by TUDC. We conclude that TUDC-induced preservation of Bsep and Mrp2 function and location in E217G-induced cholestasis involves halting of cPKC and PI3K activation by mechanisms independent of protein phosphatase-induced dephosphorylation or integrin-mediated signaling, but rather by counteracting a yet not explored E217G-activated cholestatic signaling pathways upstream of cPKC and PI3K.

FC14. Extracellular ATP regulation of Caco-2 cells.

Zaher K. Bazzi, Cora L. Alvarez, Paula Faillace, Débora Rinaldi, Pablo J. Schwarzbaum, Julieta Schachter. IQUIFIB-FFyb UBA.

In many eukaryotic cells, intracellular ATP can be released by several stimuli as mechanical stress, calcium influx and exposure to osmotic change. Candidate conduits for ATP exit include anionic channels and exocytosis. In the intestinal lumen, extracellular ATP (ATPe) can activate purinergic receptors, which can modulate ATP release. In addition, ATPe can be hydrolyzed by ectonucleotidases located on the apical domain of the epithelium. Thus, ATP release, purinergic activation and ectonucleotidases can regulate ATPe concentration.

To gain insight into ATPe regulation in the intestine epithelium, we analyzed the effects of a hypotonic shock on ATP efflux, purinergic activation and ATPe hydrolysis of the human intestinal Caco-2 cell line. ATPe kinetics and ectoATPase activity were assessed by real time luminometric detection of [ATPe] and colorimetric and radioactive techniques to quantify Pi release from ATP and other nucleotides. The presence of ectonucleotidases was assessed by immunocytochemistry.

In the absence of stimuli, Caco-2 cells displayed a stable ATPe concentration at approx. 20 ± 5 nM. Addition of exogenous ATP (0.5 - 8 µM) led to an acute [ATPe] increase, followed by a non linear decay caused by ectoATPase activity.

Next, we determined the effect of hypo-osmotic stress on ATP release. Using pharmacological tools, we observed that ATP release exhibited exocytotic and conductive components, the latter being mediated by Pannexin-1. Blockage of P2X receptors reduced ATP release by 45-50 %. On the other hand, Caco-2 cells exhibited significant ectoATPase and ectoAMPase activities, but low ectoADPase activity. Accordingly, we detected the presence of NTPDase 1, 2 and ecto-5'-nucleotidase (ectoAMPase) in Caco- 2 cells.

ATP efflux was fast and sensitive to osmotic shock. Upon activation of ATP release, the accumulated ATPe activated purinergic receptors that promoted further activation of ATP exit. Simultaneously, ectoATPase activity partially compensated these increases in [ATPe]. Thus, ATPe kinetics of Caco-2 cells resulted from the dynamic balance between ATP release, purinergic activation and ATPe hydrolysis.

FC15. Regulation by the cAMP Pathway of Primary Cilia Length in LLC-PK1 Renal Epithelial Cells

Perez PL, Scarinci N, Cantero MR & Cantiello HF. Laboratorio de Canales Iónicos, Instituto Multidisciplinario de Salud, Tecnología y Desarrollo (IMSaTeD, CONICET- UNSE)

The primary cilium (PC) is a non-motile sensory organelle whose dysfunction leads to the onset of autosomal dominant polycystic kidney disease (ADPKD). Previous studies demonstrated that the PC of renal epithelial cells express a functional polycystin-2 (PC2, TRPP2) channel, and the type-2 vasopressin receptor (V2R), whose activation mediates the local elevation of cAMP. Although PC2 contributes to the regulation of Ca^{2+} transport in renal epithelial cells, little is known as to how PC2

contributes to the regulation of PC length. In this study we explored how the Ca2+- and cAMP- signaling pathways crosstalk and regulate PC length in LLC-PK1 renal epithelial cells. The PC labeled with a specific antibody against α-acetylated tubulin and measured with the ImageJ software. Under normal Ca2+ conditions (1.2 mM), arginine-vasopressin (AVP, 10 µM) increased PC length by 15.47% respect to its control condition (4.72 ± 0.05 µm, n = 510, vs. 5.45 ± 0.09 µm, n = 120, p < 0.001). Treatment of cells with the cAMP analog, 8-Br-cAMP (1 mM) in the presence of normal Ca2+ also increased PC length by 16.31 ± 1.46% (5.49 ± 0.08 µm, n = 157, p < 0.001). Despite the elongating effect of the cAMP signals (both AVP and 8Br-cAMP) on PC length in normal Ca2+, cells exposed to high external Ca2+ (6.2 mM) had shorter PC lengths both in the absence (13,56%, 4.08 ± 0.06 µm, n = 653, p < 0.001) or presence of AVP (12.72%, 4.12 ± 0.11 µm, n = 140, p < 0.001) compared to their respective controls in normal Ca2+. Cells exposed to 8-Br-cAMP, however, reverted the effect of high Ca2+, with a 13.77% increase (5.37 ± 0.12 µm, n = 136, p < 0.001) on PC length. The encompassed evidence indicates a crosstalk between Ca2+ and cAMP signals on the PC length of renal epithelial cells. The differential effects of 8Br-cAMP and AVP in high Ca2+, however, suggests the presence of a possible secondary pathway not associated with a V2R-mediated increment in cAMP but possibly a Ca2+-associated V1R activation as well. The functional interaction between signaling pathways could be relevant in the final outcome of the control of kidney function, and the onset of mechanisms that trigger cyst formation in ADPKD.

Gastroenterology

G2. A mutation in multidrug resistance-associated protein 2 linker region prevents its internalization triggered by classic protein kinase c activation.

Camila Juliana Domínguez, Guillermo Nicolás Tocchetti, Agostina Arias, Felipe Zecchinati, Maite Rocío Arana, José Manuel Pellegrino, Juan Pablo Rigalli, María Laura Ruiz, Silvina Stella Maris Villanueva, Claudia Elena Banchio, Aldo Domingo Mottino. Instituto de Fisiología Experimental (IFISE) - CONICET UNR.

INTRODUCTION: Hepatic multidrug resistance-associated protein 2 (MRP2) is a canalicular ABC transporter contributing to bile acid-independent bile flow formation. We have previously shown that i) the activation of cPKC by thymeleatoxin (TTX) leads to internalization of MRP2 and consequently to decreased transport activity and ii) TTX induces direct phosphorylation of MRP2 in serine residues substrates of cPKC. Now we aim to identify amino acid residues (AA) targets of cPKC in MRP2 molecule and to evaluate their role in MRP2 endocytosis.
EXPERIMENTAL DESIGN: After in silico prediction of cPKC-target AA ranking, a plasmid encoding GFP-MRP2 fusion protein with a phosphorylation-defective mutation in the highest scoring serine (S908) was obtained by site-directed mutagenesis. Using human hepatic cells C3A, wild-type and mutant stable cell lines were generated. GFP-MRP2 localization was evaluated by membrane fractionation followed by immunoblotting and by confocal microscopy. MRP2 activity was assessed by quantifying the efflux of its model substrate DNP-SG. Immunoprecipitation of MRP2 was performed to evaluate its phosphorylation status.
RESULTS: TTX treatment (100 nM, 30 min) led to decreased GFP-MRP2 expression in plasma membrane-enriched fractions (-74%; p<0.05) with concomitant increase in intracellular membranes-enriched fractions (+113%; p<0.05) in wild-type cells, with no effect in mutant cells. Confocal microscopy studies confirmed that GFP-MRP2 was internalized in response to TTX in the wild-type cell line without affecting its apical localization in the mutant cell line. MRP2 activity was decreased by TTX in wild-type but not in mutant cells, agreeing well with localization studies. Finally, the mutation in S908 decreased the phosphorylation status of other serine residues substrates of cPKC in response to TTX, suggesting a prominent role of this particular AA in the overall MRP2 phosphorylation.
CONCLUSION: S908 appears to be decisive in the modulation of MRP2 localization and activity under TTX-mediated cPKC activation. Moreover, phosphorylation of this AA likely regulates phosphorylation of other serine residues substrates of cPKC.

G3. Vitamin C (VitC) reverts intestinal Mrp2 down-regulation in rats with fructose-induced metabolic syndrome (MS)

Zecchinati F, Tocchetti GN, Dominguez CJ, Barranco MM, Arana MR, Perdomo VG, Luquita MG, Mottino AD, García F, Villanueva SSM. INSTITUTO DE FISIOLOGIA EXPERIMENTAL (IFISE), CONICET.

Intestinal Mrp2 is an ABC transporter that limits the absorption of xenobiotics orally ingested, thus acting as a biochemical barrier. MS is a pathological condition characterized by insulin resistance, hyperinsulinemia and dyslipidemia as well as by chronic inflammation and oxidative stress (OS). In previous studies we observed that MS-like conditions induced by fructose in drinking water (10% v/v, during 21 days), reduced the expression and activity of intestinal Mrp2 in rats. We here evaluated the effect of VitC (100 mg/kg/day; p.o), a natural potent antioxidant, in reverting fructose-induced Mrp2 alterations. Once the MS features were settled, animals were cotreated with VitC for another 14 days. VitC reverted down-regulation of Mrp2 protein induced by MS, as detected by western blotting (C: 100%, FRU: 25%, FRU+VitC: 102%, P<0.05). Likewise, detection of mRNA levels by RT-qPCR, revealed that fructose administration decreased Mrp2 expression (-56%, P<0.05) respect to C, whereas this parameter returned to normal values in the FRU+VitC group. Mrp2 activity was evaluated using the in vitro model of everted intestinal sacs. Efflux of the Mrp2 substrate DNP-SG was decreased in FRU rats (-42%) respect to C (P<0.05), whereas Mrp2 activity in the cotreated group remained unchanged. Additionally, fructose generated OS in intestinal tissue as indicated by increasing lipid peroxidation products (+65%, P<0.05) and activity of the antioxidant enzyme SOD (+63%, P<0.05), respect to C, and by decreasing GSH/GSSG ratio (-36%) respect to C (P<0.05). Moreover, fructose increased the

intestinal mRNA expression levels of proinflammatory cytokine IL-1β (+212%, P<0.05) respect to C. Interestingly, VitC not only reverted MS parameters studied and improved the intestinal redox unbalance, but also normalized the increased intestinal IL-1β mRNA levels, generated by fructose. Our study suggests that inflammation and OS, perhaps this one with a key role, could be important mediators of Mrp2 down-regulation under MS-like conditions, and that VitC could be a potential therapeutic tool to prevent intestinal barrier impairment by counteracting the effect of such mediators.

G4. Levels of salivary immunoglobulin A in edentulous subjects

Juárez RP, Serrano CP. Physiology Department. Facultad de Odontología. FaCENA. UNNE.

Salivary immunoglobulin A, in its secretory form (s-IgA), is the main antibody class in saliva and provides the first line of defense against pathogens. Association of dental caries and gingival inflammation with s-IgA has been studied in children and young adults, but information on s-IgA in healthy edentulous older people is very limited. Multiple studies have failed to demonstrate the utility of s-IgA as a non-invasive biomarker of mucosal immunity in oral cavity. The aim of this study was to determine the changes in salivary s-IgA levels of adults with varying degrees of tooth loss. In this observational analytical cross-sectional study, unstimulated saliva samples were collected by spitting method without swallowing from 66 adults (age 18-88 years), 22 dentulous and 44 edentulous. The edentulous patients were divided into two groups with different number of tooth lost (22 partially edentulous and 22 totally edentulous). Salivary s-IgA levels were determined using immunoturbidimetry. Experiments were carried out in triplicate and the results analyzed using Student's t-test and Anova. Among edentulous patients, significantly higher s-IgA levels were concomitant with tooth loss. Level of s-IgA was highest in the group of totally edentulous patients ($p<0.01$). There were not significant differences between dentulous subjects and partially edentulous patients. These results reveal an increase in s-IgA levels in edentulous patients with conventional plate denture. Further research is needed to investigate s-IgA levels among edentulous patients with different types of prosthetic restorations in order to aid in the prediction and management of oral manifestations.

G5. Bibliometric analysis of scientific production on saliva in PubMed and Dentistry & Oral Sciences Source during the period 2000-2018.

Juárez, RP. R&D Group: Saliva as a Diagnostic Fluid. FOUNNE.

Saliva has many functions which are needed for proper protection and functioning of the human body. Bibliometrics studies related to dentistry is uncommon in the literature. Salivary flow reductions can negatively affect the oral health and quality of life of patients. From this perspective, the aim of this study was to map the scientific landscape related to saliva research worldwide between 2000 and 2018. This study adopted a bibliometric method. An observational, descriptive, retrospective investigation was carried out, with quantitative and qualitative description of the data, through the analysis of documents. PubMed (PM) and Dentistry & Oral Sciences Source (DOSS) have been selected as important online bibliometric databases for searching and retrieving documents, in order to display the structural and dynamic aspects, and the evolution of saliva scientific research. In PM, 34327 documents were registered, with 21% of publications in dental journals. The most discussed topics were related to chemistry, metabolism and salivary biomarkers. In DOSS, 6621 documents were registered, with 51% of publications in 25 journals. Dental caries and saliva analysis were the most studied topics. During the study period, international research on saliva was increased, indicating the importance of saliva as an oral health problem. However, the scope of multicenter studies was lower than expected, given the enormous burden of global health problems related to saliva. Further bibliometric studies are needed to analyze the scientific literature related to saliva as diagnostic fluid.

G6. 5-lipoxygenase (5-LOX) inhibition by zileuton reduces the amount of neutrophils that reaches the liver during liver regeneration after partial hepatectomy (PH)

Florencia Lorenzetti, Marina Cecilia Vera, Alvaro Lucci, Carla Gabriela Comanzo, Anabela Cecilia Ferretti, María Cristina Carrillo, María Paula Ceballos, Ariel Darío Quiroga, María de Luján Alvarez. Instituto de Fisiología Experimental (IFISE-CONICET)

5-LOX is the first and main enzyme that participates in the synthesis of leukotrienes. LTB4 is a leukotriene involved in inflammation and also is a potent chemoattractant of neutrophils. Previous studies from our lab showed that treatment with a 5-LOX inhibitor produces a decrease in hepatic LTB4 content and a reduction in liver proliferation after 70% PH in rats, indicating that LTB4 is necessary for liver regeneration. Additionally, it was reported that liver regeneration was impaired after neutrophils depletion. Objective: To study if the reduction in hepatic LTB4 reduces the levels of polymorphonuclear leukocytes (PMNs) post-PH which could be necessary to trigger liver regeneration at early times after PH. Methods and results: Adult male Wistar rats were subjected to sham surgery or PH (70 % liver removal). Two hours before surgery, animals received a specific inhibitor of 5-LOX, zileuton (Zi) 40 mg/kg BW or the drug vehicle. Liver samples were obtained at 3 and 5h post-PH. We studied by immunohistochemistry and qRT-PCR the myeloperoxidase (MPO) levels (marker of neutrophils) and we found a significant increment in the levels of MPO-positive cells in the hepatectomized animals treated with vehicle (control PH) respect to the sham (+300%*). On the other hand, the animals that received the 5-LOX inhibitor showed a decrease in MPO-positive cells (-62,5%# 5h post-PH) and MPO mRNA levels (-38,48%# 3h post-PH) respect to control PH rats. Also, by immunofluorescence and confocal microscopy we analyzed a specific marker of rat neutrophil leukocytes (using anti-neutrophil antibody LS C348005, LSBio) and we observed a significant reduction in the number of positive cells in PH rats treated with Zi compared with control PH rats at 3h post-PH (-36,6%#) (*p<0,05 vs. sham, # p<0,05 vs. control PH). Conclusion: Inhibition of 5-LOX by zileuton reduces the levels of LTB4 that attract PMNs cells into the liver. This phenomenon can mediate the inhibitory effect of 5-LOX inhibition on liver regeneration post PH.

G7. Molecular mechanism of P-glycoprotein induction by Genistein in rat hepatocyte primary culture

Semeniuk M(1), Bucci-Muñoz M(1), Ceré LI(1), Taborda D(1), Catania VA(1), Rigalli JP(2), Ruiz ML(1). (1) Instituto de Fisiología Experimental (IFISE)-CONICET, Facultad de Cs. Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario (UNR). (2) Department of Physiology, Radboud Institute for Molecular Life Sciences, Radboud University Medical Center, Países Bajos.

Background and Aim: Previously, we found that hepatic P-glycoprotein (P-gp), an ATP binding cassette (ABC)-transporter involved in xenobiotic excretion, was induced by genistein (GNT) treatment. GNT is a phytoestrogen that behaves as a ligand of nuclear receptors, such as estrogen receptor (ER) and pregnane X receptor (PXR), and interacts with transcription factors (e.g. Nrf2) involved in the regulation of ABC transporters. The aim of this study was to elucidate the molecular mechanism of P-gp induction by GNT.

Methods: Isolated rat hepatocytes were cultured and incubated with GNT (10 µM) for 6 h, control hepatocytes (C) were incubated with GNT vehicle (dimethyl sulfoxide, 0.1 % v/v). To evaluate ER or PXR participation in P-gp regulation by GNT, ER antagonist (ICI182/780, 1 µM) or PXR inhibitor (sulforaphane, SFN, 10 µM), both added 30 min before GNT, or vehicle, were used. Involvement of Nrf2 was evaluated by siRNA knock down (KD). P-gp encoding genes: Mdr1a and Mdr1b, and Nrf2 mRNA levels were measured by Real Time PCR. The efficiency of Nrf2 KD was evaluated by measuring mRNA levels of Nrf2.

Results: GNT (10 µM) increased Mdr1a levels (GNT: 136±15 % vs C: 100±4 %). Mdr1b levels remained unchanged. ER antagonist did not prevent Mdr1a induction by GNT (ICI182/780 + GNT: 161±8 % vs ICI182/780: 100±14 %). SFN prevented Mdr1a induction by GNT (SFN + GNT: 129±27 % vs SFN: 100±20 %). Nrf2 KD did not prevent Mdr1a induction by GNT (Nrf2 KD + GNT: 173±33 % vs Nrf2 KD: 100±29 %). All results are presented as mean ± SD, n = 3-4, $p < 0.05$.

Discussion: GNT induced Mdr1a levels in hepatocyte primary culture. Since the ER antagonist, ICI 182/780, and the KD of Nrf2 did not prevent GNT from increasing Mdr1a mRNA levels, it can be suggested that GNT is acting through an ER and Nrf2 independent mechanism. Our results suggest PXR as a potential candidate to mediate P-gp up-regulation by GNT, since its antagonist, sulforaphane, prevented Mdr1a mRNA induction.

G8. In vivo inhibition of bilirubin conjugation does not induce a loss of its protective effect in oxidative stress-induced cholestasis

1Pamela L. Martín, 1Denisse Taurizano, 1María V. Razori, 2Sandra M. Arriaga, 3Gerardo B. Pisani, 1Enrique J. Sánchez Pozzi, 1Marcelo G. Roma, 1,2Cecilia L. Basiglio. 1Instituto de Fisiología Experimental (IFISE - CONICET); 2Area Bioquímica Clínica; 3Area Morfología. Facultad de Ciencias Bioquímicas y Farmacéuticas, UNR. Rosario, Argentina

Our group proved that induction of hemeoxygenase 1 (HO1) and consequent elevation of bilirubin (BR) protect the liver from oxidative injury occurring in cholestatic diseases. To assess whether BR thus generated needs to be conjugated to exert its protective effect, we aimed to impair BR conjugation by depleting the endogenous pool of UDP, cofactor of UDP-glucuronosyl transferase (UGT), key enzyme in BR conjugation. Male Wistar rats were used throughout. Oxidative cholestatic injury was induced by tert-butyl hydroperoxide (T, 440 µmol/kg b.w., i.p.). Induction of HO1 and inhibition of UGT were achieved by administration of hemin (H, 20 mg/kg b.w., i.p.) and galactosamine (G, 200 mg/kg b.w., i.p.), respectively. Results are expressed as media±SD. Bile flow (µl/min/g liver) decreased 4-6 h post T injection (1.65±0.73 vs 3.12±0.51 for control -T vehicle-, $p<0.05$, n=6); pre-treatment with H completely prevented this reduction (2.89±0.84, $p<0.05$ vs T, n=6). G administration to HT animals did not impair the protective effect of H on bile flow (2.33±0.83, $p>0.05$ vs HT, n=6), thus evidencing that BR conjugation is not crucial for its protective effect on biliary function. Biliary excretion of BR (mg/min/g liver) decreased after T (0.48±0.31 vs 1.10±0.16 for control, $p<0.05$, n=6) and this decrease was prevented by H (0.95±0.21, $p<0.05$ vs T, n=6). As expected, pre-treatment with G induced a decrease in biliary excretion of BR (0.53±0.13, $p<0.05$ vs HT, n=6), since BR conjugation is impaired and, consequently, BR cannot be excreted into bile. Lipid peroxidation (pmol MDA/mg protein) increased after T treatment (3.53±0.38 vs 1.78±0.44 for control, $p<0.05$, n=6) while H prevented that increase (1.82±0.61, $p<0.05$ vs T, n=6), likely due to the elevated levels of antioxidant BR generated by HO1 induction. G did not produce a decline in the antioxidant protective effect of BR (2.23±0.36 $p>0.05$ vs HT, n=6), likely as a consequence of its accumulation in liver tissue. We conclude that BR conjugation is not essential for BR to exert its antioxidant properties. These results are in line with previous findings of our group as regards the in vitro antioxidant action of unconjugated BR.

G9. Hemeoxygenase 1 inhibition induces a loss of antioxidant protection in rats with bile duct ligation

1Denisse Taurizano, 1Pamela L. Martín, 1María V. Razori, 2Estefanía M. Massa, 3Gerardo B. Pisani, 2Sandra M. Arriaga, 1Enrique J. Sánchez Pozzi, 1Marcelo G. Roma, 1, 2Cecilia L. Basiglio. 1Instituto de Fisiología Experimental (IFISE - CONICET); 2Area Bioquímica Clínica; 3Area Morfología. Facultad de Ciencias Bioquímicas y Farmacéuticas, UNR. Rosario, Argentina

We had demonstrated that hemeoxygenase 1 (HO1) induction, and consequently elevated bilirubin (BR), protects the liver from oxidative stress-induced cholestatic injury in vivo. To corroborate this protective role of BR, we evaluated the effect of HO1 inhibition on endogenous BR levels and BR antioxidant capacity in animals subjected to a cholestatic insult. Wistar rats were subjected to bile duct ligation (BDL, n=6) or sham surgery (Sh, n=5). HO1 was inhibited by Zn(II) protoporphyrin IX (PP, 30 mg/Kg b.w., i.p.) administered 24h before BDL (PP+BDL, n=6) or Sh (n=5). Animals were euthanized 7 days after BDL. Blood samples were obtained before surgery (BS1) and before euthanasia (BS2) and BR, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma-glutamyltransferase (gGT) and lipid peroxidation were determined. At euthanasia, liver samples were obtained for the assessment of lipid peroxidation. Results (media±SD for BS1/BS2 in Sh; BDL; PP+BDL) were, respectively: BR(mg/dL) 0.13±0.05/0.11±0.04; 0.35±0.02/8.60±0.15a; 0.13±0.01/0.50±0.08a,b. ALT(U/L) 48±7/39±3; 136±10/188±15a; 172±32/211±21a. AST(U/L) 131±38/136±22; 385±25/474±40a; 343±49/449±36a. ALP(U/L) 570±24/707±79; 343±1/1047±84a;

510±90/1015±120a. gGT(U/L) 0.2±0.1/0.3±0.1; 0.5±0.1/12.6±3.1a; 0.8±0.1/1.5±0.2a,b; (a)p<0.05 vs BS1 and Sh; (b)p<0.05 vs BDL. After 7 days of BDL, BR levels increased far more in BDL than in PP+BDL, corroborating the inhibition of HO1. Differences in ALT, AST, ALP and gGT were not significant between BDL and PP+BDL,
evidencing the establishment of cholestasis in both groups. The increase in plasma lipid peroxidation (media ± SD) was higher in PP+BDL than in BDL (368±18% vs 177±10%, p<0.05), demonstrating that, under cholestatic conditions, oxidative damage worsens when BR production is impaired. Similar results were obtained for lipid peroxidation in liver tissue. We conclude that BR ameliorates oxidative damage induced by BDL, contributing to limit the progression of cholestatic diseases with oxidative background.

G10. Hepatic gene transfer of human aquaporin-1 improves MRP2/ABCC2 transport activity in rats with lipopolysaccharide-induced cholestasis

Julieta Marrone, Guillermo N. Tocchetti, Mauro Danielli, Aldo D. Mottino, and Raúl A. Marinelli. Instituto de Fisiología Experimental (IFISE), CONICET-UNR.

Lipopolysaccharide (LPS) induces cholestasis by downregulating the expression of key biliary transporters, i.e., multidrug resistance-associated protein 2 (MRP2/ABCC2), bile acid export pump BSEP/ABCB11, and aquaporin-8 water channel. We previously demonstrated that hepatic gene delivery of human aquaporin-1 (hAQP1) water channels improves the LPS-induced cholestasis in part by stimulating BSEP/ABCB11-mediated biliary bile salt output. We now studied whether hepatocyte-expressed hAQP1 is also able to promote MRP2/ABCC2 transport activity. Adenoviral vector encoding hAQP1 (AdhAQP1) or control vector was administered to rats by retrograde intrabiliary infusion. LPS significantly reduced (P<0.05) bile flow and biliary glutathione output by 30% and 65%, respectively. AdhAQP1-treatment normalized both bile flow and biliary glutathione output in LPS-induced cholestasis. Hepatocyte canalicular MRP2/ABCC2 expression was assessed by liver immunostaining and immunoblotting in purified plasma membranes. MRP2/ABCC2 was largely located to canalicular membranes and its protein expression was downregulated by around 70% (P<0.05) in cholestasis triggered by LPS. AdhAQP1 delivery did not alter canalicular MRP2/ABCC2 protein expression. MRP2/ABCC2 transport activity was estimated by determining spectrophotometrically the concentration of dinitrophenyl-S-glutathione (DNP-SG), the glutathione derivative of 1-chloro-2,4-dinitrobenzene (CDNB, 10 µmol/kg, i.v.), into bile. Cumulative biliary DNP-SG output in LPS-treated rats was restored to normal values after AdhAQP1 hepatic transduction (C:353±14;
C+AdhAQP1:368±44; LPS:151±24*;
LPS+AdhAQP1:268±2 nmol/100 g body weight; *P<0.05), suggesting a hAQP1-induced gain in MRP2/ABCC2 transport activity specific for cholestasis. As MRP2/ABCC2 activity is critically dependent on membrane cholesterol content, the findings may be linked to the fact that hAQP1 expression restores reduced canalicular cholesterol content (C:0.32±0.02; C+AdhAQP1:0.31±0.03; LPS:0.18±0.02*; LPS+AdhAQP1:0.35±0.04 µmol cholesterol/mg protein; *P<0.05). Our results suggest that hAQP1-induced MRP2/ABCC2 activation contributes to the improvement of LPS-induced cholestasis.

G11. Thioacetamide impairs hepatocyte ureagenesis from ammonia, but not from glutamine or alanine: Involvement of mitochondrial aquaporin-8

Alejo M. Capiglioni, María de Luján Alvarez, Raúl A. Marinelli. Instituto de Fisiología Experimental (IFISE), CONICET-UNR.

Urea is synthetized by hepatocytes as the major end product of nitrogen metabolism and urea production is used as a key marker for hepatocyte functional capacity. Thioacetamide (TAA) is a well-known hepatotoxic agent. Nevertheless, studies on TAA toxicity in rat hepatocytes under similar conditions have reported either reduced or not altered urea synthesis. To clarify this issue, we studied the effect of TAA (0–30 mM for 24 h) on urea synthesis from ammonia, glutamine or alanine in primary cultured rat hepatocytes. It is of note that free ammonia has to diffuse into mitochondria, while amino acids represent an intramitochondrial source of ammonia. TAA significantly impaired urea synthesis from ammonia (Control: 100 ± 4%; TAA-10mM: 81 ± 8%; TAA-20mM: 69 ± 9%*; TAA-30mM: 69 ± 9%*; data are means ± SEM; n=6; *P<0.05) without affecting that from glutamine or alanine. Thus, ureagenesis in TAA-treated hepatocytes seems to be functional but dependent on the specific source of precursors. In addition, we found that TAA significantly reduced protein expression of mitochondrial aquaporin-8 (mtAQP8) (Control: 100 ± 18%; TAA-10mM: 68 ± 16%; TAA-20mM: 38 ± 7%*; TAA-30mM: 33 ± 17%*; data are means ± SEM; n=3; *P<0.05), a channel protein that facilitates ammonia diffusion into mitochondria to supply urea cycle. In conclusion, our data indicate that in rat hepatocytes, TAA can specifically impair ammonia-derived ureagenesis by a mechanism that would involve a reduced mtAQP8-dependent mitochondrial ammonia uptake. This knowledge may contribute to the understanding of the mechanisms of drug-induced liver dysfunction.

G12. Effect of Insulin growth factor 1 binding protein 2 mRNA-binding protein 1(IGF2BP1) knock down on the expression of multidrug resistance proteins in HepG2 cells.

Bucci Muñoz, M 1; Semeniuk, M 1; Livore, V 1; Banchio, C 2;Mottino, AD 1; Ceballos, MP 1; Ruiz, ML 1. 1-Instituto de Fisiología Experimental (IFISE)-CONICET, Facultad de Cs. Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario (UNR). 2-Instituto de Biología Molecular y Celular de Rosario (IBR)-CONICET, Facultad de Cs. Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario (UNR).

Background and aim: Multidrug resistance associated protein 3 (MRP3/ABCC3) and Breast cancer resistance protein (BCRP/ABCG2) are export pumps that extrude endo- and xenobiotics out of the cells, such as chemotherapeutic drugs. MRP3 and BCRP overexpression leads to chemoresistance in tumoral cells. IGF2BP1 is an oncoprotein highly expressed in tumoral cells, which modulates protein expression by binding and stabilizing mRNAs. The aim of this study is to evaluate if IGF2BP1 regulates MRP3 and BCRP expression in HepG2 cells. Methods: A specific shRNA targeting human IGF2BP1 was designed and cloned into pSR-GFP/Neo. The control shRNA (scrambled, SC) was designed by scrambling the nucleotides of the specific sequence. HepG2 cells were

cultured and transiently transfected with plasmids encoding IGF2BP1 shRNA (IGF2BP1-KD) or SC shRNA for 72 h with Lipofectamine® 2000. The KD of IGF2BP1 was evaluated by Western blotting. MRP3 and BCRP protein levels were evaluated in IGF2BP1 KD cells and SC cells by Western blotting.
Results: IGF2BP1 protein expression was decreased in HepG2 cells transfected with IGF2BP1 shRNA (SC: 100 ± 9 %, IGF2BP1 KD: 68 ± 2 %*). MRP3 and BCRP protein expression also decreased in HepG2 cells transfected with IGF2BP1 shRNA (MRP3: SC: 100 ± 3 %, IGF2BP1 KD: 80 ± 5 %*. BCRP: SC: 100 ± 14 %, IGF2BP1 KD: 47 ± 3 %*). All results are presented as mean ± SD, n = 5-6, *p < 0.05 vs. SC.
Discussion: The IGF2BP1 knock down resulted in a downregulation of MRP3 and BCRP. This finding suggests that targeting IGF2BP1 could be a useful tool to modulate multidrug associated resistance proteins, at least BCRP and MRP3, in order to minimize the development of chemoresistance in tumoral cells.

G13. KIR6.2 protein defficency affects normal liver regeneration after partial hepatectomy in mice

Florencia Lorenzetti 1; Marina Vera 1; Francisco Heit Barbini 2; Lautaro Hesse 2; Carla Comanzo 1; Alvaro Lucci 1,3; Gerardo Bruno Pisani 3; María Paula Ceballos 1; Ariel Quiroga 1,2,3; María de Luján Alvarez 1,2,3. 1 Instituto de Fisiología Experimental (IFISE) Facultad de Ciencias Bioquímicas y Farmacéuticas, CONICET, UNR. 2 Centro de Altos Estudios en Ciencias Humanas y de la Salud (CAECIHS) Sede Regional Rosario, Universidad Abierta Interamericana. 3 Área Morfología, Facultad de Ciencias Bioquímicas y Farmacéuticas, UNR

Liver regeneration (LR) after two-thirds partial hepatectomy (PH) in rodents has become a useful paradigm for studying the unique capacity of the organ to regenerate. Many signals come into play to drive normal LR, among them hepatic ATP content needs to be conserved. Kir6.2/K-ATP channels are expressed in the liver and they play critical roles in the cellular responses for tissue protection under stress. Previous results from our group showed that Kir6.2 knockout mice (Kir6.2-/-) displayed a significant diminution in hepatic PCNA and cyclin D1 protein levels at 48 h post-PH, indicating a possible participation of Kir6.2 in LR. Aim: to evaluate the role of Kir6.2 in LR after PH. Methods and Results: Male C57/B6 wild type (WT) and Kir6.2-/- mice were subjected to PH. LR was evaluated at the different phases of LR: priming (1, 2 and 3h), proliferation (48 and 96h) and termination (168 h) phases after PH. Liver to body weight ratio (LW/BW) showed a reduction in Kir6.2-/- mice at the studied times (1h post-PH: WT= 2.04±0.15; Kir6.2-/-= 1.61±0.10*; 2h post-PH: WT= 2.16±0.15; Kir6.2-/-= 1.70±0.07*; 3h post-PH: WT= 2.11±0.15; Kir6.2-/-= 1.58±0.10*; 48h post-PH: WT= 3.10±0.02; Kir6.2-/-= 2.55±0.19*; 96h post-PH: WT= 3.58±0.26; Kir6.2-/-= 2.62±0.29*; 168h post-PH: WT= 4.82±0.19; Kir6.2-/-= 3.89±0.09*; data are expressed as percent values and are means ± SEM; n=5; *p<0.05). On the other hand, plasma levels of alanine and aspartate aminotransferases and alkaline phosphatase did not show differences between WT and Kir6.2-/-. It was reported the importance of hemodynamic changes at the initial times after PH. Differences in LW/BW during the first hours post-surgery may indicate that hepatic blood supply is altered in Kir6.2-/- mice. At later times, liver proliferation is also altered in Kir6.2-/- mice, since LW/BW was reduced and it did not reach normal values even a week after surgery. In conclusion, our data indicate that Kir6.2 protein is involved in mouse LR after PH and its deletion affects the normal time-course of the process. Understanding the mechanisms of LR is needed to prevent loss of liver function and liver failure.

Metabolism and Nutrition

MN1. Grape pomace extract supplementation activates FNDC5/irisin in muscle and promotes white adipose browning in rats fed a high-fat diet.

Cecilia Rodriguez Lanzi1,2, Diahann J. Perdicaro1,2, Julián Gambarte Tudela2, Victoria Muscia1,2, Ariel R. Fontana3, Patricia I. Oteiza4, Marcela A. Vazquez Prieto1,2. 1Laboratorio de Nutrición y Fisiopatología de la Obesidad, Instituto de Medicina y Biología Experimental de Cuyo (IMBECU)- CONICET, Mendoza, Argentina. 2Facultad de Ciencias Médicas, Universidad Nacional de Cuyo, Mendoza, Argentina. 3Laboratorio de Bioquímica Vegetal, Instituto de Biología Agrícola de Mendoza (IBAM), Facultad de Ciencias Agrarias, Universidad Nacional de Cuyo CONICET, Mendoza, Argentina. 4Departments of Nutrition and Environmental Toxicology, University of California, Davis, USA.

Increased visceral adiposity plays an important role in the physiopathology of obesity and is related to altered glucose tolerance. Thus, promoting strategies to prevent these complications might be relevant. Concerning to this, phenolic compounds (PC) are molecules widely distributed in fruits and vegetables with proved beneficial effects. In addition, grape pomace extract (GPE) is a concentrate product obtained from a winemaking-residue with a relative high concentration of PC, (-)-epicatechin (EC) is one of the most relevant. On the other hand, Irisin is a novel myokine activates by PGC-1α in exercising muscles, that is released into the bloodstream after cleavage of fibronectin type 3 domain containing protein 5 (FNDC5). Irisin is related with adipose tissue "browning" and increased glucose tolerance. Recent evidence suggest that PC are able to activate Irisin. The aim of this study was to evaluated the effects of GPE supplementation: i. in the activation, secretion and possible underling mechanism of FNDC5/Irisin pathway on muscles of HFD-rats and L6 myotubes and ii. on epididymal adipose tissue (eWAT) "browning", adiposity and glucose tolerance on HFD-rats. Results: GPE activates the FNDC5/Irisin pathway and enhances Irisin secretion in vivo and in vitro, at least in part via PGC-1α activation. GPE prevents HFD-induced adipocyte hypertrophy and insulin resistance. In adittion, GPE activates eWAT "browning" evidence by increased protein levels of PGC-1α, PPARγ, PRDM16 and UCP-1. Finally, GPE induced AMPK-phosphorylation on muscles. Conclusions: Together, our findings demonstrate that GPE activates the FNDC5/Irisin pathway, which is associated with eWAT "browning". This can in part underlie the GPE

capacity to prevent HFD-induced adiposity and insulin resistance.

MN2. Vitamin K2 blocks the inhibitory effects of interferon alpha 2b on migration and invasion of liver cancer cells.

Marina Cecilia Vera, Álvaro Lucci, Florencia Lorenzetti, Carla Gabriela Comanzo, Francisco Javier Heit Barbini, Anabella Ferreti, María Paula Ceballos, María de Luján Álvarez, Ariel Darío Quiroga y María Cristina Carrillo. Instituto de Fisiología Experimental (IFISE-CONICET).

IFN-α is the primary choice for viral hepatitis treatment and a promising therapy for hepatocellular carcinoma (HCC). Vitamin K2 (VK2) exerts growth-inhibitory effects in several human cancer cells, including HCC. We previously reported that VK2 blocks the beneficial effects of interferon alpha 2b (IFN-α-2b) administered on the early stages of liver cancer development in rats. However, little is known on the mechanism. Objective: to further evaluate the effects of IFN-α-2b and VK2 in a human hepatic cancer cell line. Methods and Results: We used the human liver cancer cell line SK-Hep 1. The cells were treated with 10000 U/l of IFN-α-2b, 25 μM of VK2. Treatments were performed alone (IFN-group, VK2-group) or combined (IFN-VK2-group). The length of each treatment varied according to the experiment to be carried out. We performed the MTT assay to determine cell viability 24 hours after treatment, the wound healing assay to determine migration was performed 20 hours after treatment, and the invasion test in transwell chambers was performed 48 hours after initiation of treatments. No changes in cell viability were found with any of the treatments. On the other hand, IFN-treated cells showed a decrease (-15 %*) on migration, whereas VK2 and IFN-VK2 groups did not show any differences respect to control group. In line, IFN-group showed a significant diminution (-25%*) on invasion and VK2 treatment blocked the effect of IFN in the combined group. VK2 alone had no effects in this assay. Conclusion: there is no beneficial effect in treating human liver cancer cells with VK2. Moreover, VK2 blocks the positive effects of IFN-α-2b in cell migration and invasion. This is in agreement with our in vivo studies and seems to be clear that VK2 interferes with the beneficial cellular actions exerted by IFN-α-2b, ultimately leading to undesired final outcomes.

MN3. High-fat diet and fructose overload modify aortic vascular morphology and type C natriuretic peptide expression in rats.

Lee, Hyun Jin 1, 2; Cantú, Silvana M. 1, 2; Álvarez Primo, María 1; Donoso, Adriana S. 1, 2; Cao, Gabriel 3; Choi, Marcelo R. 1, 2, 3; Puyó, Ana M. 1, 2. 1. Universidad de Buenos Aires. Facultad de Farmacia y Bioquímica. Departamento de Ciencias Biológicas, Cátedra de Anatomía e Histología. Buenos Aires, Argentina. 2. Universidad de Buenos Aires. Facultad de Farmacia y Bioquímica. Instituto de Fisiopatología y Bioquímica Clínica (INFIBIOC). Buenos Aires, Argentina.

Metabolic and cardiovascular diseases are closely linked. Experimental models such as high-fat diet (HF) and/or fructose-overload (F) in rats resemble human metabolic syndrome (MS). Type C natriuretic peptide (CNP) produced in endothelial cells exert vasodilatory effects and a variety of metabolic actions. Therefore, we analyze the effects of F, HF diets and the combination of both (HF+F) on aortic vascular morphology and CNP immunoreactivity. Four groups of male Sprague-Dawley rats were studied for 9 weeks: control (C): standard diet (SD) and tap water to drink; fructose-overloaded (F): SD and fructose solution (10% w/v) to drink; high-fat diet (HF): 50% (w/w) bovine fat added to SD and water to drink; fructose plus high-fat diet (HFF), both treatments. We evaluated CNP in aorta tissue by immunohistochemistry, expressed as integrated optical density (IOD), and aortic morphology measuring the wall thickness/lumen diameter ratio. Visceral adiposity index was calculated as mesenteric vascular bed fat weight/body weight x 100. All groups with dietary modification showed elevated insulinemia (ng/ml, F: 4.3±0.5, HF: 5.3±0.4, HFF: 4.3±0.2, vs C: 1.2±0.1; p<0.01), triglyceridaemia (mg/dl, F: 163±10, HF: 170±9, HFF: 188±16, vs C: 65±9; p<0.01), systolic blood pressure (SBP, mmHg, F: 149±1, HF: 151±1, HFF: 153±1, vs C: 119±2; p<0.01), visceral adiposity index (VAI, %, F: 1.1±0.1; p<0.05, HF: 1.8±0.1, HFF: 1.8±0.1, vs C: 0.7±0.1; p<0.01) and aortic wall thickness/lumen diameter ratio (um/mm, F: 62.6±3.1, HF: 63.3±2.6, HFF: 64.0±3.0, vs C: 44.7±1.3; p<0.01) compared to control group. This ratio also positively correlates with SBP (r=0.91, p<0.05) and VAI (r=0.82, p<0.05). Besides, the modified diets decrease CNP immunoreactivity in the aortas of all experimental groups (DOI, F: 3,19±0,40, HF: 4,82±0,71; p<0.05, HFF: 2,29±0,03 vs C: 7,72±0,26; p<0.01). In conclusion, HF, F and HF+F diets increased adipose tissue and produced vascular wall modifications, increasing wall thickness and diminishing CNP expression. All these alterations would contribute to the development of visceral obesity-related hypertension directly through the reduction of vasodilatory mechanisms.

MN4. Chronic administration of antioxidant substances in experimental metabolic syndrome improves dyslipidemia and hepatic lipoperoxidation

Reyes Toso CF, Linares LM, Scacchi Bernasconi PA, Zuccarella V, Duarte M, Balcarce P, Bortolazzo GC, Ponzo OJ. Facultad de Medicina. Dep. de Fisiología. Unidad Académica 2. UBA.

Introduction: Increased intake of fructose has been linked to increased prevalence of Metabolic Syndrome (MS) and fatty liver in humans. Furthermore, consumption of a high-fructose-fat diet (HFFD) in rats promotes the development of pathological characteristics associated with experimental MS, such as increased systolic blood pressure (SBP), dyslipidemia, insulin resistance (IR), and visceral adiposity. The present work was designed to evaluate the effects of chronic administration of antioxidant substances (AS) on experimental MS alterations.

Material and Methods: Male rats were exposed for 20 weeks to a standard diet –SD- or HFFD with or without antioxidants (each subgroup n=8). The antioxidant supplement was composed of oligoelements (selenium and zinc) and vitamins E and C. Metabolic variables (fasting blood glucose, triglycerides, serum cholesterol levels, insulin and thiobarbituric acid reactive substances –TBARS-) were assayed during and at the end of the experiment. Body weight and SBP were also measured. Lipid peroxides in plasma were estimated by evaluating (TBARS). After euthanasia, abdominal white adipose tissue (AWAT) and liver were extracted and weights were expressed as a percentage of body weight. Lipid peroxidation was estimated by evaluating TBARS in liver homogenates. Data were analyzed by two-way ANOVA

tests adjusted by Bonferroni correction. The significance level was set at p≤0.05.
Results: Diet-induced weight gain in HFFD-treated group was not found. Significant differences were observed in SBP ($p<0.01$), fasting serum triglycerides levels ($p<0.01$), fasting serum cholesterol levels ($p<0.05$), insulin ($p<0.05$), TBARS in plasma ($p<0.001$), AWAT ($p<0.001$) and liver ($p<0.01$) weight and TBARS in hepatic tissue ($p<0.01$). AS administration reduced the increase of SBP ($p<0.05$), serum triglycerides ($p<0.01$), cholesterol ($p<0.05$), TBARS in plasma ($p<0.01$) and in hepatic tissue ($p<0.05$). Weight gain in AWAT was reduced ($p<0.05$) but not in liver.
Conclusions: This study show some evidence that chronic AS intake may have a role in counteracting the effect of HFFD probably through the scavenging property of O2- accumulation.

MN5. Prolactin modulation of breast cancer resistance protein and P-glycoprotein expression in a murine model of obesity induced by a high fat diet

Sedlmeier, María Guillermina; Ceré, Lucila Inés; Arboatti, Ainelén Soledad; Lmbertucci, Flavia; Monti, Juan; Catania, Viviana; Ronco, María Teresa; Francés, Daniel. Instituto de Fisiología Experimental (IFISE-CONICET).

Obesity is a metabolic disease characterized by low-grade inflammation as well as alterations in serum levels of different hormones. Prolactin (PRL) is a pleiotropic peptide hormone that participates at different levels to regulate the metabolism. Obese patients have shown alterations in the biliary excretion of different exogenous compounds, including therapeutic drugs, affecting their pharmacokinetics and efficacy. Differential expression of canalicular transporters would be involved. Previously, we have shown that mice fed with High-Fat Diet (HFD) displayed obesity hallmarks and elevated plasma levels of PRL that were associated with an increased canalicular expression of the ABC transporters Breast Cancer Resistance Protein (Bcrp) and P-glycoprotein (P-gp). In this regard, we suggest that the restoration of plasma levels of PRL could contribute to normalizing the expression and activity of transport systems. We aimed to inhibit pituitary PRL secretion using bromocriptine (Brc) and evaluate the canalicular expression of ABC transporters in our murine model. Five-week-old C57BL/6 mice were fed with regular chow diet (CHOW, n=8) or a 40% high-fat diet (HFD, n=8) for 16 weeks. Mice were injected subcutaneously either with Brc (2 mg/kg or 4 mg/kg, n=4 each group) or vehicle (veh, n=4) for three days. PRL plasma levels were measured using an ELISA kit. PRL plasma levels were not modified when we used the lower dose of Brc, but the dose of 4 mg/kg led to a significant decrease of PRL (-45%; $p<0.05$) in HFD Brc-treated group. In line with this, enhanced canalicular expression of Bcrp and P-gp after HFD was attenuated (-30%; $p<0.05$ and -20%; $p<0.05$, respectively) by Brc treatment. Also, the evaluation of hepatic PRL receptor (PRLR) mRNA expression levels by RT-qPCR showed an increase in HFD when compared to CHOW group (+64%; $p<0.05$), suggesting that HFD could sensitize liver to PRL action, reinforcing its effect. In conclusion, the restoration of PRL plasma levels could contribute to normalizing the expression and activity of canalicular transport systems, with favorable consequences on the efficacy of drugs used by obese patients.

MN6. Study of the metabolic impact of hyperlipemic diets in peripheral blood mononuclear cells: preliminary results

Elías, María Lucía1; Heredia, Rocío1; Avena, Maria Virginia1; Mussi Stoizik, Jessica1; Colombo, Regina2; Fornés, Miguel Walter2; Saez Lancellotti, Tania Emilce2; Boarelli, Paola Vanina1. 1-Universidad Juan.A Maza, Laboratorio de Enfermedades Metabólicas (LEM), Mendoza. 2-Instituto de Histología y Embriología de Mendoza – CONICET (IHEM)

Metabolic disorders associated with the diet are of great impact on health, due to their relation to chronic noncommunicable diseases. Peripheral blood mononuclear cells (PBMC) have an important role as early biomarkers in the study of the impact of fat-rich diets on lipid metabolism. These allow deepening the knowledge of the pathogenesis by non-invasive methods. Therefore, the objective is to study PBMC as a research tool for gene expression in alterations of lipid metabolism. Samples were obtained of ten New Zealand rabbits, divided in control group (N=5) fed with balanced feed (C), and case group (N=5) fed with the same feed supplemented with a 17% bovine fat (G). Fat groups do not receive fructose overload, keeping constant the concentration of carbohydrates and proteins, typical of the basic balanced food. Biochemical tests were performed to determine the levels of blood glucose (Gl), triglycerides (TG) and total cholesterol (CT). In PBMC, immunohistochemical tests (IHQ) were performed for SREBP1c and SERBP2 (Protein binding to sterile regulatory elements). Similar values of Gl (C: 140 ± 28.4 mg/dl vs. G: 118.3 ± 12.0 mg/dl) and TG (C: 144.1 ± 15.5 mg/dl vs. G: 135.6 ± 8.3 mg/dl) can be observed in preliminary biochemical studies of both groups, while group G shows an increase in CT (42.8 ± 21.6 mg/dl) compared to the group C (27.1 ± 4.5 mg/dl). However, some animals of group G have similar values to group C for CT (21.7 ± 2.4 mg/dl), normocholesterolemia group (NG). Thus, these animals do not show biochemical changes despite the intake of fat as occurs with others. In addition, liver tissue studies showed steatosis (oil red O stain), as well as the presence of SREBP1c (perinuclear/nuclear ratio: C: 2.0, G: 0.71, NG: 1.81) and SERBP2 (perinuclear/nuclear ratio: C: 3.29, G: 0.85, NG: 0.57) in PBMC. In conclusion, these results would indicate an activation of gene regulation without changes at the biochemical level. These results indicate that it is possible to study gene expression in PBMC, because it can observe the presence of specific molecules related to lipid metabolism.

MN.7 Natriuretic peptide system in the adipose tissue: the effect of high fat diet.

Martínez Tambella, Joaquín1; Soria, Damián1; Saravia, Melisa1; Magaldi, Rocío1; Sosa, Agustina1; Romero, Mariana1; Zago, Valeria3; Schreier, Laura3; Tomat, Analía12; Elesgaray, Rosana12; Arranz, Cristina12; Caniffi, Carolina12. 1Universidad de Buenos Aires. Facultad de Farmacia y Bioquímica. Cátedra de Fisiología. Buenos Aires, Argentina. 2CONICET – UBA. Instituto de Química y Metabolismo del Fármaco (IQUIMEFA). Buenos Aires, Argentina. 3Universidad de Buenos Aires. Facultad de Farmacia y Bioquímica. Laboratorio de Lípidos y Lipoproteínas.

Obesity and overweight are risk factors for the development of cardiovascular diseases. Natriuretic peptides (NP) modulate cardiovascular homeostasis and have lipolytic effects on adipose tissue (AT). The bioavailability of these peptides depends on the neprilysin

enzyme (NEP) which is responsible for the degradation of NP.
Objective: To evaluate the expression of components responsible for the degradation of NP in retroperitoneal AT (rAT) of rats fed high-fat diet (HFD) for 11 weeks.
Experimental design: Wistar rats received, from weaning until the 14th week of life, HFD (60% of total calories correspond to fat) or control diet (CD). At the end of the experimental period, body weight (BW) and systolic blood pressure (SBP) were measured, and oral glucose tolerance test (OGTT) was performed. The animals were sacrificed, and rAT morphology was evaluated by hematoxylin-eosin staining. NEP expression were evaluated (RT-qPCR) in rAT. Results are expressed as mean±SEM. Statistical analysis: Student test (n=6 rats/group; *p<0.05; **p<0.01; ***p<0.001 vs CD).
Results: BW, content of rAT, and area of adipocytes (AA), were increased in HFD rats at the end of the experimental protocol (BW(g): CD=486.4±11.5, HFD=550.8±13.4**; rAT/tibial length(g/cm): CD=1.83±0.14; HFD=3.15±0.18***; AA(µm2): CD=7456±594; HFD=12920±567*). PAS (mmHg) was similar between both groups (CD=126±4; HFD=131±3). Both area under the curve (AUC) and glycemia at 180 minutes from OGTT were greater in HFD rats, showing alterations in carbohydrate metabolism in this group (AUC(min.mg/dL): CD=24200±1151, HFD=28310±959*;
Glycemia(mg/dL): CD=114.4±5.8; HFD=147.8±7.0**). The expression of NEP was increased in HFD group compared to CD rats (NEP/GAPDH: CD=0.78±0.14; HFD=1.43±0.17*).
Conclusion: The HFD promotes the increase of NEP, a component of NP system involved in the degradation of NP in rAT. Considering that the peptides have lipolytic effects, their decrease could be involved in the development and / or maintenance of obesity and associated cardiometabolic diseases.

MN8. Protein malnutrition during the critical developmental stage induces type 2 diabetes in adulthood.

Echarte, Stella Maris; Motta, Estela; Campisano, Sabrina; Anabela La Colla, Chisari, Andrea. Department of Chemistry, Faculty of Exact and Natural Sciences, National University of Mar del Plata.

Individuals who suffer malnutrition during gestation, lactation and childhood have an elevated predisposition to develop metabolic diseases in adulthood.
The aim was study the effects of protein malnutrition during the stage of development (pregnancy, lactation and childhood) until 120 days of age as an inducer of insulin resistance and obesity in the offsprings.
Pregnant rats were fed with a control diet (CD) or a low protein diet (LPD). Female offspring rats received a LPD during gestation, lactation and until they were 120 days of age (MM group), a late LPD that began after 60 days (CM), or a LPD administrated during gestation, lactation and up to 60 days, followed by a CD (MC). The control group received only a CD.
The Glucose Tolerance curve showed to have significantly larger areas in MC than MM and MC ($p < 0.05$). Urinary excretion of C-peptide (co-secreted insulin molecule) relative to food intake has also been used as a more physiological indicator of IR. in group MC was greater than MM and CC ($p < 0.05$).
The glucose, triglyceride, cholesterol, HOMA, insulin, leptin, ghrelin values were significantly higher in the MC group than MM and CC ($p < 0.05$).
These results suggest that protein malnutrition during the development predisposes to the occurrence of fatty liver and the increment of inflammation and oxidative stress markers in adulthood. The disease of nonalcoholic fatty liver and nonalcoholic steatohepatitis is the hepatic manifestation of metabolic syndrome

Nephrology

R1. Urinary excretion of the organic anion transporter 5 (Oat5u) as a diagnostic biomarker of obstructive nephropathy.

Romina V. Campagno, Evangelina C. Nosetto, Anabel Brandoni, Adriana M. Torres. Área Farmacología. Facultad de Ciencias Bioquímicas y Farmacéuticas. Universidad Nacional de Rosario. CONICET.

At present, an array of peptides and proteins are studied and evaluated as potential biomarkers of renal injury able to early diagnose the renal dysfunction, in comparison to the conventional clinical indicators (plasma, urea or creatinine), that are low sensitive and non-specific. The Organic anion transporter 5 (Oat5) is an apical protein located in the proximal tubule cells that recognizes a wide spectrum of substrates:
estrone/dehydroepiandrosterone sulfate, toxins, and therapeutic drugs. The purpose of this study was to evaluate Oat5u as a biomarker of obstructive nephropathy and compared it with traditional markers of renal function, and with the urinary excretion of Neutrophil Gelatinase-Associated Lipocalin (NGALu), a novel biomarker already described for this pathology.
Bilateral ureteral occlusion (B) was induced in male Wistar rats, by ligation of both ureters for 1 h (B1, n=5), 2 h (B2, n=7), 5 h (B5, n=6) and 24 h (B24, n=6). The studies were performed after 24 h of ureteral releasing. Parallel to each group, a Sham one (Sh, n=11) was processed. Urea (Up) and creatinine (Crp) plasma levels were determined. Oat5u and NGALu were evaluated by electrophoresis and Western blotting. Results: Mean ± SE. Data were analyzed with ANOVA plus Newman Keuls $P<0.05$: (a) vs Sh, (b) vs B1, (c) vs B2, (d) vs B5, (e) vs B24. Up(g/L): Sh=0.43±0.03, B1=0.56±0.02, B2=0.67±0.05, B5=0.77±0.08, B24=4.32±0.32a,b,c,d; Crp(mg/L): Sh=5.73±0.16, B1=5.73±0.19, B2=7.47±0.46, B5=7.95±0.56, B24=41.54±3.54a,b,c,d; NGALu(%): Sh=100±2, B1=93±4, B2=90±5, B5=131±10a,b,c, B24=301±19a,b,c,d; Oat5u(%): Sh=100±3, B1=271±11a, B2=295±27a, B5=359±13a,b,c, B24=900±26a,b,c,d. Up and Crp significantly increased only in B24. NGALu significantly increased in B5 and B24. On the contrary, Oat5u increased in all experimental groups, even in those with short obstruction time periods (B1 and B2), in the absence of modifications of the other parameters of renal function analyzed. These preliminary results indicated that the urinary excretion of Oat5 could be considered a potential early diagnostic biomarker of

obstructive nephropathy, more sensitive than others to predict obstructive renal damage.

R2. Renal organic anion transporter 1 (Oat1) is primarily localized in caveolae and is redistributed into non-caveolar domains in extrahepatic cholestasis.

Evangelina C. Nosetto, Romina V. Campagno, Adriana M. Torres, Anabel Brandoni. Area Farmacología. Facultad de Ciencias Bioquímicas y Farmacéuticas. Universidad Nacional de Rosario. CONICET.

Oat1 is a transporter localized in the basolateral membrane of renal proximal tubular cells, responsible for the secretion of various endogenous compounds and xenobiotics. Previously, we have reported an increase in the expression and function of Oat1 in extrahepatic cholestasis (EC). Caveolae are cholesterol-enriched membrane microdomains implicated in processes like signal transduction and intracellular trafficking. The aim of this study was to examine the expression of Oat1 in different cellular fractions under physiological conditions as well as the effects of EC on the distribution of Oat1 between membrane microdomains. Cellular and membrane fractions of cortical cells were obtained from control Wistar rats to evaluate the expression of Oat1 and caveolin 2 (Cav-2, a structural component of caveolae) by immunoblotting. Another set of rats were subjected to 21 hour of bile duct ligation (L, n=6). Sham rats served as control (S, n=6). Caveolar (R) and non-caveolar (NR) domains were obtained from renal cortex of S and L rats. The membrane distribution of Oat1 between R and NR domains was determined by immunoblotting. Cav-2 expression as well as cholesterol levels in microdomains, assayed spectrophotometrically, marked the purity of R fraction. Under physiological conditions, Oat1 was mainly localized in membranes, concentrated in the caveolar fraction. Relative expression levels of Oat1 between R and NR fractions in S and L was calculated. Oat1 (%): S: R=72±4, NR=28±4; L: R=40±3*, NR=60±3* (*$p<0.05$ versus fraction isolated from S group). The pathology induces a significant redistribution of Oat1 from R to NR domains, indicating that the increase for Oat1 expression and function previously observed in L rats occurs together with the movement of the transporter to the NR fraction. Oat1 could shift from R to NR fraction to avoid being internalized by the caveolae-mediated pathway, which would result in more Oat1 being stably expressed in the membrane. Oat1 redistribution may have direct consequences in the increase of the transporter expression and activity and thus increase the transport of toxic substances that the liver cannot eliminate in this pathology.

R3. Is sex chromosome complement (SCC) responsible for sex differences in kidney V2 receptor (V2R) expression and desmopressin-induced antidiuresis?

Gonzalez, Lihue; Porcari, Cintia; Godino, Andrea; Vivas, Laura; Caeiro, Ximena. INIMEC-CONICET-Universidad Nacional de Córdoba.

An important number of experimental and clinical studies indicate a clear sexual difference in the antidiuretic response to desmopressin (V2R agonist). Women have a greater sensitivity to desmopressin and are more likely than men to develop hyponatremia in response to similar doses. Furthermore, in intact rats (without neutering) females express higher levels of renal mRNA and protein V2 receptor (V2R) levels, as well as a major urinary osmolarity response to desmopressin when compared to males. Moreover, in vitro studies have demonstrated that the X-linked V2R gene has high probability of escaping X-inactivation.

Our present study aimed to explore the role of the SCC (XX/XY) and/or the organizational hormonal effects of gonadal steroids in the sexually dimorphic response to the antidiuretic desmopressin administration on urinary osmolarity as well as in the relative gene expression of renal V2R. For this purpose, we used male (XX and XY) and female (XX and XY) mice of the “four core genotype” model, in which the effect of gonadal sex and SCC are dissociated, allowing comparisons of sexually dimorphic traits among XX and XY females, as well as in XX and XY males.

Mice aged 60-65 days old, were gonadectomized and forty-two days later were subcutaneously injected with vehicle solution or desmopressin (1 mg/kg). In a different group of animals kidneys were excised for V2R mRNA evaluation by qPCR.

As expected desmopressin administration induced a significant effect of treatment {$F(1,37)=439,63$} however no SCC nor organizational hormonal effects were observed in absence of the activational hormonal effects. In coincidence with this data the analysis of V2R mRNA levels showed no differences between male and female mice as well as among XX and XY mice. All this data in conjunction with previous studies highlights the importance of analyzing in further studies the contribution of the activational hormonal effects as well as the interaction with SCC and organizational hormonal effect in the potential mechanisms involved in sex-specific differences in kidney V2R expression and desmopressin-induced antidiuresis.

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R4. Text mining applied to PubMed searches on Hemolytic Uremic Syndrome.

Ricardo Dorr, Claudia Silberstein, Cristina Ibarra, Roxana Toriano. Instituto de Fisiología y Biofísica "Bernardo Houssay" (IFIBIO "Houssay") UBA-CONICET y Dpto. de Cs. Fisiológicas, Facultad de Medicina, UBA.

Human Hemolytic Uremic Syndrome (HUS) is characterized by the simultaneous development of nonimmune hemolytic anemia, thrombocytopenia, and acute renal failure. Different causes lead to the syndrome, but the more frequent is the infection caused by Shiga toxin-producing Escherichia coli (STEC), present in food and water supplies. STEC causes human gastrointestinal infections and the developing of HUS in 15% of the cases. According to the World Health Organization, Argentina has the highest global incidence rate of HUS in children under five. HUS can cause death and is the leading cause of acute renal failure in pediatric patients. From the first publication in 1955 a great number of papers have contributed to the understanding of HUS. At the same time, with the exponential increase in the number of articles published each year on biomedical topics, it is raised as a necessity in science to build automated systems to extract information from them. Our hypothesis holds that text-mining on scientific databases offers a powerful

tool to analyze behaviors, track tendencies and make predictions. To test, we have carried out an in-depth data-mining analysis on the results of a search on HUS of all publications indexed in MEDLINE up to 2018. Our main goal was to analyze the underlying text at the level of the descriptors used in searches and to discover information structures and non-explicit (often hidden) patterns. Different informatic tools were applied: Knime Analytics Platform; Voyant tools and AntConc. We show the text mining results on a set from 7989 original articles with more than 3.2 x 106 words. We analyzed 5949 abstracts, 40851 authors and 6191 affiliations to obtaining new valuable information in the study of HUS. Also, we worked on bag of words to analyze temporal frequencies and to do forecasting, and we applied unsupervised computing techniques in topic extraction. As a conclusion, we believe that text mining is an important tool to enriching understanding and promoting disease prevention. Suporting by PUE 2017 #22920170100041CO - CONICET; UBACyT 2017-2020 #20020170100733BA UBA; NVIDIA Corporation for donation of the Titan Xp GPU used for our research.

R5. Renal expression of RhoGTPases and its modulator, ICMT, in the evolution of tubular epithelial renal expression of RhoGTPases and its modulator, ICMT, in the evolution of tubular epithelial response to acute renal damage.

Buono, Gabriel M.1, Fussi, María F. 1; Marquez, Susana B.2; Rivabella Maknis, Tomas1; Girardini, Javier E3.; Larocca M. Cecilia4; Monasterolo, Liliana A.1,5,6; Molinas Sara M.1,6. 1 Área Farmacología. Facultad de Cs. Bioquímicas y Farmacéuticas. Universidad Nacional de Rosario. 2 Anatomía y Fisiología Patológica. Facultad de Cs. Médicas. Universidad Nacional de Rosario. 3 IDICER–CONICET. 4 IFISE-CONICET, 5 CIUNR. 6 CONICET.

Acute kidney injury (AKI) is still a significant medical problem. Renal ischemia-reperfusion (IR) injury is one of the main causes of AKI. RhoA and Cdc42 are RhoGTPases involved in the maintenance of epithelial integrity in renal tubules. Our aim was to study the signaling pathways involved in the development of AKI by characterizing RhoGTPase pathway in the IR model. Male Wistar rats (n=6 per group) were subjected to 40 min of unilateral renal ischemia followed by 1 (I1) or 7 (I7) days of reperfusion, or sham operation. Histological studies showed extensive tubular damage in I1 kidneys associated with decreased glomerular filtration rate (GFR). In agreement with studies performed at shorter reperfusion times, we found that cortical expression of RhoA (-65% $p<0.05$) and Cdc42 (-55%, $p<0.05$), evaluated by Western Blot, were decreased in I1. We also found an increment in cortical a-SMA (mesenchymal phenotype marker), evidencing an early tissue repair response. In I7, GFR was recovered and kidneys showed marked tubular regeneration together with an overexpression of cortical a-SMA and fibronectin (fibrosis marker). RhoA returned to control levels whereas Cdc42 levels were increased (+144%, $p<0.05$). RhoGTPases are posttranslationally regulated by prenylation. Since the isoprenylcystein carboxyl methyltransferase (ICMT) could modulate prenylated RhoGTPase membrane association and its inhibition increased RhoA and Cdc42 protein turnover, we studied its expression. We showed that cortical ICMT mRNA and protein levels, evaluated by RT-qPCR and Western Blot, respectively, were reduced in I1 and increased in I7. In conclusion, we demonstrated that, in the IR model, the early stage of renal damage is associated with a decrease in RhoA, Cdc42 and ICMT and the stage of marked regeneration is associated with an increment of these proteins. ICMT could have an important role in the response of injured kidney by modulating RhoA and Cdc42 expression and function, which are necessary to signal tissue repair events. Further studies are necessary to elucidate if this pathway may have a role in the development of fibrosis that could lead to chronic kidney disease.

R6. RhoGTPases and end-binding protein 1 (EB1) expression associated with apelin-induced proximal tubule function improvement in the post-ischemic kidney.

Sara M. Molinas(1,2), Florencia Hidalgo(3), Florencia Barsi(1), María Eugenia Taborra(1), Tomás Rivabella Maknis(1), Javier Girardini (4), Cecilia Larocca(3), Liliana A. Monasterolo (1,2,5). (1)Farmacología, Facultad de Ciencias Bioquímicas y Farmacéuticas. Universidad Nacional de Rosario. (2)CONICET. (3)IFISE-CONICET. (4)IDICER-CONICET. (5)CIC-UNR.

Understanding the mechanisms of kidney repair after ischemic injury is critical in the research of new therapeutic strategies. The repair process is accomplished by migration of new cells into the region, reconstituting the functional tubular epithelium. Ischemia–reperfusion induces changes in expression and distribution of Rho GTPases in proximal tubules. Similarly to Rho GTPases, EB1 is an important regulator of microtubule and actin dynamics, essential for kidney tubular cell-migration and epithelial remodelling. Furthermore, in certain scenarios, EB1 regulatory function overlaps with Rho GTPases signalling. Increasing evidence indicates that the adipokine apelin and its receptor, APJ, may be potential target for the treatment of kidney disease. The aim of this study was to evaluate the effects of apelin on functional parameters and the expression of RhoGTPases and EB1 in the post-ischemic kidney. Male Wistar rats (n= 4 per group) underwent 40 min unilateral renal ischemia followed by 24 h reperfusion (IR) or sham surgery (C). Apelin 50ug/Kg/d i.p. was administered during 3 days prior to IR (IR+Ap). Increased fractional glucose excretion in IR was prevented by Ap treatment (C: 0.0042 +/- 0.001%; IR: 0.25 +/- 0.06*; IR+Ap: 0.0097 +/- 0.004#). Western blot analysis of cortical plasma membrane fractions showed diminished levels of the RhoGTPases Cdc42 (C: 1 +/- 0.24; IR: 0.19 +/- 0.02*; IR+Ap: 0.64 +/- 0.10#) and RhoA (C: 1 +/- 0.19; IR: 0.11 +/- 0.05*; IR+Ap: 0.43 +/- 0.18#; *$p<0.05$ vs C, #$p<0.05$ vs IR) in IR which were restored with apelin treatment. EB1 expression pattern in total cortical homogenates was similar to Rho GTPases pattern. These findings show that apelin pretreatment in IR preserves proximal tubular function and prevents the decrease in RhoGTPases and EB1 expression, providing novel insights into the apelin/APJ system as potential therapy target for kidney injury. The disturb in Cdc42, RhoA and EB1 expression during injury and its recuperation in a functionally improved situation gather evidence for the role of these proteins in underlying mechanisms involved in the injury/repair process of renal tubular epithelium.

R7. Effect of vitamin K2 supplementation on vascular calcification in patients on hemodialysis.

Pérez Abúd R (1); García Bustos MF (1); Singh C (1), León de la Fuente R (2); Wangensteen Fuentes R (3); Moya A (4); Moreno D (4); Tamayo SR (1). 1: Escuela Universitaria en Ciencias de la Salud, Universidad Católica de Salta. 2: Instituto de Cardiología y Cirugía Cardiovascular de la Fundación Favaloro. 3: Universidad de Jaén, España. 4: Centro de Diálisis Salta.

Introduction: Vascular calcification (VC) is the leading cause of death in patients with end-stage renal disease (ESRD) on hemodialysis (HD). The vitamin K-dependent Gla protein matrix is a VC inhibitor and is inactive in renal patients. The objective of the study was to evaluate the effect of vitamin K2 on vascular calcification in patients with ESRD on HD. Experimental design: Prospective, randomized study. Men and women ≥ 18 years were included in HD ≥ 6 months. The study subjects (n = 59) constituted a control group: n= 24 (1000 µl of physiological serum) or treated group: n= 35 (1000 µl with 1000 mg of Vitamin K2). Vitamin K2 was administered intravenously three times a week using the same HD vascular access. Calcium and phosphorus were determined. The product Ca x P was obtained. The VC was evaluated before and after 6 months of treatment through EchoDoppler of the intimate medium thickness of the carotid arteries. Factorial ANOVA was performed for paired data. The StatGraphics Centurion XVII statistical package was used. Results: The treated group before treatment had 30.43% of cases above 55 mg/dl of Ca x P product, and after treatment, 26.09% above this value vs. untreated patients who showed no variation. The EchoDoppler showing the right carotid thickness (RCT) or left carotid thickness (LCT) were; Baseline parameters: Treated group 0.752 mm ± 0.06 (RCT), 0.811 mm ± 0.06 (LCT) vs untreated group 0.793 mm ± 0.05 (RCT) and 0.778 mm ± 0.04 (LCT). After Vitamin K2 treatment: Treated group 0.718 mm ± 0.18 (RCT); 0.831mm ± 0.16 (LCT) vs untreated group 1.01 mm ± 0.19 (RCT), 1,005 mm ± 0.21 (LCT), p = 0.0002. Conclusion: Treatment with Vitamin K2 significantly decreased the Ca x P product and vascular calcification in the treated group. Vitamin K2 could be a beneficial therapy for ESRD patients on HD.

Neurobiology

N1. Anesthetic effects in rats: interaction alpha2 agonists with ketamine hydrochloride

Joaquín Cogo Pagella, María Fernanda Rodríguez, Rosario López y Claudio O. Cervino. Instituto de Fisiología y Neurociencias (IFiNe-SeCyT, UM). Morón.

Dexmedetomidine (DM) and xylazine (XZ) are α2-noradrenergic receptor agonists. XZ combination with ketamine hydrochloride (KH) has been classically used in animal anesthesia. Currently, there is a growing interest in the use of DM in veterinary and human anesthesiology.

Objectives. a) Evaluate XZ and DM doses in combination with KH as an anesthetic agent in rats. b) Compare duration and effectiveness of the anesthetic state, and evaluate cardiac and respiratory changes under different anesthesia regimens.

Material and Method. Doses of DM (50 and 100 microg/kg IP), XZ (3 mg/kg IP) and KH (35 and 70 mg/kg IP), alone (control and drugs) or combined (KH35+XZ3, KH70+XZ3, KH35+DM50, KH70+DM50, KH35+DM100 and KH70+DM100) were tested in rats (N= 60). The effect and depth of anesthesia was evaluated through righting and withdrawal reflexes. Four states of anesthesia were defined: induction, AIT; general, GAT; surgical, SAT and full recovery, RTT. During the anesthesia, ECG and respiratory rate were simultaneously recorded. Data was statistically analyzed.

Results. The 12 different treatments showed: (a) control, KH35 and XZ3 did not produce anesthetic effects; b) KH70, DM50, DM100 and KH35+XZ3 produced only general anesthesia; c) KH70+XZ3, KH35+DM50, KH70+DM50, KH35+DM100 and KH70+DM100 produced general and surgical anesthesia. DM50 or DM100 showed a three-fold increase of the GAT in comparison to XZ3, when it was combined with KH35 or KH70. The SAT was represented (over total GAT) by 85% in DM100 combined with KH35 or KH70 treatments, by 60% in DM50 combined with KH treatments and by 30% with XZ3+KH70. During the SAT (considering the beginning of the GAT) marked bradycardia (50% HR reduction) and respiratory rate decrease were found under DM effects.

Conclusions. The DM (compared to XZ) combined with KH not only prolongs GAT and SAT, but also: a) increases the SAT ratio; b) reduces anesthetic recovery time. Together, our results confirm DM have better anesthetic qualities than XZ when is combined with KH, resulting in an increase in analgesic effect (SAT).

N2. Combined action of melatonin and alpha2 agonist for anesthesia in rats

Joaquín Cogo Pagella, María Fernanda Rodríguez, Fabricio Frisina and Claudio O. Cervino. Instituto de Fisiología y Neurociencias (IFiNe-SeCyT, UM). Morón.

Dexmedetomidine (DM) is an α2-noradrenergic receptor agonist used as sedative in combination with other drugs for pre-anesthesia or as continuous intraoperative infusion drug in both veterinary and human medicine. Ketamine hydrochloride (KH) is a fast-acting dissociative anesthetic that generates a deep analgesia state until anesthesia and presents a wide safety range. Alpha2 agonists have been used in combination with KH to provide a more effective state of general anesthesia. In rats and humans, melatonin (ML) has been shown to increase the effects of several anesthetic drugs. Objectives. a) Demonstrate that ML and DM enhance their anesthetics action. b) Compare the duration and effectiveness of the anesthetic state between ML+DM vs ML+KH.

Material and Method. Doses of DM (100 microg/kg IP), ML (50 and 100 mg/kg IP) and KH (35 and 70 mg/kg IP), alone (control-ML and drugs) or combined (ML100+KH35, ML100+KH70, ML50+DM100, ML100+DM100) were tested in rats (N= 50). The duration and depth of anesthesia was evaluated through righting and withdrawal reflexes. Four states of anesthesia were defined: induction, AIT; general, GAT; surgical, SAT and full recovery, RTT. Data was statistically analyzed (mean±SD).

Results. The 10 different treatments showed: (a) control-ML, ML50 and KH35 did not produce anesthetic effects; b) DM100, KH70 and ML50+DM100 produced only general anesthesia; c) ML100, ML100+KH35, ML100+KH70 and ML100+DM100 produced general and surgical anesthesia. ML100 showed a short GAT and SAT (11±4 and 2±4 min, respectively), which increased (112±20 and 30±2 min; 184±34 and 74±27 min, respectively) when it was combined with KH35 or KH70. ML100+DM100 combination showed 232±1 and 136±33 min GAT and SAT, respectively.
Conclusions. Our group has evaluated the anesthetic effects of ML administration when associated with KH and DM. Each drug alone does not generate an effective surgical anesthetic state, however when they are combined their effects are enhanced achieving adequate SAT. We demonstrate the ML100+DM100 combination effectiveness witch overcomes ML100 combination with KH35 or KH70.

N3. Effect of prenatal and postnatal zinc deficiency on central nervous system and anxiety-like behaviour

Mendes Garrido F1, Torres Suárez MP2, Pedrón V3, Varani A3, Moriondo M4, Caniffi C1, Elesgaray R1, Gobetto N1, Balerio G3, Tomat A1, Arranz C1. 1. Cátedra de Fisiología, Facultad de Farmacia y Bioquímica (UBA) - IQUIMEFA (CONICET) - 2. ICAAP, Facultad de Medicina (UBA) - 3. ININCA (UBA-CONICET) - 4. ININFA (UBA-CONICET).

Introduction: Moderate zinc deficiency during fetal and postnatal life programs cardiovascular, renal and metabolic disorders in male adult rats. Zinc is an essential micronutrient that plays a key role in neurogenesis and emotional behaviour.
Objective: We evaluated Central Nervous System histology and oxidative stress, and anxiety-like behaviour responses in adult male rats exposed to fetal and postnatal zinc deficiency.
Methods: Female Wistar rats received during pregnancy up to weaning low (L: 8ppm) or control (C: 30ppm) zinc diet. After weaning, male L offspring fed low (l) or control (c) zinc diet during 60 days (Ll, Lc). C offspring fed control (c) diet (Cc) for 60 days. At day 81, anxiety-like behaviour was measured by the elevated plus maze (EPM) test for 15 minutes (n=11-17/group). Then animals were sacrificed and brain sections were stained with Nissl to evaluate limbic system areas (hippocampus CA3 region and amygdala) histology (n=6). Glutathione peroxidase activity (GPx) and glutathione content were measured in brain homogenates (n=6). Statistics: ANOVA, Bonferroni post-test, mean±SEM, *p<0.05 vs Cc.
Results: EPM test showed a decrease in the percentage of entries into the open arms in Ll and Lc rats at 10 minutes (Cc:15.1±3.4; Ll:9.3±2.0*; Lc:4.4±2.6*%) and there were no changes in the time spent in open arms (Cc:5.1±1.4; Ll:5.6±1.7; Lc:3.2±1.9%). Zinc restriction did not induce changes in neuron size (soma area CA3, Cc:147±18; Ll:141±11; Lc:140±11 microm2) or density (CA3, Cc:78±9; Ll:83±2; Lc:91±9 - Amygdala, Cc:68±1; Ll:76±7; Lc:75±5 neurons/area). A pro-oxidant state was observed in zinc-deficient rats (GPx, Cc:3.7±0.7; Ll:1.3±0.4*; Lc:1.2±0.2* nmol/min/mg - Glutathione, Cc:145±0,7; Ll:80±7*; Lc:128±7 microg/mg prot).
Discussion: Rats exposed to zinc deficiency during fetal life, lactation and/or postweaning growth showed an anxiety-like behaviour related to a decrease in antioxidant systems, indicative of increased oxidative stress, but not to histological changes in limbic system areas.

N4. Glyphosate exposure induces neurobehavioral alterations in developmental rats

Luna Sebastián, Borgatello Conrado, Rosso Silvana. Laboratorio de Toxicología Experimental (LATOEX). Área Toxicología. Facultad de Ciencias Bioquímicas y Farmacéuticas. Universidad Nacional de Rosario. PROFISIO. Facultad de Medicina. Universidad Nacional de Rosario.

The right functioning of the brain relies on the precise connectivity of neuronal networks. Nowadays, there is growing evidence of the high vulnerability of developing nervous system to environmental contaminants. Glyphosate-containing herbicides are the most used agrochemicals in the world, particularly on genetically modified plants. Previous studies have demonstrated that glyphosate (Glyph) induces neurotoxicity in mammals. Therefore, in this work, we studied the potential effect of Glyph on nervous system during development. Wistar rats postnatal day (PND) 7 were exposed subcutaneously to Glyph (35 or 70 mg/Kg) or vehicle (PBS) every 48 hours for 3 weeks (critical period of synaptogenesis). Then, we performed behavioural evaluation through different tests, such as, Open Field for locomotor activity and Novel Object Recognition and Water Maze for learning and memory examination. Finally, animals were sacrificed on PND35 and hippocampi were dissected for biochemical analysis. We found that Glyph exposed rats showed a decrease in motor activity ($p<0.05$; $p=0.0146$) and also learning and memory impairments, observed by memory recognition assay ($p<0.05$; $p=0.0464$) and hippocampus-dependent spatial learning test ($p<0.05$; $p=0.0438$). Surprisingly, we observed a significant decrease in the expression of synaptic proteins in the hippocampus from Glyph exposed rats. Particularly, we detected a decrease in PSD-95 ($p<0.001$; $p=0.0001$) (a post-synaptic marker) and Synapsin I ($p<0.05$; $p=0.041$) (a presynaptic marker). In conclusion, these findings suggest that subletal doses of glyphosate exposure alter nervous system functionality impairing complex cognitive behavior, neuronal connectivity and synaptic activity.

Oncology

O1. Myeloid differentiation primary response protein 88 (Myd88) knockout mice are more susceptible to develop liver cancer

Heit Barbini Francisco J., Comanzo Carla G., Vera Marina C., Lorenzetti Florencia, Lucci Alvaro, Ferretti Anabela C., Ceballos Maria P., Alvarez Maria L., Carrillo Mar'ia C., Quiroga Ariel D. IFISE-CONICET. CAECIHS - Universidad Abierta Interamericana. Area Morfologia-Facultad de Ciencias Bioquimicas y Farmaceuticas - UNR

Inflammation is an important component of tumorigenesis. Myd88 is involved in Toll-like receptor and IL-1 receptor signaling pathways in the innate immune

response. We claim that signaling through Myd88 plays a key role in liver cancer development in mice. Adult C57BL/6 wild-type (WT) and Myd88-/- mice (23-25 g) were subject to a model of early liver cancer development. This was induced by administration of 2 i.p. doses of diethylnitrosamine (75 mg/kg bw) 2 weeks apart. One week after the last injection, mice received 20 mg/kg bw of 2-acetylaminofluorene by gastric probe 3 days a week for 3 weeks. All studies were performed before the initiation of treatment and showed no difference between genotypes. After cancer development, Myd88-/- mice showed lower body but higher liver weights than WT mice. We confirmed the complete absence of liver Myd88 protein expression by immunoblotting, as well as Myd88 mRNA expression, when evaluated by qPCR. Liver histology analysis showed scatter alterations on hepatocyte architecture, with accumulation of cytosolic lipid droplets (+23%) and increased inflammatory infiltration (+45%) in Myd88-/- mice compared to WT mice. Hepatic enzymes aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were slightly increased (+15%, and +12%, respectively) in plasma of Myd88-/- mice compared to WT mice, indicating a mild liver damage. Then, we evaluated proliferation and apoptosis by immunoblotting. We found that Myd88-/- mice presented with decreased protein expression of proliferation cell nuclear antigen (PCNA) (-35%), with a slight decrease in caspase-3 expression and no changes in Bax and cytochrome c expressions in total liver homogenates. These studies represent the first steps in the evaluation of the role of Myd88 in liver cancer development, and demonstrate that Myd88 is involved in prevention of chemical hepatocarcinogenesis; exposing, once again, the tight relationship between the immune system and the de development of cancer.

O2. Epidermal growth factor receptor (EGFR) activation induces the expression of multidrug resistance associated protein 4 (MRP4/ABCC4) in a pancreatic cancer human cell line

Rodrigo Lagos1, Ismael Barosso2, Ana Sahores1, Julieta Imperiale1; José E Manautou3, Carlos Davio1, Carolina Ghanem1,4. 1. Instituto de Investigaciones Farmacológicas (ININFA), Facultad de Farmacia y Bioquímica. Universidad de Buenos Aires. CONICET. Buenos Aires, Argentina. 2. Instituto de Fisiología Experimental (IFISE), Facultad de Cs. Bioquímicas y Farmacéuticas Farmacia y Bioquímica. Universidad Nacional de Rosario. UNR. Santa Fe, Argentina. 3. Department of Pharmaceutical Sciences, University of Connecticut, Storrs, CT, United States.

Pancreatic ductal adenocarcinoma (PDAC) is the most frequent type of pancreatic cancer and has one of the worst prognosis. The poor overall survival is associated with the overexpression of epidermal growth factor receptor (EGFR), a known member of the ErbB family of receptor tyrosine kinases, and the multidrug resistance associated protein 4 (MRP4/ABCC4). Our previous results show that high levels of MRP4 are associated with an increase in tumor cell proliferation, metastatic invasion and up-regulation of EGFR protein levels in PDACs cell lines. The aim of our study was to evaluate the regulation of MRP4 by EGFR activation in a pancreatic cancer cell model. To accomplish our objective, we treated the pancreatic cancer cell line BxPC-3 with EGF (0.1ng/µl). EGFR activation was confirmed by ERK phosphorylation at 5, 10, 20, 30, and 40 min after EGF treatment. MRP4 protein expression was evaluated by western blot using whole cell extracts following incubation with EGF for 0, 24 and 48 h, using histone as loading control. MRP4 expression levels were also assessed 48 h after treatment with EGF alone or in combination with the EGFR inhibitor CL 387-785 (1µM). Our results confirm that EGFR is quickly activated upon incubation with EGF, as evidenced by a 4-fold increase in the pERK/total ERK ratio detected ($P<0.001$) at 5 min and normalized at 40 min. Maximal induction of MRP4 expression (86%, $P<0.001$) was observed in cells treated with EGF for 48 h. Furthermore, EGF-mediated MRP4 induction was abolished by co-treatment with CL387-785 ($P<0.05$), while its expression did not change by treatment with this EGFR inhibitor alone. These data demonstrate that EGFR activation produces increments in MRP4 protein levels in a PDAC cell line. In summary, it is possible that MRP4 and EGFR, both PDAC poor prognosis markers, are co-regulated by a positive feed-back which ultimately enhances their effect upon each other.

O3. Antiproliferative and apoptotic effects of combined treatment of interferon alpha-2b (IFN) and vitamin e (vit E) on SK HEP-1 cells

Lucci A, Comanzo C, Vera M, Lorenzetti F, Ferretti A, Ceballos MP, Quiroga AD, Alvarez ML, Carrillo MC. Instituto de Fisiología Experimental-CONICET.

Our group demonstrated that IFN is an effective antitumor agent in the prevention and treatment of hepatocellular carcinoma (HCC) given its apoptotic and antiproliferative effects. Besides, it was shown that vit E exerts inhibitory effects on liver cancer due to its apoptotic, antiangiogenic and antiproliferative activities. Previously, we observed that combined treatment of IFN with vit E significantly decreased cell viability, migration and invasion of human HCC cell line SK HEP-1, in comparison with single treatments.

The aim of the present work is to deepen the study of the effects of combining IFN and vit E, with particular focus on proliferation and apoptosis. Methods: SK-HEP 1 cells were treated with 10000 U/l IFN and 25 uM δ-Tocotrienol (an isomer of vit E). Treatments were performed for 72 h using single drugs (IFN-group and E-group) and their combination (IFN-E-group). Also, a control group treated with drugs vehicles was included. We performed: a) annexin V-FITC assay to determine total apoptosis by flow citometry, b) western blot studies to analyze the expression of PCNA (marker of proliferation), proapoptotic Bax and antiapoptotic Bcl-Xl proteins, and c) dichlorofluorescein assay to evaluate reactive oxygen species (ROS) production. Results: IFN-E-group showed a higher increase in total apoptosis (+520%*&), compared with monotherapies (IFN-group: +75%*; E-group: +90%*). Also, IFN-E-group showed a significant decrease in PCNA expression (-35%*&) together with an increase in Bax protein expression (+65 %*&) and a decrease in Bcl-Xl expression (-85%*&), compared with monotherapies (IFN-group: PCNA: -17%*, Bax: +23%*, Bcl-Xl: -55%*; E-group: PCNA: -20%*, Bax: +25%*, Bcl-Xl: -60%*). On the other hand, IFN-E-group showed a significant increase in ROS production (+480%*&) compared with monodrug therapies (IFN-group: +50%*, E-group: +150%*) (*p<0.05

vs control; &p<0.05 vs IFN-group and E-group). Conclusion: combination of IFN and vit E in SK HEP-1 treatment enhances antiproliferative and apoptotic effects of monotherapies. Despite preliminary, the proposed combination could be taken into account in the development of new antitumor strategies against HCC.

O4. The flavonoid 2'nitroflavone inhibits proliferation, survival and migration of human triple-negative breast cancer cells

Vachetta VS, Marder M, Elola MT, Troncoso MF. Instituto de Química y Fisicoquímica Biológicas "Profesor Alejandro C. Paladini" (IQUIFIB, UBA-CONICET), Facultad de Farmacia y Bioquímica, UBA

Triple-negative breast cancer (TNBC) is one the most aggressive subtype of mammary tumors. As TNBC cells lack estrogen receptor, progesterone receptor and human epidermal growth factor receptor 2 (HER2), neither endocrine nor anti-HER2 molecular targeting therapy work. Flavonoids are a group of polyphenolic compounds considered potential chemopreventive candidates for cancer treatment. We previously described that the synthetic flavonoid 2'nitroflavone (2'NF) induces LM3 murine breast cancer cell apoptosis and acts as an anti-tumor agent in vivo. Our aim was to evaluate 2'NF effect on human TNBC cell proliferation, survival and migration. 2'NF exerted a dose- and time-dependent inhibitory effect on MDA-MB-468 (Basal-like TNBC subtype), MDA-MB-231 (Claudine-low TNBC subtype) and BT-549 (Claudine-low TNBC subtype) cell viability (MTT assay). The half maximal inhibitory concentrations (IC50 mean, 95% confidence interval) were 3.3 (2.7-4.2), 2.1 (0.6-7.0) and 2.9 (2.4-3.5) µM at 96 h for MDA-MB-468, MDA-MB-231 and BT-549 cells, respectively. Significant inhibitory effects on colony formation were observed in 2'NF-treated TNBC cells (Clonogenic survival assay). Colony formation at 7 days was: 74±9, 54±6% for MDA-MB-468 cells; 46±19, 12±7% for MDA-MB-231 cells; 79±19, 29±10% for BT-549 cells versus the corresponding control cells (100%) after 3-day treatment with 1.2 and 2.5 µM 2´NF, respectively. Similar results were founded after 6-day treatment. At conditions which do not affect cell viability, 2'NF inhibited claudine-low TNBC cell migration (Wound-healing assay) in a dose-dependent manner. In the presence of 1.2 and 2.5 µM 2'NF, cell migration at 48 h significantly decreased versus vehicle (100%): 80.0±4.5 and 27.4±0.1% for MDA-MB-231 cells, 65.5±4.1 and 52.3±10.2% for BT-549 cells, respectively. Basal-like TNBC MDA-MB-468 cell migration was not affected by 2'NF at concentrations 0.02-0.2 µM, while higher doses inhibited cell proliferation. In conclusion, 2'NF inhibits proliferation, viability and survival of MDA-MB-468, MDA-MB-231 and BT-549 cells, and cell migration in claudine-low TNBC cells. Thus, we propose 2'NF as a novel potential candidate for TNBC therapy.

O5. Role of AMPK-p53 pathway in the antimigratory effects of alpha lipoic acid in hepatocellular carcinoma cells.

Florencia Hidalgo, Emilia Baffo, Anabela Ferretti, Alejandro P. Pariani, María Cecilia Larocca, Cristián Favre. IFISE, CONICET-UNR.

Alpha lipoic acid (ALA) is a natural compound, which acts as a cofactor of mitochondrial enzymes and selectively induces mitochondrial dysfunction and cytotoxicity in tumor cells. In addition, ALA antitumor role is associated to its capacity of AMPK activation. We are focused on studying less explored effects of ALA on cell migration/invasion in hepatocellular carcinoma (HCC). In previous work we demonstrated that ALA increases cell death and decreases cell migration and invasion in HCC cells in which an increase in p-AMPK levels is also observed. Some of these effects were enhanced by glucose starvation (GS). It is proposed that energy stress activates p53 via AMPK in diverse cell types and that AMPK can induce p53 transcription and/or phosphorylate it and hence stabilize it postranscriptionally. In this part of the study, our aim was to analyze to what extent the antimigratory effect of ALA could depend on this kinase and if p53, which is related to some effects of ALA, participates. For these purposes, we used, on the one hand, AMPKa-silenced HCC cells (AMPK-KD) and on the other hand, p53 null cells, Hep3B. In HCC cell line with wild type p53 (HepG2/C3A) the levels of p53 expression were measured (mRNA by qPCR) after cell culture in DMEM without (C), or with 1mM ALA (ALA) for 24 h, and both also in conditions of glucose starvation (GS). We found that mRNA levels were increased (ΔΔCt in arbitrary units C: 1; ALA: 5.85; GS: 6.3; ALA GS: 7.65, p<0.01), and greater nuclear localization of this protein was also observed by confocal immunofluorescence microscopy after 24 h treatments. Wound-healing assay showed that AMPK knock down blocked the effect of ALA treatment observed in scramble-control (SC) HCC cells (SC: ALA: 43+/-3 (p<0.001); AMPK-KD: ALA: 81+/-2, % of migration vs. C). The lack of p53 also impeded this effect of ALA in Hep3B cells. We conclude that AMPK is essential for ALA antimigratory effects, and that its target p53 is upregulated by ALA. Elucidation of the complete downstream mechanism explaining the reduced migration of the cells requires further studies.

O6. The sirtuins inhibitor cambinol reverses sorafenib resistance in human hepatocellular carcinoma HepG2 cells

Delprato Carla Beatriz; Livore Verónica Inés; Carmeli Barberis Eugenia; Ferretti Anabela Cecilia; Lucci Alvaro; Comanzo Carla Gabriela; Quiroga Ariel Darío; Alvarez María de Luján; Mottino Aldo Domingo; Carrillo María Cristina; Ceballos María Paula. IFISE-CONICET

Sorafenib (SFB) is the only approved drug for hepatocellular carcinoma (HCC) treatment but it only prolongs patients' median survival by nearly 3 months. The main reason underlying the impaired sensitivity to SFB is multidrug resistance (MDR). MDR often results from upregulation of ABC transporters, like P-glycoprotein (P-gp) and multidrug resistance-associated protein 3 (MRP3). Sirtuins 1 and 2 (SIRTs1/2) are overexpressed in HCC and are associated with tumoral progression and MDR. Previous results from our group showed that the SIRTs1/2 inhibitor cambinol (CAMB) diminished P-gp and MRP3 expression in HepG2 cells and exacerbated the effects of SFB on cellular viability and migration in 2D and 3D cultures of these cells. Aim: to analyze whether SIRTs1/2 activities blockage overcomes MDR during SFB treatment. Methods: 2D and 3D cultures of the HepG2 cell line were treated for 72 h with SFB (2 µM) in presence or absence of CAMB (50 µM). Proliferation (2D: PCNA-western blot; 3D: Ki67-immunofluorescence), apoptosis (2D and 3D: CellEventCaspase 3/7 reagent), invasion (2D: transwell

assay) and ABC expression (2D: P-gp and MRP3-western blot) were assayed. Results: In 2D and 3D cultures, cells treated with SFB and CAMB showed a greater fall in cellular proliferation (2D: SBF -17%, SFB+CAMB -98%*#) and presented with more apoptosis than cells treated with SFB alone. In 2D cultures, treatment with SFB and CAMB diminished invasion compared to SFB treatment (SBF -70%*, SFB+CAMB -85%*#). Whereas SFB induced ABC expression in 2D cultures (P-gp: +154*, MRP3: +92*) treatment with CAMB+SFB reduced ABC protein levels (P-gp: -50*#, MRP3: -44*#) compared to control cells. *p<0.05 vs. control; #p<0.05 vs. SFB. Conclusions: cambinol improved the sensitivity of HepG2 cells to sorafenib probably by reducing P-gp and MRP3 protein levels. This resulted in a reduction of proliferation and invasion and in an increment of apoptosis. Results from 3D cultures, that mimic tumor features in vivo, reinforce the clinical relevance of the current data. Findings support a potential application of SIRTs1/2 inhibitors in combination with sorafenib to reverse MDR during HCC therapy.

O7. Effect of alkaline gradient on clear renal cell carcinoma mortality: role of isoform 1 of Na^+/H^+ exchanger function.

Mazzocchi M, Di Giusto G, Pizzoni A, Beltramone N, Ford P, Capurro C, Rivarola V. Universidad de Buenos Aires. Facultad de Medicina. Departamento de Fisiología. Laboratorio de Biomembranas. Buenos Aires, Argentina. CONICET-Universidad de Buenos Aires. Instituto de Fisiología y Biofísica "Bernardo Houssay" IFIBIO-HOUSSAY. Buenos Aires, Argentina. vrivarola@yahoo.com

The association between cell death and intracellular pH elicits the possibility that extracellular pH (pHe) may modify cell survival. Moreover, as tumor extracellular acidity is a hallmark of cancers, is probable that pHe affects differently cancer or normal cells. Our previous studies showed that cells derived from renal cell carcinoma (RCC) were more sensitive to 24h alkalosis than normal cells. The aim of this study was to investigate further the best alkaline condition to kill cancer cells without affecting normal ones. We use three renal cell models: HK2, derived from normal human proximal epithelial cells, 786-O and Caki-1, both derived from human RCC. We exposed cells to media with different alkaline pHe during 48 to 96h. Then, we estimated cell survival by acrydin orange–ethidium bromide experiments. Our results show that normal HK2 cells could survive up to 72h in very mild alkaline conditions (4.8mM NaOH, + 0.05 pH units) without being affected (94±16 % of living cells respect to control, n=6). At the same conditions, both RCC derived cell lines had significant fewer % of living cells respect to control (64±5 % for 786-O and 72±7 % for Caki-1 cells, both with n=6). With 72h exposition to 9.6 mM NaOH (+ 0.1 pH units) the percentage of living RCC cells respect to control became minimal (23±8 % for 786-O and 16±5 % for Caki-1 cells, both with n=6). However, at this condition HK2 cells were also affected (28±5 % of living cells respect to control, n=6). NHE1 isoform of Na^+/H^+ exchanger favors apoptotic process in some experimental models. Then, we inhibited the transporter during the 72h exposition to 9.6 mM NaOH. NHE1 inhibition partially reverted the effects of alkalosis only in normal HK2 cells (% of living cells respect to control: +NHE1 28±5 vs –NHE1 54±6, p<0.01, n=12). In summary, the combination of alkali plus NHE1 treatment might improve tumor control with less normal tissue damage.

AWARDS

Selected for Camilion de Hurtado Award

CH1. A defective sarcoplasmic reticulum Ca2+ cycling is linked to the increased susceptibility to Ca2+ alternans of the hypertrophied myocardium

Juan Ignacio Elio Mariángelo, Luis Gonano, Leticia Vittone, Cecilia Mundiña-Weilenmann, Matilde Said. Centro de Investigaciones Cardiovasculares - Facultad de Ciencias Médicas - Universidad Nacional de La Plata.

One of the earliest cardiovascular alterations produced by hypertension is the left ventricular hypertrophy (LVH).This abnormal increase in LV mass is recognized as an independent risk factor for poorer cardiovascular outcome. Among other complications, LVH shows an increased propensity to cardiac alternans. Clinically, these alterations create a highly arrhythmogenic substrate. At the cellular level, alternans is a cyclic beat-to-beat oscillation in contraction amplitude (mechanical alternans), action potential duration (APD alternans) and/or cytosolic Ca2+ transient amplitude (Ca2+ alternans) at a constant heart rate. The emerging consensus is that cardiac alternans is ultimately linked to a defective sarcoplasmic reticulum (SR) Ca2+ cycling. In previous studies in ex vivo hearts, we demonstrated an increased propensity to mechanical alternans in the hypertrophied myocardium of spontaneously hypertensive rats (SHR) vs. normotensive rats (W). To further characterize these beat-to-beat fluctuations, isolated cardiac myocytes from SHR and W rats were loaded with the fluorescent Ca2+ probe FURA2-AM and paced between 0.5 and 5Hz to induce alternans. The threshold frequency for Ca2+alternans was significantly lower in SHR myocytes than in W myocytes (3.19±0.29Hz, n=8 vs 4.03±0.22Hz, n=8). SR Ca2+ uptake, indirectly evaluated by the frequency-dependent relaxant effect (half time for Ca2+ decline), was not different between both strains. The refractoriness of SR Ca2+ release was evaluated by the Ca2+ transient restitution at different time interval (Ti) between electrical stimulation. Ti at 50% restitution was significantly prolonged in SHR vs W myocytes (in msec: 339.0±6.7, n=14 vs 311.1±5.9, n=8). Confocal microscopy studies confirmed Ca2+ alternans in SHR myocytes. Our results indicate that the increased susceptibility to cardiac alternans in SHR rats was associated to a defective SR Ca2+ cycling, based on a slowed down recovery from refractoriness of SR Ca2+ release, without alteration in SR Ca2+ uptake.

CH2. The activation of the G protein-coupled estrogen receptor (GPER) prevents and regresses cardiac hypertrophy.

Di Mattia, Romina A.; Mariángelo Juan Ignacio E.; Blanco, Paula G.; Portiansky, Enrique L.; Mundiña-Weilenmann, Cecilia B.; Aiello, Ernesto A.; Orlowski, Alejandro. Centro de Investigaciones Cardiovasculares Dr. "Horacio E. Cingolani"
Introduction: The G protein-coupled estrogen receptor (GPER) has been associated to non-genomic actions of estrogen in the heart. The activation of GPER by its synthetic ligand G1 has been shown to be cardioprotective. Thus, the aim of this work was to evaluate the role of GPER activation in both in vitro and in vivo models of cardiac hypertrophy.
Experimental design: For the in vitro model, neonatal rat cardiomyocytes (NRCM) were treated for 48 hours with Aldosterone (Ald), G1, or the combination of both. The mineralocorticoid receptor (MR) and the GPER were blocked by pharmacological and molecular tools (transfecting cells with specific siRNAs). Cell area was measured in all cases. The in vivo model consisted of Spontaneous Hypertensive Rats (SHR) treated with G1, administered with osmotic mini pumps for 28 days. Echocardiographic studies were performed and evaluation of cardiac hypertrophy parameters was assessed.
Results: In NRCM, the hypertrophy induced by Ald was prevented by the co-treatment with G1. Both, the reduction of the expression of MR by the siRNA and the MR blocker, eplerenona, totally prevented the hypertrophic effect of Ald in NRCM. In addition, G1 was unable to prevent Ald-induced hypertrophy when the cells were transfected with siGPER or treated with its synthetic antagonists, G15 and G36. Echocardiographic studies showed a reduced left ventricular mass index in SHR treated during 28 days with G1 (normalized by tibia length in mg/cm; Wistar: 250.8±15.75, n=5; SHR*: 376.5±16.67, n=6; SHR Veh*: 377.3±20.93, n=6; SHR G1: 263.4±41.52, n=7; *p<0.05 vs Wistar), which was corroborated by measuring of cross-sectional area on the cardiomyocytes (in µm2; Wistar: 299,1±11,1, n=5; SHR*: 353,6±20,2, n=5; SHR Veh*:333,7±7,2, n=6; SHR G1: 257,1±19,5, n=8; *p<0,05 vs Wistar) and with quantification of mRNAs of fetal genes, such as BNP and ANP.
Conclusion: Overall, the present data indicate that the MR-dependent Ald-induced hypertrophy is prevented by GPER activation by G1. Furthermore, G1 reduced cardiac hypertrophy in an in vivo model, suggesting the possibility of considering this receptor a novel therapeutic target for the treatment of this pathology.

CH3. Transient gene therapy with baculoviral vector encoding mutant HIF-1A induces collateral vessels formation in rabbits with peripheral arterial disease
Giménez CS1; Castillo MG1; Nuñez Pedrozo CN1; Simonin JA2; Belaich MN2; Bauzá MR1; López AE1; Locatelli P1; Mendiz O3; Crottogini A1; Cuniberti L1; Olea FD1. Instituto de Medicina Traslacional, Trasplante y Bioingeniería (IMETTYB-Universidad Favaloro-CONICET). 2- Universidad Nacional de Quilmes. 3- Hospital Universitario de la Fundación Favaloro.
Introduction: Peripheral artery disease (PAD) is an ailment characterized by decreased arterial blood flow to the lower limbs. We hypothesized that the administration of a baculovirus (Bv) encoding mutant HIF-1A is safe and induces neovasculature in the ischemic limb. Methods: 1) HIF-1A expression was evaluated in transduced and non-transduce muscle cells (SkM) by RT-qPCR and western blot. 2) In vitro tubulogenesis assay was performed with supernatants of SkM and SkM-mHIF-1A cultures. 3) Rabbits underwent sterile excision of the femoral artery of the left posterior limb. Seven days later, rabbits were randomized in two protocols: A) Tracking assay: Rabbits with PAD were injected with 1E9 copies of Bv.GFP and sacrificed at 3, 7 and 14 days (n=2 per time). Two animals injected with PBS were used as controls. The presence of viral DNA or GFP mRNA in the injected limb and remote tissues biopsies were analyzed by RT-qPCR. GFP protein was observed by fluorescence microscopy. B) Therapeutic efficacy: Rabbits were randomized to receive 1E9 copies of the Bv.mHIF-1A or Bv.null (n=6 per group). Two weeks post-treatment digital angiography and immunohistology were performed in the posterior limbs. Results: HIF-1A mRNA levels in transduced SkM were 1000-fold higher than SkM ($p<0.05$, t-test). HIF-1A protein levels were also overexpressed. SkM-mHIF-1A induced 2-fold higher tubular networks than SkM ($p<0.01$, t-test). Viral DNA and GFP mRNA were detectable only at 3 and 7 days after injection in hind limbs. The virus was not detected in remote tissues. GFP protein was detectable in hind limbs at all-time points. The angiographies showed that visible collaterals density was higher in Bv.mHIF-1A-treated rabbits than in Bv.null-treated animals (8.12±0.42 vs. 6.13±1.15 collaterals/cm2, $p<0.05$, t-test). The same profile was observed by immunohistological techniques (27.9±7.0 vs. 15.3±4.0 arterioles/mm2 and 341.8±109.9 vs. 208.8±87.7 capillaries/mm2, $p<0.05$, t-test). Conclusion: Bv.mHIF-1A induced angiogenesis and displayed a good safety profile in rabbits with PAD at 14 days post-treatment. Complementary studies to evaluate its potential usefulness in the clinic are needed.

CH4. Cardioprotective effects of n-methylacetazolamide mediated by inhibition of l-type Ca2+ current
Alejandro Ciocci Pardo, Luisa F González Arbeláez, Leandro Díaz, Alejandro M. Ibáñez, Romina G. Díaz, Ernesto A Aiello, Susana M Mosca. Centro de Investigaciones Cardiovasculares Dr. Horacio E. Cingolani.
Our objective was to examine the effects of N-methylacetazolamide (NMA), a non-carbonic anhydrase inhibitor, on the myocardial post-ischemic alterations. For it, isolated perfused rat hearts were assigned to the following groups: 1) Non-ischemic control (NIC):110 min of perfusion and 2) Ischemic control (IC): 30 min of global ischemia and 60 min of reperfusion (R). Both groups were repeated in presence of NMA (5 µM), which was administered during the first 10 min of R. Infarct size (IS) was measured by TTC staining. Developed pressure (LVDP) and end-diastolic pressure (LVEDP) of the left ventricle were used to assess systolic and diastolic function, respectively. The content of phosphorylated forms of Akt and PKCε were also evaluated. The changes of pHi in papillary muscle by immunofluorescence were determined. In isolated ventricular myocytes the L-type Ca2+ current (ICa) with the whole-cell configuration of patch-clamp technique and intracellular Ca2+ transients (estimated by the Fura 2AM fluorescence ratio) simultaneously monitored with cell shortening were measured. In NIC group the NMA decreased the contractility a 20 % vs 3 % in non-treated hearts. In IC group, NMA decreased IS (22 ± 2 % vs 32 ± 2 %, $p < 0.05$) and improved the post-ischemic recovery of myocardial function. At the end of R, LVDP was 54 ± 7 % vs 18 ± 3 % and LVEDP was 23 ± 8 vs. 55 ± 7 mmHg ($p < 0.05$). The P-Akt and P-PKCε levels increased 20 % in hearts treated with NMA compared to IC non-treated hearts. In papillary muscle, NMA did not modified the pHi recovery from sustained intracellular acidosis. Peak I(Ca) density recorded at 0 mV was smaller in myocytes treated with NMA than non-treated (-2.01 ± 0.26 pA/pF vs -2.41 ± 0.27

pA/pF, p < 0.05). Ca2+ transient amplitude and cell shortening, explored at 1 mM extracellular Ca2+, decreased but not significantly after NMA treatment.
These data demonstrated that NMA cardioprotects the myocardium against ischemi-reperfusion injury. The diminution of L-type Ca2+ current appears as the responsible mechanism, participating Akt and PKCε-dependent pathways.

Selected for SAFIS Award

S1. Actin filaments modulate the electrical activity of brain microtubules

Brenda C. Gutierrez, María del Rocío Cantero, and Horacio F. Cantiello. Laboratorio de Canales Iónicos. Instituto Multidisciplinario de Salud, Tecnología y Desarrollo (IMSaTeD, UNSE-CONICET), Santiago del Estero.

The cytoskeleton of eukaryotic cells contains networks of actin filaments and microtubules (MTs) that are jointly implicated in various cell functions, including cell division, morphogenesis and migration. In neurons, this synergistic activity drives both the formation of axons during neuronal development and synaptic activity in mature neurons. Both actin filaments and MTs are also highly charged polyelectrolytes that generate and conduct electrical signals. However, information on an electrical crosstalk between these two cytoskeletal networks is presently unavailable. Herein we tested the effect of actin polymerization on the electrical oscillations spontaneously generated by two-dimensional sheets of MTs from bovine brains. Voltage-clamped bovine brain MT sheets displayed electrical oscillations with a fundamental frequency of 38 Hz that represented a synchronous 224% change in MT conductance. To explore whether actin may affect the electrical activity of the brain MT sheets, monomeric (G-) actin was added to the sample from the bathing solution. Addition of a critical concentration for polymerization of G-actin (0.25 microM) produced no immediate effect on the MT oscillations. However, the amplitude of the oscillatory signals started to rise with a lag-time of approximately 2 min. G-actin addition increased the amplitude of the oscillations, and thus, the charge transferred by 72.3% (7.15 ± 2.85 nC, n = 5), but not the frequency of oscillation. Addition of the G-actin-binding protein DNAse I (100 microM) prevented the G-actin effect. Addition of pre-polymerized F-actin, in contrast, had a rapid onset (<10 sec) and a higher effect on the MT sheets (~100% increase, 8.25 nC). The data indicate that an interaction with actin filaments increases the electrical activity of brain MTs, in what seems to be an electrostatic effect between polymers. This may have important implications in neuronal function, such as the processing of intracellular signaling and the gating and activation of actin cytoskeleton-regulated excitable ion channels.

S2. Acute modulation of intestinal multidrug resistance-associated protein 2 localization and activity by nutrients.

Guillermo Nicolás Tocchetti, Camila Juliana Domínguez, Felipe Zecchinati, Maite Rocío Arana, Marcelo Gabriel Luquita, Juan Pablo Rigalli, María Laura Ruiz, Silvina Stella Maris Villanueva, Aldo Domingo Mottino. Instituto de Fisiología Experimental (IFISE) – CONICET-UNR.

INTRODUCTION: Intestinal absorption of food toxicants is limited by multidrug resistance-associated protein 2 (MRP2), an ATP-dependent pump localized to the brush border membrane (BBM) of the enterocyte. We have demonstrated that this intestinal barrier can be rapidly modulated by changes in MRP2 localization between BBM and intracellular domains. We here explored whether the intake of specific nutrients stimulates the insertion of MRP2 into BBM and hence restricts absorption of food contaminants. The mechanism underlying such regulation was also explored. EXPERIMENTAL DESIGN: Nutrients were administered into distal rat jejunum and, after 30 min, MRP2 barrier activity was assessed in proximal jejunum using the model substrate DNP-SG. MRP2 localization was determined by quantitative confocal microscopy. Extracellular mediators were evaluated using selective inhibitors and by immunoneutralization. Intracellular pathways were explored in vitro in Caco-2 cells, a model of intestinal epithelium. RESULTS: Oleic acid (10% V/V) and glucose (100 mM) significantly increased MRP2 activity (+202±22% and +188±45%, respectively; p<0.05) whereas glutamine (10-100 mM) and glucose (10 mM) did not. Immunoneutralization of enteroglucagon (GLP-2) prevented oleic acid effect, demonstrating mediation of this intestinotrophic hormone. Use of selective inhibitors demonstrated that extracellular adenosine production and its subsequent binding to enterocytic adenosine receptor A2B follow GLP-2 action. Moreover, i.v. administration of GLP-2 or adenosine boosted MRP2 activity to similar levels of those produced by oleic acid. In vitro studies confirmed participation of A2BAR/cAMP/PKA intracellular axis in MRP2 modulation. All reported changes were associated with increased MRP2 localization in BBM. CONCLUSION: We postulate that selective nutrients trigger GLP-2 secretion by enteroendocrine cells. Next, GLP-2 stimulates enteric neurons to secrete adenosine, which binds to basolateral A2BAR in enterocytes, ultimately leading to AMPc/PKA-dependent insertion of MRP2 to BBM. This constitutes a novel physiological mechanism aimed to reduce the absorption of toxic compounds after food intake.

S3. An intact caveolar structure is necessary for the proper formation of placental microvasculature.

Reppetti Julieta1, Sierra Matías N.2, Anud Carolina2, Erlejman Alejandra3, Damiano Alicia E.1,2, Martínez Nora1. 1 Laboratorio de Biología de la Reproducción. Instituto de Fisiología y Biofísica Bernardo Houssay (IFIBIO Houssay) UBA-CONICET. Buenos Aires, Argentina. 2 Cátedra de Biología Celular y Molecular. Departamento de Ciencias Biológicas, Facultad de Farmacia y Bioquímica, UBA. Buenos Aires, Argentina. 3 Laboratorio de Biología Molecular y Celular. Departamento de Química Biológica, Facultad de Ciencias Exactas y Naturales, UBA. IQUIBICEN-CONICET. Buenos Aires, Argentina.

Introduction: A proper development of the placental vasculature is necessary for a successful pregnancy. Two types of endothelial cells form the human placenta: the microvascular endothelial cells which comprise the fetal capillaries of chorionic villi and are involved in the oxygen and nutrients exchange, and the macrovascular endothelial cells which are the large conduit vessels of the umbilical cord. These cells differ in morphology, gene expression and function.

Caveolae are a type of lipid rafts, coated with caveolin-1 (Cav-1) protein. On the other hand, Aquaporin-1 (AQP1) is a transmembrane water channel which moves water in response to osmotic gradients. Recently, it was suggested that AQPs andCav-1 are also indispensable for efficient cell migration.
Objective: To evaluate the role of caveolae in the formation of placental microvasculature and its interaction with AQP1.
Materials and Methods: This study was approved by the ethics committee of the Hospital Nacional Prof. Dr. A. Posadas. Placental microvascular endothelial cells (hPMEC) and EA.hy926 cell line (ATCC® CRL-2922™) were used. Gene expression of Cav-1 and AQP1 was evaluated by RT-PCR. Protein expression and localization were studied by Western Blot and immunofluorescence. Co-localization of Cav-1 with AQP1 was assessed by immunoprecipitation assay. Cells were treated with 5 mM methyl-β-cyclodextrin (MβCD) to disrupt caveolae and with Tetraethylammonium chloride (TEA) to block AQP1. Cell migration was evaluated by wound healing assay.
Results: Cav-1 and AQP1 co-localized in the cell membrane of placental endothelial cells. MβC D significantly reduced cell migration in EA.hy926 cells ($n=4$; $p<0.05$) and in hPMEC cells ($n=4$; $p<0.0001$), while no changes were observed when cells were treated with TEA.
Conclusions: Although cell migration is not related to AQP1, an intact caveolar structure is required for the appropriate migration of placental endothelial cells. Any perturbations might result in aberrant angiogenesis leading to serious pregnancy disorders such as preeclampsia or fetal growth restriction.

NOTAS