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Resúmenes Científicos XLI Congreso Anual Sociedad de Farmacología de Chile

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PANEL DE EDITORES

La Revista de Farmacología de Chile tiene un panel de editores conformado por connotados farmacólogos nacionales que son miembros de la Sociedad de Farmacología de Chile y académicos de las principales universidades chilenas.

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MISIÓN

La Revista de Farmacología de Chile es considerada el órgano oficial de difusión científica y de opinión de la Sociedad de Farmacología de Chile. En un principio esta revista nació como un remozado libro de resúmenes del XVIII Congreso Latinoamericano de la Asociación de Farmacología realizado en Chile el año 2008. Desde 2009 y hasta ahora la Revista de Farmacología de Chile ha recibido varios trabajos originales de investigación y diversas revisiones de temas farmacológicos relevantes.

La Revista de Farmacología de Chile aborda temas relacionados con la farmacología básica y experimental, así como investigaciones clínicas. Las áreas temáticas principales son: farmacocinética, farmacodinamia, farmacología cardiovascular, farmacología pulmonar, farmacología endocrina, neurofarmacología, farmacología clínica, estudios preclínicos, estrés oxidativo, fitofarmacología, ciencias farmacéuticas, química-médica y toxicología. También la revista actualmente permite divulgar opiniones sobre los principales temas de salud relacionados con medicamentos en Chile, la presentación de líneas de investigación de laboratorios nacionales en donde se realizan investigaciones farmacológicas, información de curso y programas de postgrados nacionales en farmacología y la publicación de resúmenes científicos del Congreso Anual SOFARCHI.

Audiencia:

La Revista de Farmacología de Chile esta dirigida a farmacólogos nacionales e internacionales interesados en la divulgación de la farmacología. También está dirigida a estudiantes de pregrado de carreras universitarias del área de la salud y ciencias biomédicas, y a estudiantes de postgrado que cursen maestrías y doctorados en farmacología.

Periodicidad:

Se editarán 3 números anuales (Abril, Agosto y Diciembre) en formato digital e impreso. El número de Diciembre incluirá trabajos originales y los resúmenes del Congreso Anual de la Sociedad de Farmacología de Chile.

Temas a Publicar:

- Artículos originales en Farmacología Básica, Farmacología Clínica, Farmacoterapia y Toxicología.
- Artículos originales de investigación nacional e internacional en Farmacocinética y Farmacogenética.
- Artículos de revisión de temas farmacológicos importantes sobre las diversas temáticas de la disciplina.
- Artículos de Información de nuevos fármacos incorporados al arsenal terapéutico nacional.
- Opiniones oficiales de la sociedad sobre los aspectos regulatorios y nuevas políticas de medicamentos.
- Artículos sobre nuevas metodologías docentes, aplicadas en Farmacología.
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- Libros y revistas de los temas.
- Promoción de actividades académicas, congresos y cursos nacionales e internacionales en farmacología.
- Publicitar las ofertas de trabajo de inserción académica en Universidades Chilenas y extranjeras, así como las oportunidades de inserción laboral en la industria privada ligada al desarrollo de fármacos.

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ABSTRACTS FOR XLI OF THE CHILEAN SOCIETY OF PHARMACOLOGY

CONFERENCES

1. THE EMOTION OF SCIENTIFIC DISCOVERY.
Antonio G. García
Instituto Teófilo Hernando, Departamento de Farmacología y Terapéutica, Facultad de Medicina, Universidad Autónoma de Madrid, Spain

Stephen Hawking said that "science is not only a matter of reason; it is also a matter of romance and passion". This was already written by Santiago Ramón y Cajal in his famous book "Reglas y Consejos sobre Investigación Científica", a hundred years ago. More recently, the National Academy of Sciences of the USA wrote a short guide entitled "ON being a scientist", that summarizes the emotions that a scientist may feel along his carrier when pursuing a problem and finding the response sometimes after years of hard work. I will illustrate the way science is practiced with some experimental findings on the topic of calcium signaling and exocytosis in adrenal chromaffin cells. In doing so, I will focus first on basic science, describing how we arrived to the concept of a functional triad that includes the voltage-gated calcium channels (VGCCs), the endoplasmic reticulum (ER) and mitochondria (MIT). Such triad shapes the cytosolic calcium signals that control both pre-exocytotic and exocytotic responses, the basis of the fight-or-flight stress response of W. Cannon. Then, I will focus on more recent translational research done in chromaffin cells from mouse models of neurodegenerative diseases. I will comment on dysfunctions of Ca2+ and exocytosis occurring even at pre-symptomatic disease stages. I will next make some comments on the failure of clinical trials in AD, to end with some hints on the pressure to "publish-or-perish" and how science is becoming just a mere business for editorials and else.

Recent and ongoing work from AGG'S laboratory is being founded by
1. European Union Horizon 2020 Research and Innovation Programme under the Marie Skoldowska-Curie Grant Agreement 766124);
2. Grant SAF-2016-48892-R, from Ministerio de Ciencia, Innovación y Universidades, Spain; and
3. Fundación Teófilo Hernando, Madrid, Spainen un contexto general y discutiré su importancia con relación a la salud y a la enfermedad.

2. THE PLEASURE OF SCIENCE: MY LIFE IN PHARMACOLOGY.
Salvador Moncada
Manchester Cancer Research Centre, The University of Manchester, U.K.

I will describe the work that opened several fields of investigation. From the mechanism of action of non-steroidal anti-inflammatory drugs, to the discovery of thromboxane synthase and prostacyclin, to the identification of nitride oxide and its metabolic pathway of synthesis. I will finish with a reference of the role of mitochondria in oxidative stress. I will put all this work in a general context and its importance in health and disease will be discussed.

3. DIRECT C–H FUNCTIONALIZATION OF CYTISINE. NICOTINIC RECEPTOR SELECTIVITY AND MECHANISM OF ACTIVATION.
Tim Gallagher
University of Bristol, UK

The talk will cover the application of subtype-selective nicotinic partial agonists to manage nicotine addiction, with a focus on the chemistry of cytisine. Already used for smoking cessation, cytisine (and varenicline) target subsets of nicotinic receptors, and the opportunity to generate novel structural variants of cytisine raises the question of whether more subtype selective ligands are available and of value or indeed are even desirable. The talk will cover recent work in this area, much of which is underpinned by development of C-H functionalisation chemistry that provides very direct and efficient access to new C-10 cytisine derivatives, which in turn, offer more selective subtype profiles. Recent studies (in collaboration with Henry Lester and Dennis Dougherty) have probed the influence of steric vs. electronic factors in determining the binding mode of cytisine. We have also pursued extensive molecular dynamics simulations to probe the mechanism (timing) of signal propagation through the protein scaffold that occurs on ligand association to the receptor, and addressed the question of the applicability and generality of that mechanism across other receptors.

4. GENETIC, PROTEIN AND PHARMACOLOGICAL MODULATION OF HUMAN α7 NICOTINIC RECEPTORS.
Cecilia Bouzat
Instituto de Investigaciones Bioquímicas de Bahía Blanca, INIBIBB (CONICET-UNS), Departamento de Biología, Bioquímica y Farmacia, Universidad Nacional del Sur, Bahía Blanca, Argentina.

The α7 nicotinic acetylcholine receptor is a pentameric ligand-gated ion channel. It is widely expressed in the central nervous system where it is involved in cognition, attention, and memory. It is also expressed in many non-neuronal cells and its activation has anti-inflammatory and neuroprotective roles. Enhancement of α7 activity is emerging as a therapeutic strategy for cognitive, neurodegenerative and inflammatory

disorders. We have focused on understanding α7 function and its different mechanisms of modulation associated to physiological, pathological and therapeutic situations. By single-channel recordings we determined that positive allosteric modulators (PAMs) enhance α7 activation by increasing open-channel lifetime and inducing prolonged activation episodes, and we also identified novel PAMs. Although α7 has been considered the homomeric member of the family, heteromeric α7β2 receptors have been detected in human brain. We generated α7β2 receptors with different stoichiometries and determined how the β2 subunit modifies α7 kinetics and its allosteric modulation. This information is required to decipher the role of α7β2 receptors in native cells. In humans, there is a truncated α7 subunit (dupα7) that lacks part of the ACh-binding site and results from partial duplication of the α7 gene. We demonstrated that dupα7 acts as a negative modulator and can assemble with α7 into functional heteromeric receptors. Deciphering the molecular basis underlying α7 function has implications for the design of novel therapeutic compounds as well as for clarifying its pleiotropic actions.

5. IRON AND FERROPTOSIS IN AGING AND AGE-RELATED NEUROLOGICAL DISEASES.

Ashley Bush
The Melbourne Dementia Research Centre, The Florey Institute of Neuroscience and Mental Health and the University of Melbourne, Australia.

Recent research has implicated increased brain iron as a trait that can propel various neurodegenerative diseases, including Alzheimer's disease, Parkinson's disease, Motor Neuron disease and the complications of stroke. Ageing itself causes iron to increase in the brain to a point where it is "too much of a good thing" and can set up conditions that lead to neurodegeneration. During childhood and reproductive life, iron recruitment is geared towards avoiding iron deficiency, but there is no natural mechanism for off-loading excess iron. After reproductive life the systems that harvest iron so efficiently do not turn off, and lead to accumulation in tissues that are not normally shed, like brain. In the C. elegans model of ageing, we find that such iron elevation limits lifespan. In Alzheimer's disease brain iron elevation is associated with the rate of cognitive loss, lipid peroxidation products and features of the regulated cell death mechanism, ferroptosis. Anti-ferroptosis agents have been effective in animal models of neurodegenerative disease, and a recent phase 2 clinical trial of the anti-ferroptotic chelator deferiprone in Parkinson's disease lowered nigral iron and improved clinical readouts. We are currently testing this drug in a phase 2 RCT in Alzheimer's disease. CuATSM, has recently reported benefits in phase 1 studies of Parkinson's disease and Motor Neuron Disease, and we have identified that it possesses potent anti-ferroptotic properties.

References:

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Ayton et al. Brain iron is associated with accelerated cognitive decline in people with Alzheimer pathology. Molecular Psychiatry, in press doi: 10.1038/s41380-019-0375-7.
Southon et al. CuII(atsm) inhibits ferroptosis: implications for treatment of neurodegenerative disease. British Journal of Pharmacology, in press.

SYMPOSIA

1. PURINERGIC SIGNALLING: FROM STRUCTURE-ACTIVITY TO APPLICATION IN PATHOLOGIES

CONTRIBUTIONS OF MOLECULAR DYNAMIC CALCULATIONS TO STRUCTURAL BIOLOGY UNDERSTANDING, THE CASE OF IVERMECTIN AND ALFAXOLONE AS P2X4R MODULATORS.

Huidobro-Toro J.P.; Latapiat V.; Alveal N.; Montenegro F.; Barrera N.
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Multiple molecules modulate the electrophysiological activity of P2X receptors (P2XR). In particular, trace metals such as Cu(II) or Zn(II), modulate P2X4R concentrated in neurons by selective and specific cationic transporters; the putative site of action of these trace metals is apparently restricted to the extracellular receptor domain. Drugs such as the antiparasitic antibiotic ivermectin (IVN) or alfaxolone (A) a synthetic neuro-steroid, with hypnotic properties, also modulate the P2X4R but at intracellular sites, near the transmembrane P2X4R domain. In order to reveal and understand the site and mechanism of the modulator effect, as well as the allosteric agonism of A, we strategically used molecular dynamic simulations/calculations to visualize the docking and mode of action of these modulators. A rP2X4R homology model was built; docking of either IVM or A revealed selective binding sites confined to the transmembrane domain as anticipated based on drug lipophilicity. In addition, molecular dynamic simulations in the APO and HOLO P2X4R states revealed allosteric-induced stability. Pore and lateral P2X4R fenestrations measurements of the different receptor states, in the absence and next in the presence of either IVM or A, showed that both IVM and A can elicit a larger pore opening that proved larger in the presence of ATP, as expected for allosteric modulators. Interestingly, in the case of A, consonant and consistent with an allosteric agonist, the hole analysis demonstrated an even larger opening. The present findings reveal the power and strategy of using simulation studies to understand molecular aspects of the binding and intricate molecular mechanism of allosteric agonist studies. Based on these findings, dynamic simulation calculations offer opportunities to design novel, P2X4R ligands not based on purines.

Pharmacology area: Farmacología molecular (Molecular Pharmacology)
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Acknowledgments: FONDECYT grants 117-0842 and Basal 0807 (CEDENNA).

P2X2 RECEPTOR: NEW PHARMACOLOGICAL TARGET TO AD.

Fuentealba, J.
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Soluble oligomers of amyloid beta peptide (SOAβ) have been considered as central factors in Alzheimer's disease (AD). Aβ peptide is generated through the sequential cleavage of the amyloid precursor protein (APP), a process that requires the previous endocytosis of APP and that can be modulated by the multidomain adaptor protein Fe65. This protein is able to regulate the transcription of key genes directly related to AD pathogenesis, encoding proteins like APP and BACE 1. On the other hand, we have described that chronic SOAβ treatment induces an increase in the expression of the P2X2 purinergic receptor in PC12 cells and hippocampal neurons. Additionally, it has been described that the P2X2a isoform has an intracellular domain that can interact with Fe65, a segment which is absent on the P2X2b isoform. We found that SOAβ treated cells displayed an increase in evoked ATP currents (C: 100 ± 50%; SOAβ: 231 ± 70%; n=9). Additionally, immunocytochemistry (ICC) experiments demonstrated that these cells exhibited an increase in their P2X2R immunoreactivity (C: 100 ± 1 %; SOAβ: 149 ± 15%; n=5). Moreover, cells treated chronically with SOAβ showed a reduction in the Fe65 nuclear-cytoplasmic (N-C) ratio (C: 100 ± 6%; SOAβ: 80 ± 4%; n=5). A similar behavior was observed in PC12 cells transfected to express the P2X2a isoform, but not in those transfected with P2X2b (C: 100 ± 5%; P2X2a: 70 ± 6%; P2X2b: 95 ± 6%; n=3). Colocalization analyses demonstrated that SOAβ decreased the colocalization of Fe65 with APP (C: 100 ± 17%; SOAβ: 47 ± 12%; n=5); results that correlate with the increase observed in the colocalization of APP with clathrin (C: 100 ± 8%; SOAβ: 127 ± 8%; n=4) and Rab5 (C: 100 ± 6%; SOAβ: 132 ± 16%; n=5). In conclusion, these results suggest that chronic SOAβ treatment promotes the endocytosis of APP, potentiating its amyloidogenic processing. Additionally, the calcium dyshomeostasis/overload induced by P2X2R overexpression, alter the activation and localization of CAMKIIα, in the context of AD. Using molecular biology techniques, we observed that after chronic SO-Aβ treatments, mice hippocampal neurons showed an increase on the levels of P2X2R compared to the control cells (C: 100.0 ± 6.4%; SOAβ: 130.1 ± 10.7%, n=5). This was correlated with increased Ca2+ signal evoked by ATP (C: 100.0 ± 12%, SOAβ: 194 ± 24%, n=4). Immunocytochemistry approaches on mice hippocampal neurons, showed that the overexpression of P2X2R induced changes on the immunorreactivity pattern of pCAMKIIα (in soma and neurites), which induced alterations on the cells morphology, and electrophysiological recordings assessed by Sholl Analysis and Patch Clamp, respectively. These results suggest that P2X2R overexpression can potentiate the toxicity of SO-Aβ, due to the chronic Ca2+ overload and inactivation of CAMKIIα, and thus, altering the mechanisms of neuronal plasticity, the basis of the pathophysiological mechanism of AD.

Pharmacology area: Neuropharmacology
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Acknowledgments: Proyecto Fondecyt Nº1161078

ROLE OF PURINERGIC SIGNALING AND IN GASTRIC CANCER.

Coddou C.1; Castro P.2; Cerda D.1; Reyna-Jeldes M.1; De la Fuente E.1.
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Gastric cancer (GC) is the one of the most prevalent cancers worldwide. Here, we studied in detail purinergic signaling in several gastric adenocarcinoma-derived cell lines: AGS, MKN-45 and MKN-74, and compared them to a non-tumoral epithelial cell line: GES-1. In GC-derived cells, we detected the expression of several purinergic receptors, and found important differences as compared to GES-1 cells. Functional pharmacological studies with calcium imaging and proliferation assays revealed a strong contribution of P2YRs, especially P2Y2Rs to increases in cell proliferation and an antiproliferative effect induced by the activation of P2X4Rs. Also, we detected a tonic purinergic response that is probably a reflect of the paracrine and autocrine nucleotide signaling, because to sole application of purinergic antagonists changed the basal proliferation of GC-derived cells. In tumor-derived biopsies, we found an increase of P2Y2R and a decrease in P2X4R expression; however, we found high variability between the biopsies and their respective adjacent healthy gastric mucosa. Even so, we found a correlation between the expression levels of P2Y2R and P2X4R and survival rates of gastric cancer patients. Our latest experiments suggest that purinergic signaling also can contributes to epithelial to mesenchymal transition in CG-derived cells and that transactivation of P2Y2/HER2 can also contribute to these effects. Taken together, these results demonstrate the involvement of purinergic signaling in GC, and that the changes in expression and nucleotides release observed in GC could direct nucleotide signaling from anti-proliferative effects in healthy tissues to proliferative effects in cancer.

Pharmacology area: Farmacología gastrointestinal (Gastrointestinal Pharmacology)
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Acknowledgments: FONDECYT 1161490 FONDEQUIP EQM140100

2. TOXICOLOGY AS A MULTIDISCIPLINARY SCIENCE

TOXICOLOGY AS A MULTIDISCIPLINARY SCIENCE.

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The development of the Toxicology is strong related to the human history and its relationship with the environment, as well as geographic, social or politic environment. In the primitive phase of the toxicology as health topic, the principal focus of the knowledge was the description of the toxic events on humans, and its accidental or intentional character. Many of the early register of intoxications were based on the contact of plants, minerals or animals with the human bodies and it deleterious results. This description of events summarized the botanic, chemical, zoological and medical sciences. With the human enrichment on scientific information, the description of intoxications are more specific and included clinical symptoms, treatment of the intoxicated patients or forensic evidences in the body. For that are applied the physiopathology, forensic medicine, clinical medicine and pharmacology. The actual toxicology is related to many complex and heterogeneous knowledge, as well as analytical, molecular, computational, clinical, legal, epidemiological and pharmacological methods. In a more general aspect, toxicology is implicated in diverse applied areas, as clinical toxicology, forensic and legal toxicology, occupational toxicology, ecotoxicology and environmental toxicology. Therefore, toxicology is clearly a multidisciplinary science, it may be used in other areas, and toxicology needs information of other different disciplines as pharmacology.

Pharmacology area: Toxicología (Toxicology)
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STATE OF THE ART OF TOXICOLOGY IN CHILE.

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The first scientific meeting of Sociedad de Toxicología de Chile (SoTox) was held in november 2012. The event brought together toxicologists from academia, regulatory agencies and industry that represented the practice of toxicology in Chile. Only a few months earlier, SoTox had acquired its legal personality becoming Chile´s first scientific society in toxicology, so the meeting was an unprecedented opportunity to talk about the state of the art of basic and applied toxicological sciences in Chile. In general terms, it was acknowledged that toxicology played an important yet undefined role in the Chilean society, mostly due to the lack of professionals who had received formal academic training in toxicology. Seven years later, SoTox continues its efforts to position toxicology and toxicologists as an important discipline developed by professionals who can contribute to better decision making in the many different areas needed for the growth of the country. In this talk I will: i) introduce a modern perspective of what toxicology should be; ii) describe the state of development of the science in the country and iii) communicate SoTox´s goals and achievements.

Pharmacology area: Toxicología (Toxicology)
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EPIDEMIOLOGY OF INTOXICATIONS IN CHILE. 2018 ANNUAL REPORT OF THE TOXICOLOGICAL INFORMATION CENTER OF THE PONTIFICIA CATHOLIC UNIVERSITY OF CHILE (CITUC).

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Centro de Información Toxicológica, Facultad de Medicina, Pontificia Universidad Católica de Chile (CITUC).

The epidemiology of poisonings in countries is relevant when addressing public health guidelines, evaluating exposure profiles and defining treatment and prevention strategies. This presentation describes the characterization of exposures to potentially dangerous substances based on the cross-sectional descriptive study of the universe of calls that entered the CITUC toxicological emergency center during 2018. The variables analyzed were: sex, age, circumstance of the exposure, agent (s), interlocutors of the call, location of the interlocutor and the incident, routes of exposure, symptomatology and severity. This work collects information regarding calls for toxicological emergencies that entered CITUC, from the 15 regions, 54 provinces and 346 communes of Chile. During the telephone

call, the emergency center professionals collect all available information provided by the caller, required for the evaluation of the case considering data of the agent, the circumstances of the exposure and the patient. After the background evaluation, the technical recommendations for exposure management are communicated. The data is collected in the manual registration form and subsequently all the data is entered into the electronic Registration System called "Call Registry System CITUC SRL". The Central of toxicological emergencies provides free telephone assistance with qualified professionals 365 days a year in continuous hours (24/7), answering questions from health professionals, authorities, emergency personnel and the general public. The commitment and dedication of the professionals, together with the excellence in the service, guarantee the 27 years of existence of the center and the 35,000 calls on average that CITUC receives annually.

Pharmacology area: Toxicología (Toxicology)
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CLINICAL MANAGEMENT OF POISONINGS: SPECIALISTS NEEDED.
Müller C.
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In Chile, the number of acutely poisoned patients, admitted into emergency medicine departments, is increasing every year. Under this circumstance, medical staff need to be prepared, in terms of proving poisoned patients, with adequate clinical treatment. According to the National Poison Control Center (CITUC), 57% of phone calls received at the center are from healthcare settings. This may be indicative of existing limitations related to the clinical management of poisoned patients. Thus, availability of basic up-to-date knowledge about clinical management of poisoning events would optimize treatment regimes, economic burden, and also improve patients' prognosis. A classic example of this is the use of gastric decontamination techniques, such as gastric lavage and activated charcoal, when patients expose to chemicals through the oral route. However, there are some limitations when employing these maneuvers. Specifically, the frame time elapsed between the exposure and the use of the technique (up to 60 minutes). After that time, there is a significant decrease of effectiveness, and the procedure itself turns, not only very traumatic to the patient, but also in unnecessary expenses to the healthcare setting. This inappropriate practice has become common in several health institutions, which are characterized by a lack of specialized medical staff with knowledge in clinical toxicology.

Pharmacology area: Toxicología (Toxicology)
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3. TRANSLATIONAL OPTIONS FOR THE TREATMENT OF DRUG-ABUSE DISORDERS: OPPORTUNITIES, SUCCESSES AND PITFALLS

INTRODUCTORY REMARKS.
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Synthesized molecules and drugs can be used for rapidly achieving a high pleasant and/or euphoric mood, bypassing the homeostatic pathways for pleasure and reward. That can be escalated by repeating and increasing the drug experience, abusing of the shortcutting pathway to pleasure, arriving to dependence and addiction to the substances providing that shortcut, changing the physiological substrate for perpetuating an addicting behaviour, impacting on the social and familiar environment for the compulsion of "experiencing a new trip". Much has been investigated about the physiological and neuronal framework sustaining that condition in mammals, including humans, arriving to the pivotal neurocircuitry of pleasure, identifying a role for dopamine, glutamate and opioid neuropeptides. The obtained knowledge is enormous, but that has not led to a consensus for treating a medical issue, which is not only menacing the individual, but is destroying the society. We have hereby sampled a group of international leaders and experts, who have devoted a research life to investigate the issue, both at basic and clinical levels, to discuss why preclinical results have not translated into the clinic. Thus, Gaetano Di Chiara (Cagliari), who first proposed a role for accumbens dopamine release as a common substrate for addictive substances will discuss about translational approaches for treating cocaine addiction. Rainer Spanagel (Mannheim), a leader on neuropeptides and addiction will discuss on the role of corticotropin-releasing pathways for sustaining drug abuse and addiction. Yedy Israel (Santiago), an international referent on alcohol and alcoholism will discuss on a neuroinflammatory-oxidative stress cycle sustaining chronic alcohol intake.

Pharmacology area: Neuropsicofarmacología (Neuropsycopharmacology)
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Acknowledgments: Acknowledgments. Supported by FONDECYT# 1180042, 1190562.

TRANSLATIONAL APPROACHES TO THE TREATMENT OF COCAINE ADDICTION.
Gaetano Di Chiara
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Cocaine addiction treatment is probably the most challenging and paradigmatic example of the difficulties in translating neurobiological knowledge into addiction therapy. Thus, to date, in spite of the advances in the knowledge of the neural basis of cocaine addiction, none, among the translationally-based treatments proposed, has been approved by national or international agencies. Clearly, the difficulties with cocaine addiction might be a case of a general difficulty in translating experimental results obtained in animals into human therapy. Although drug addiction is recognized as a brain disease, it is an exceedingly complex one and it is unclear to which extent, as in the case of schizophrenia and dementia, animal models are able to model the human condition. Therefore, critical analysis of the defaillances with cocaine can provide important clues as to the models to utilize. As far as the main lines of research,

simple approaches targeting DA transmission, the indirect site of cocaine action, with DA receptor agonists and antagonists or with low abuse liability DAT blockers have been discouraging, although the development of allosteric DAT ligands is currently a major line of research at NIDA. Acknowledgment of the critical role of neuroplastic changes at the level of the glutamatergic/dopaminergic cortico-ventral striatal circuit is the basis for pharmacological and physical treatments (DBS and TMS), aimed at reversing the neural changes induced by long lasting drug exposure. Preliminary, small scale observations in humans indicate that this might be the right way to go.

Pharmacology area: Neuropsicofarmacología (Neuropsycopharmacology)
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CORTICOTROPIN-RELEASING HORMONE RECEPTOR 1 (CRHR1) AND ADDICTION: WHY THE PRECLINICAL RESULTS DID NOT TRANSLATE INTO THE CLINIC?.

Spanagel, R.
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ALCOHOL-INDUCED NEUROINFLAMMATION-OXIDATIVE STRESS CYCLE: INFLAMMATORY AND ANTIOXIDANT DRUGS INHIBIT CHRONIC ALCOHOL INTAKE AND BLUNT RELAPSE BINGE-DRINKING.

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Brain of UChB rats chronically consuming over 10 g ethanol/kg/day show (i) a 200% increase in hippocampal oxidative stress, determined as the ratio of oxidized/reduced glutathione (GSSG/GSH), and (ii) marked neuroinflammation, shown as 60% increases in astrocyte glial-fibrillary acidic protein (GFAP) and increases in microglial density (Iba-1). Noteworthy, these changes remain long after ethanol intake is discontinued; in line with a proposed self-perpetuation (vicious cycle) of oxidative stress and neuroinflammation. Administration of a low dose of the antioxidant N-acetyl cysteine (40 mg/kg) reduces brain oxidative stress and neuroinflammation and inhibits chronic alcohol intake by 50-60%. The co-administration of N-acetyl cysteine with low doses of aspirin (ASA 15 mg/kg) inhibit alcohol intake by 75%, showing a significant synergism of both drugs. Following chronic ethanol intake, co-administration of N-acetyl cysteine plus the anti-inflammatory drug during a 2-week alcohol deprivation period block neuroinflammation and oxidative stress and inhibit by 85% the relapse binge-like drinking ("ADE") prompted by the subsequent ethanol re-access. As will be shown, studies tie neuroinflammation-oxidative-stress and hyper-glutamatergic conditions as the likely mechanisms that perpetuate chronic alcohol intake and promote intoxicating relapse drinking, and also indicate the pharmacological agents that block this condition. Studies suggest that anti-oxidant and anti-inflammatory agents may add significantly to interventions aimed at reducing alcohol-use disorders.

Pharmacology area: Neuropsicofarmacología (Neuropsycopharmacology)
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4. STRATEGIES FOR THE DESIGN AND DEVELOPMENT OF NEW DRUGS

DRUG DESIGN STRATEGIES BASED ON THE MOLECULE.

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Resistance to antibacterial agents is a growing problem of global public health, which affects the treatment of infectious diseases, reducing the effectiveness of available antibacterials, causing an increase in mortality and morbidity of patients. If innovative initiatives that seek to solve this problem are not generated, it is projected that, worldwide, in the year 2050 there will be around 10 million deaths caused by resistant microorganisms, with costs estimated at 100 trillion dollars. Unfortunately, the pharmaceutical industry has been leaving research in this area which is reflected in the approval of only ten drugs by the FDA, during the last ten years, none of them with new mechanisms of action. Due to this is why it is necessary to promote and encourage the development of a greater quantity of antibacterial compounds, different from those already known. For this reason, the discovery and development of new molecules is of vital importance. Thus, different approaches have been used for the discovery of active compounds, such as the High-throughput screening, the extraction of compounds from natural products or the design based on a biological target. But, what can be done when an isolated molecule (or series) is available and its pharmacological target is unknown? How to know which area of the molecule can be modified and what type of modification can be made to obtain compounds that are more active? How to know the relationship structure activity of this family of molecules? In this work, we show the experience of development of a new family of antibacterial drugs using different strategies of traditional medicinal chemistry. References W. H. Organization, Antimicrobial resistance: global report on surveillance. World Health Organization, 2014. J. Campanini-Salinas, J. Andrades-Lagos, J. Mella-Raipan, and D. Vasquez-Velasquez, "Novel classes of antibacterial drugs in clinical development, a hope in post antibiotic era.," Curr. Top. Med. Chem., 2018. J. Campanini-Salinas et al., "A New Kind of Quinonic-Antibiotic Useful Against Multidrug-Resistant S. aureus and E. faecium Infections," Molecules, vol. 23, no. 7, 2018.

Pharmacology area: Otros (Others)
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INTEGRATION OF STRUCTURAL BIOINFORMATICS AND CHEMICAL BIOLOGY FOR THE DISCOVERY OF NOVEL DRUGS.

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The discovery and design of drugs is increasingly incorporating structural bioinformatics techniques to model and analyze proteins of biological or therapeutic interest, perform large-scale virtual screening programs to identify lead compounds and evaluate molecular interactions through molecular dynamics simulations. These techniques are fast, cost effective and complementary to the existing experimental techniques of chemical biology. In this presentation, we will discuss some examples of strategies that combine different structural bioinformatics approaches with chemical biology tools to successfully discovery of novel drugs, focusing on the analysis of the inherent strengths and limitations on the use of structural bioinformatics tools, as well as complementary biological assays.

Pharmacology area: Farmacología molecular (Molecular Pharmacology)
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Acknowledgments: OpenEye Scientific Software, ChemAxon, CORFO 13CTI-21526-P1, USS-QYFA-P11, NHLPC (ECM-02).

DESIGN STRATEGIES FOR NEW CLASSES OF ANTIBACTERIAL DRUGS, A HOPE IN A POST-ANTIBIOTIC ERA.

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Bacterial resistance is a growing problem worldwide and is estimated that deaths by infectious diseases associated with resistant pathogens will generate 10 million deaths per year in 2050. This problem becomes more serious due to the low level of research and development of new drugs, which has fallen drastically in the last 40 years. For example, in the last decade of a total of 293 new drugs approved by FDA, only 9 corresponded to antimicrobial drugs and none constituted a new structural class. The majority of the molecules in the clinical phase II or III, coming from modifications of drugs in clinical use, this strategy make easier the bacterial susceptibility to generate resistance through the mechanisms expressed for their drug predecessors. Under this scenario, is urgent to generate the most novel strategies for the development of antibacterial compounds with new targets or mechanism of action, without structural relationship with the antibiotic drugs predecessors. Under this look, the present work addresses the development of the latest antibacterial drugs in clinical phases II and III, analyzing the design strategies by which these new molecules were obtained and the structure-activity relationship of these new families of antibiotics, in order to define the state of the vanguard antibacterial drugs in the post-antibiotic era.

Pharmacology area: Otros (Others)
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PRECLINICAL DEVELOPMENT OF A NEW CLASS OF ANTIBACTERIALS, A NATIONAL EXPERIENCE.

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The rapid emergence of resistant bacteria is occurring worldwide, endangering the efficacy of antibiotics, reducing the therapeutics arsenal for treatment of infectious diseases. According this, it is a urgent need the development of new antibacterial drugs. In this study, we develop a new class of compounds obtained with a simple synthesis in two single-step. These compounds were screened for in vitro antibacterial activity against ATCC strains and clinical isolates, using the broth microdilution method. In addition, a series of trials were conducted to gather information about the effectiveness and safety of derivatives, such as how; toxicity in mammalian cells and galleria mellonella, assays of potential for induction of mutations, among others. The compounds exhibited MICs of 1-32 µg/ml against Gram-positive ATCC strains. The MIC50 for compound 7 against the MRSA isolates tested were 2 mg/L, compound 16 exhibit 2 mg/L. For the VREF isolates the compound 7 showed MIC50 and MIC90 values of 2 and 4 mg/L, and the compounds 16 obtain values of 4 and 4 mg/L. The compounds were bactericidal in all isolates tested. Both compounds were bactericidal in all clinical isolates tested. Neither compound affected cell viability in any of the mammalian cell lines and Galleria mellonella larvaes, at any of the concentrations tested. These in vitro data indicate that compounds 7 and 16 can advance in assay on murine models of infection and determination of pharmacokinetics parameters.

Pharmacology area: Otros (Others)
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5. ALZHEIMER'S DISEASE: NEW TARGETS AND DRUGS.

INTRODUCTION.

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Alzheimer's disease (AD) is becoming a devastating health, social, and economical problem. Burden to society will increase

as population ages. The search of disease-modifying drugs has focused on over 30 distinct targets, most of them linked to amyloid beta (Aβ) aggregation or hyperphosphorylated tau. Anti-oxidant, anti-inflammatories, neurotransmitter receptors, or growth factors have been explored as targets to develop a medicine to delay disease progression. Ligands for those targets have been explored in cell and murine models of AD. Although many of them have shown efficacy in this preclinical set-up, they have failed in dozens of clinical trials performed during the last 20 years in AD patients. It is interesting that the Alzheimer's Foundation for Drug Discovery (AFDD) is not supporting any more clinical trials with compounds targeted to Aβ or tau. So, new targets and ideas are urgently needed. In this symposium on new targets and drugs for AD, four scientists from the Institute "Teófilo Hernando for Drug Discovery", at the Universidad Autónoma de Madrid, Spain, will present their work on new approaches to the search of new targets beyond conventional (Manuela García López), multitarget compounds (Rafael León Martínez), and Phosphatase PP2 (Raquel López Arribas). A last communication focus on altered neurotransmission processes in AD (Luis Gandía Juan). It is expected that only with these and other new strategies, we can find out the way to a medicine capable of slowing down the natural course of the disease; and what it is even more challenging, if administered at presymptomatic AD stages, in patients at risk diagnosed with biomarkers, this medicine be capable of delaying disease.

NON CONVENTIONAL TARGETS FOR THE TREATMENT OF ALZHEIMER´S DISEASE.

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Alzheimer´s disease (AD) is the most common form of dementia with still no effective treatment. From a histopathological point of view, AD is characterized by extracellular aggregates of betaamyloid and, intracellular neurofibrillary tangles composed of hyperphosphorylated tau protein. During the last twenty years great effort has been made to develop therapeutic strategies, mostly based on beta-amyloid pathology, but without success. On the other hand, AD shares with other neurodegenerative diseases pathological mechanisms like oxidative stress, subchronic inflammation, mitochondrial dysfunction and proteinopathy. Given this scenario, our group seeks to identify new therapeutic targets focused on the regulation of oxidative stress and neuroinflammation, processes that precede the accumulation of aberrant proteins and cognitive impairment. We have therefore centered out attention on two targets: (1) the NADPH oxidases enzymes (NOXs), which are the enzymes responsible for the production of reactive oxygen species such as superoxide and hydrogen peroxide, and more specifically, in its NOX4 isoform and, (2) in the transcription factor NRF2 (Nuclear factor (erythroid-derived 2) -like 2), master regulator of the antioxidant response, which also regulates the expression of genes that participate in the anti-inflammatory response and autophagic processes. To validate NOX4 as a possible target, we have used transgenic mice that do not express this enzyme and a model of taupathy by injecting i.c.v. adeno viruses containing the human tau protein mutated in P2301L, under the promoter synapsin. In this model we have been able to determine that animals that do not express NOX4 have less oxidative stress and less neuroinflammation which results in an improvement in the cognitive tests. For our second target, we are looking for compounds that inhibit the interaction Keap-1 (NRF2 repressor protein) and NRF2. To do this, we have performed an in-silico screening of large libraries using docking and molecular dynamics, as well as the synthesis of new compounds. In the latter case, we want to obtain multitarget compounds with complementary activities to the induction of NRF2, with the aim of interfering on different nodes of Alzheimer's pathophysiology. In this project we are following a sequential screening protocol based on studying the Nrf2 induction, antioxidant, anti-neuroinflammatory and neuroprotective properties of the compounds. As a last step, those compounds with a more favorable toxicological, pharmacokinetic and pharmacodynamic profile will be evaluated in in vivo models of AD.

Pharmacology area: Otros (Others)

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ALTERATIONS IN NEUROTRANSMISSION RELATED TO THE PROGRESSION OF ALZHEIMER'S DISEASE.

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Alzheimer´s disease (AD) is the most common form of dementia, being aging the main risk factor for the development this disease. The alteration of several neurotransmitter systems has been reported in AD, which could be correlated with changes in the synthesis, storage or release of these neurotransmitters. In this study, we tested how aging affects ionic currents, cell excitability and last steps of exocytosis under physiological and pathological conditions. For this purpose, we used a triple transgenic model of AD (3xTg-AD) that contains mutations in the gene encoding the amyloid precursor protein (APPSwe), presenilin-1 (PS1M146V) and tauO301L, which mimics the development of the disease on Alzheimer's patients, and B6129SF mice (wild type). By using amperometric techniques, we have found significant changes in the exocytosis of catecholamines occurring in mice of 6 and more than 12 months of age, where the pathology is already established, when compared with prepathological mice (2 months), in particular, an increase of the number of amperometric spikes, although the quantal catecholamine content on individual spikes is lower. Kinetic analysis of secretory spikes shows that as the disease progresses

amperometric spikes are faster in triggering and shorter in duration. Patch-clamp technique was also used to measure the different ionic currents involved in the physiological release of catecholamines. We observed a decrease in sodium currents and an increase in potassium currents in 3xTg-AD compared with controls. Nicotinic currents exhibited a similar pattern throughout the age in both control and transgenic mice. Finally, we found an increase in calcium currents in 3xTg-AD mouse with age that was not observed in wild type mice. These findings suggest that throughout the development of 3xTg-AD mice and as Alzheimer's disease is established there is a change in chromaffin cell excitability, which causes neurotransmission to accelerate. These alterations could have an impact on the response that the organism offers in a stressful situation.

Pharmacology area: Neuropsicofarmacología (Neuropsycopharmacology)
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MULTITARGET DRUG DESIGN FOR THE TREATMENT OF ALZHEIMER'S DISEASE.

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Neurodegenerative diseases (NDDs) are currently considered a worldwide pandemia with a prevalence of about 47 million people. It is estimated that, in 2050, two billion people will be over 60 years old, thus the number of people affected is expected to triple. Therefore, the search for effective drugs capable of controlling neuronal cell death is one of the great challenges of this century.
AD is associated with several neuronal abnormalities in energy metabolism such mitochondrial dysfunction, a decline in glucose uptake, dysfunction in Ca^{2+} homeostasis. It has been shown that oxidative damage occurs before the onset of significant Aβ plaque formation. For instance, the free radical theory of ageing implies progressive ROS cell damage with age, leading to enhanced mitochondrial DNA mutations, futile mitochondrial Ca^{2+} cycling with excess ATP consumption and ensuing mitochondrial dysfunction. On the other hand, it is now increasingly recognized that inflammation also strongly contributes to extensive oxidative stress found in AD brains. We therefore hypothesize that mitochondrial dysfunction could be the potential link between neuroinflammation and neurodegeneration. Another common characteristic is the interconnection between these pathways that causes feedback pathological loops that accelerates the advance of the disease. Therefore, their therapeutic approach must be directed to several pathological nodes, as the design of multitarget drugs capable of stopping different pathological pathways at the same time.
In this sense, the intrinsic cellular defense pathway, the Nrf2-ARE pathway, has been proposed as a therapeutic alternative for the development of effective drugs. Therefore, we are developing new multitarget compounds that combine the Nrf2 induction activity with other specific targets capable of reducing oxidative stress, neuroinflammation and the formation of protein aggregates, besides activating neuronal survival pathways that could be of potential therapeutic relevance to afford neuroprotection in Alzheimer's disease.

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SYNTHESIS AND PHARMACOLOGICAL EVALUATION OF NEW COMPOUNDS DIRECTED TO PP2A, A PROMISING THERAPEUTIC TARGET FOR ALZHEIMER'S DISEASE.

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Introduction: Alzheimer's disease (AD) is the most common cause of dementia. Nowadays, there is no cure for AD or a way to stop or slow its progression. Main histopathological hallmarks of AD are senile plaques, and neurofibrillary tangles, generated by aggregation of the microtubule associated protein tau. Over the past two decades, most scientific efforts in the development of new drugs to treat AD have focused on inhibiting the degradation of acetylcholine and avoiding amyloidogenesis. However, less interest has aroused the therapeutic approach consisting in preventing neurofibrillary death by inhibiting the abnormal hyperphosphorylation of tau. In this sense, pharmacological strategies have been almost completely oriented to inhibit the activity of tau kinase enzymes, with discouraging results. Our research group proposes to address the aberrant phosphorylation of tau by restoring the activity of its main phosphatase enzyme, protein phosphatase 2A (PP2A), which is decreased in the brains of patients with AD, mainly due to the increase in the expression of the endogenous inhibitors I1PP2A and I2PP2A/SET . Hypothesis: The study of the structure-activity relationship of okadaic acid (OA), a toxin with selective inhibitory activity of PP2A, has allowed us to design and synthesize new analogue molecules of OA that lack such inhibitory capacity. In this sense, our starting hypothesis states that these compounds, due to their binding to the catalytic subunit of PP2A, would be able to compete with the endogenous inhibitors of PP2A, and thus, to restore the compromised phosphatase activity in AD. Material and results: Our molecules are able to reduce OA-induced neurotoxicity and some of them also present a good profile in a model of oxidative stress in SH-SY5Y cells and in a model of excitotoxicity in cortical neurons. The new compounds maintained serine/threonine phosphatase activity, depressed by the action of two PP2A inhibitors: OA and cytostatin. Molecular docking studies indicated that the compounds studied are capable of binding PP2A in a similar manner to OA, but does not interact with the catalytic site, confirming our initial hypothesis. Conclusions: Our compounds have a

potential indication for the treatment of neurodegenerative diseases based on the maintenance of PP2A activity, which avoids tau hyperphosphorylation.

6. MEDICINAL CHEMISTRY: RATIONAL DESIGN AND STRUCTURE ACTIVITY RELATIONSHIP, A SYNTHETIC APPROACH OF NEW BIOLOGICALLY ACTIVE SUBSTANCES.

SYNTHESIS OF NEW INDOL DERIVATIVES AND THEIR ACTIVITY ON CHOLINERGIC AND SEROTONERGIC SYSTEMS AND IN BETA-AMYLOID DEPOSITION. A MULTIFUNCTIONAL APPROACH TO ALZHEIMER'S DISEASE.

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Alzheimer's disease is the most diffuse form of senile dementia, and is among the most devastating brain disorder an individual can face. It involves progressive and irreversible decline in cognitive functions including memory, judgment, decision-making, orientation to physical surroundings and language. Despite substantial efforts in drug development and an increased understanding of the underlying pathology of Alzheimer's disease, no effective treatment has yet been achieved. The main goal of this research is to contribute to the knowledge of the medicinal chemistry in the neurochemistry field, through the design of novel ligands functioning as multitarget agents in Alzheimer' disease (AD). In this proposal, we describe the synthesis and in vitro biological evaluation of novel indole derivative as single chemical entities to simultaneously modulate multiple targets which comprises i) binding-affinity and potential agonist properties of serotonin 5-HT4R ii) acetylcholinesterase inhibition, iii) serotonin transport re-uptake inhibition and iv) inhibition of β-amyloid deposition.

Pharmacology area: Otros (Others)
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DESIGN OF NEW BENZIMIDAZOLES WITH BETA-3 ADRENERGIC AFFINITY.

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The human receptor β3-adrenergic has been the target of recent studies due to its potential to modulate various physiological aspects of the organism involved in numerous pathologies such as diabetes, hypertension, overactive bladder, heart problems, depression, and cancer, among others. In this context, our efforts are focused on the design, synthesis and biological evaluation of new heterocyclic compounds capable of binding to the human receptor β3-adrenergic. Since the beta-3 receptor is not crystallized, we have performed extensive studies based on ligands (3D-QSAR, CoMFA, and CoMSIA), which have allowed us to generate a pharmacophoric model that we use as a basis for the rational design of our compounds. The routes of synthesis of the heterocycles proposed by our group follow a similar route to that used to obtain Mirabegrón, the only drug currently available that acts on the beta-3 receptor indicated in the treatment of overactive bladder.

Pharmacology area: Otros (Others)
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NEONICOTINOIDS, SEARCHING FOR NICOTINIC RECEPTOR LIGANDS OF ALPHA4BETA2 NACHR SUBTYPE AND ITS APPLICATION AS NEW ANTI-ADDICTIVE SUBSTANCES.

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Addiction is a chronic and compulsive drug seeking, producing detrimental consequences, and long-lasting changes in the brain. It is considered a brain disorder and a mental illness. Addiction is the most severe form of substance use disorders, caused by repeated misuse of a substance. Neuronal Nicotinic Acetylcholine Receptors (nAChR) are involved in nicotine addiction and emerging evidence suggests that nAChR could be acts as pharmacological target to be considered in alcohol abuse. Two of the most common addictive substances used and accepted by the society. Nicotinic ligands have been designed, synthesized and tested on nicotinic receptor for decades, but the focus of the design has been full and partials agonists with good therapeutics results on nicotine addiction (i.e. cytisine and varenicline). However, there are little evidences indicating that nicotinic antagonist could expert anti-addictive effects over nicotine addiction and alcoholism. In our lab, we had designed and synthesized simple nicotinic analogues with agonist or antagonist properties on alpha4beta2 nAChR subtype and using zebrafish as a behavioural model we have identified a new nicotinic antagonist, named UFR2709 that revert the effect of nicotine using a homologous CPP for zebrafish. Additionally, we tested UFR2709 on Wistar-derived University of Chile alcohol-preferring UChB rats a well-known model to evaluate ethanol consumption. Our results show that UFR2709 are able to decrease the ethanol intake using a two-bottle choice paradigm assay. UFR2709, an alpha4beta2 nAChR antagonist shows an anti-addictive effect on nicotine addiction and ethanol consumption and open a new way for drug design and the treatment of nicotine and ethanol addictions.

Pharmacology area: Otros (Others)
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7. ANTIMICROBIAL ACTIVITY OF HERBAL EXTRACTS AGAINST CLINICALLY RELEVANT PATHOGENS

NATURAL EXTRACTS AND THEIR ROLE IN THE SEARCH FOR NEW THERAPEUTIC ALTERNATIVES TO TREAT INFECTIONS.

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In May 2015 the World Health Assembly adopted a global action plan against antibiotic resistance, since deaths related to multidrug resistant bacteria have increased in alarming speed in the last decades. One of the goals of this plan is to support research and development of new antimicrobial drugs, since classic or conventional antibiotic drugs have been proposed to become obsolete in a few decades from now. This scenario is not exclusive for bacterial infections, since the lack of new clinically effective drugs is also a problem for fungal and parasite infections. In this context, natural products have emerged as a valid alternative for the discovery of new antimicrobial agents with new mechanisms of action and in some cases even in absence of current resistance mechanisms. Plants are affected by several microorganisms, so they count with high content of secondary metabolites with antibacterial, antifungal and antiparasitic effects such as flavonoids, tanins, terpenoids and alkaloids. However, herbal extracts can vary among the same species due to different extraction methods, different geographical location and even season collection. So, it is important to count with adequate characterization methods in order to achieve reproducible results and standardized extracts respect to their chemical composition and the proportion of active principles that can be related to their antimicrobial activity.

Pharmacology area: Farmacología de Productos Naturales (Natural Products Pharmacology)
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REVEALING ANTIMICROBIAL ACTIVITY OF NATURAL PRODUCTS USING CHEMICAL SUBTRACTION AS NEW STRATEGY TO PREPARE KNOCK-OUT AND KNOCK-IN EXTRACTS.

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Chilean plants have biased and incomplete chemical-pharmacological studies. The main reason for that has been the low availability of enough quantities to make biological tests and structural elucidation. Moreover, the isolation of specific constituents often omits the residual complexity existing in plants, in which it is not uncommon to find highly active compounds. In this work is presented the application of a new strategy to investigate antimicrobial activity of medicinal and food plants. This tool unifies different pharmacological/phytochemical approaches using the liquid-liquid methodology named Centrifugal Partition Chromatography (CPC) to obtain DESIGNER extracts. These extracts could be "knock-out" (selective removal of one or a group of compounds) or “knock-in” (selective addition of one or a group of compounds). In the first example, we prepare "knock-out" from propolis and Buddleja globosa (Matico) and assess their antimicrobial activity. Propolis without caffeic acid phenyl ester (CAPE) shown similar antimicrobial activity compared to raw extract, suggesting that other compounds present in its residual complexity are responsible for such activity. On the other hand, Matico "knock-out" (selective removal of verbascoside), displayed minimal antimicrobial activity. In this last example, the re-incorporation of verbascoside recovered the biological activity. Finally, we perform a double knock-out in Peumus boldus extract, removing cytotoxic compounds (e.g. ascaridole) and isoquinoline alkaloids (e.g. boldine). This Boldo DESIGNER extract reduce significantly cell injury in H. pylori-infected AGS cells without the cell toxicity observed in the raw extracts. To confirm the protective properties of this extract in vivo, we used a continuous liquid-liquid separation by True Moving Bed system (TMB-500). Hence, a dose of 100 mg/kg/day of Boldo DESIGNER extract was able to prevent H. pylori SS1 infection in Mongolian gerbils.

Pharmacology area: Farmacología de Productos Naturales (Natural Products Pharmacology)
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EFFECT OF LAVANDULA DENTATA ESSENTIAL OIL AGAINST BIOFILMS FORMATION OF RESISTANT CANDIDA ALBICANS

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There is an increase in fungal infections owing to the appearance of resistant fungi to different drugs. Candida albicans is part of the resident microbiota of the oral cavity but is also the most frequent fungal pathogen, whose biofilms formation represents one of the main resistance mechanisms. In the oral cavity, Candida albicans biofilms are extremely resistant to antifungals and together with the absence of new, effective and safe antifungals, the search for pharmacological alternatives is warranted. It has been described that essential oils from Lavandula dentata, and endemic plant in Chile, possess antimicrobial and antifungal activity against several microorganisms including Candida albicans. We described the antifungal and antibiofilm effect of Lavandula dentata essential oil on the inhibition of Candida albicans Fluconazole-resistant strain (ATCC 10231), to adhere to abiotic surfaces and to form biofilms. After the chemical characterized of the essential oil by Gas Chromatography and the determination of minimal

inhibitory concentration (MIC), we evaluated the effect of this essential oil on the adhesion ability through crystal violet assay and the antibiofilm effect through the viability of biofilm formation and scratch assay. The MIC was able to inhibit adhesion and biofilm formation in an abiotic surface for the resistant strains assayed (ATCC 10231). In conclusion, this study demonstrates that this essential oil from Lavandula dentata could be a promising strategy against biofilms from resistant Candida albicans strains. Since phytodrugs present many active compounds, who makes then difficult to generate resistance, they can be used in conjunction with conventional antifungal, sensitizing the pathogens and decreasing its adhesion and later formation of biofilms.

Pharmacology area: Farmacología de Productos Naturales (Natural Products Pharmacology)
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PARASITES AND PLANTS: ELUCIDATING THE ANTIPARASITIC ACTIVITY OF CICHORIUM INTYBUS (CHICORY).

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Parasitic helminths and protozoa affect billions of people worldwide and are amongst the most prevalent infections in livestock. Due to increasing parasite drug resistance towards the limited therapeutic arsenal available, novel antiparasitics are urgently needed. Plants with reported antiparasitic activity have been traditionally used and may provide new lead compounds. One antiparasitic plant increasingly investigated is chicory (Cichorium intybus; Asteraceae), a perennial herb distributed worldwide and commonly cultivated as crop for human and livestock consumption. Chicory has attracted research interest for its effects against parasitic nematodes in livestock, which have been linked with its content of sesquiterpene lactones (SLs). Previous in vivo studies have confirmed that chicory-fed animals have a reduced parasite burden, but detailed identification of responsible compounds has not been, until recently, thoroughly explored. By integrating parasitological studies and metabolomic analyses, we have investigated the anthelmintic activity and phytochemical profile of SL-extracts from chicory material sampled in different geographical regions. The in vitro activity of chicory SL-extracts was first evaluated using the free-living nematode Caenorhabditis elegans model and further confirmed in the parasitic pig nematode Ascaris suum, which is closely related with the human parasite A. lumbricoides. Marked differences in anthelmintic potency were observed between SL-extracts from different chicory material. Bioactivity-based molecular networking analyses suggest that some but not all SLs are linked with the anthelmintic activity of chicory. In addition, we have explored the antiprotozoal activity of chicory against Trypanosoma cruzi, the etiological agent of Chagas disease. Chicory SL-extracts induced potent concentration-dependent trypanocidal activity against T. cruzi trypomastigotes at concentrations that are not toxic to mammalian cells. Isolation and testing of individual chicory SLs are undergoing to evaluate their antiparasitic mechanisms.

Pharmacology area: Farmacología de Productos Naturales (Natural Products Pharmacology)
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8. BIOTECHONOLOGY ASPECTS OF ASPARAGINASE CLINICAL AND INDUSTRIAL DEVELOPMENT

PRODUCTION OF EXTRACELLULAR L-ASPARAGINASE: FROM BIOPROSPECTING TO THE ENGINEERING OF AN ANTILEUKEMIC BIOPHARMACEUTICAL.

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In 2013 the production of L-Asparaginase, a biopharmaceutical widely used in the treatment of acute lymphoblastic leukemia (ALL), was suspended by the foreign manufacturer who supplied the drug (Elspar®) to Brazil since the 1980s. The interruption of this supply led to life threatening delays in treatment which forced the Brazilian Ministry of Health to find emergency alternatives, including importation of a more expensive alternative (Aginasa®). Later, in 2017 and following an international public tender, MS started importing the medicine Leuginase®, from China. Such frequent changes in the supply of L-Asparaginase has provoked intense and controversial debate in Brazil regarding the quality of the imported medicine. This motivated our group to develop a technology platform for the production of L-asparaginase with more advantageous characteristics than the imported formulations. Brazil is considered a weak player on the World stage in biopharmaceutical discovery, development and production of biopharmaceuticals, and the present project proposes a union between several scientific and technological competences for the development of industrially viable L-Asparaginase production process. This new proposal is a continuation of the FAPESP Thematic Project (2013 / 08617-7) that has provided promising results, since it enabled the development of new recombinant strains of bacteria and yeast with the capacity to produce L-asparaginases with longer half-life, greater stability, lower toxicity, and lower side effects in comparison to the biopharmaceuticals currently in clinical use not just in Brazil, but worldwide. As a continuation of the previously initiated studies, this thematic project proposes the development of processes for the production, under GLP and GMP of 4 antileukemic biopharmaceuticals with different characteristics and with important potential to be produced nationally and even for export, including: 1) Escherichia coli BL21 (DE3) – a recombinant wild-type E. coli ASNase, overexpressed in epichomal vector pet28a with a resistance marker for kanamycin; 2) Escherichia coli BL21 (DE3) – a recombinant wild-type ASNase from Erwinia chrysanthemi ASNase, overexpressed in epimasomal vector pet28a with a resistance marker for kanamycin; 3) Escherichia coli BL21 (DE3) – a recombinant E. coli ASNase resistant to human serum

proteases - overexpressed in epichomal-vector pet28a with a resistance marker for kanamycin; 4) Recombinant Pichia pastoris – a recombinant wild-type E. chrysanthemi crisantaspase with humanized glycosylation (expressed in pJAG-s1 in the Superman5-Glycoswitch yeast from Biogrammatics™). To improve aspects of stability, bioavailability, toxicity and hyperallergenicity, which are problems observed with current formulations, nanotechnological approaches such as pegylation and encapsulation in polymer vesicles will be used. The project aims to develop a production process from optimization of microbial cultures to purification, pegylation and final formulation (lyophilized product), in sufficient quantity to carry out subsequent preclinical studies. The entire study will be accompanied by technical and economic evaluations (Quality by Design).

Acknowledgments: FAPESP (Proc. 2012/08617-7); and CNPq (404791/2018-2; 302431/20187; 3699145321548998; and 7466243552658485).

DEVELOPMENT OF BIOTECHNOLOGICAL PROCESS FOR THE PRODUCTION OF THE ANTILEUKEMIC BIOPHARMACEUTICAL L-ASPARAGINASE (ASNASE) USING GENETICALLY MODIFIED MICROORGANISMS.

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There is a strong global tendency to find alternative ways to produce pharmaceutical active principles from biotechnology process. In this scenario, Latin American countries show a small expression in both research and production. Worsening the situation, international suppliers of biopharmaceuticals to this region are losing interest in the market and discontinuing production, especially those related to onco-hematologic treatment. In this context, the union of different scientific and technological skills have joined to achieve a viable industrial process to biotechnologically produce L-Asparaginase, a biopharmaceutical broadly used in the treatment of leukemia. Two major research fronts are being studied with the objective of finding a promising antileukemic biopharmaceutical: the optimization of endogenous and heterologous production processes of the enzyme, with bioprospecting groups of fungi of the most varied biomes; and the rational engineering of proteins that will utilize as scaffold the S. cerevisiae and E. coli L-Aparaginases for comparative studies with the bacterial isoforms currently employed in therapeutics. To improve aspects of stability, bioavailability, toxicity and allergenicity, problems observed with bacterial formulations, several nanotechnological approaches are being used, such as pegylation and encapsulation in polymeric vesicles, and the project aims to generate a biopharmaceutical to be produced industrially. Fungi from different biomes, such as cerrado, caatinga, marine environment, and Antarctica, have been isolated and several of them have been evaluated for the production of the enzyme in shaker and in 3- or 7-Liter bioreactors, and by solid state cultivation. Biochemical and kinetic characteristics are being determined for all isolated L-asparaginases. The studies aim at obtaining recombinant E. coli and Pichia pastoris to produce of L-asparaginase with potentially improved characteristics (longer half-life, higher stability, lower toxicity and lower side effects) in comparison those that are in clinical use in the World. A recombinant E. coli with the capacity to produce L-asparaginase resistant to two plasma proteases was obtained and the toxicity studies shows important potential for starting preclinical studies. A recombinant P. pastoris strain with the ability to produce L-asparaginase with humanized glycosylation was also obtained, with great potential to reduce to immunogenic reactions and, therefore, safer for patients. Pegylation and nanoencapsulation studies of the novel L-asparaginases are being conducted and the results have shown that site-directed pegylation has the potential to generate a biopharmaceutical with better characteristics than the pegylated form on the market. In addition, polymer encapsulation studies have been conducted with promising results, especially since it is a new alternative in the nanobiotechnology process. The project is underway with the development of processes of production, in GLP (good laboratory practice) and GMP (good manufacturing practice), of new L-asparaginases with higher characteristics and with important potential to be produced nationally and even for exportation.

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ASPARAGINASE ENGINEERING IN THE OBTAINMENT OF BIOBETTERS OF THIS ANTITUMOR ENZYME.

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Asparaginase (ASNase), an enzyme biotechnologically produced in bacteria, is one of the most important compounds in the polychemotherapy to treat acute lymphoblastic leukemia (ALL) in children. There are only three options available as medicine: native enzyme from Erwinia chrysanthemi (ErA) or extracted from Escherichia coli (EcA) and formulated as native or PEGylated (PEG-EcA). However, these options yet present some problems in patients, such as to elicit hypersensitivity and allergenic reactions, neurotoxicity, and hyperammonemia. Aiming to avoid some of these problems, our research group has developed several different mutant proteoforms, expressed in bacteria and yeast, in periplasmic or secreted to extracellular space; with improvement in specific activity, kinetic parameters, and stability; different oligomerization states, glycosylated or not, through engineering of genes from E. coli and E. chrysanthemi. We obtained mutants from E. coli ASNase more resistant to human proteases and less immunogenic. In relation to E. chrysanthemi enzyme, our mutants present higher asparaginase activity than

the native form, with improved kcat. In addition, we obtained strains of Pichia pastoris that express glycosylated ASNases from bacteria. Our results suggest several biobetters options developed in this study.

Pharmacology area: Tecnología farmacológica (Pharmaceutical Technology)
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PRODUCTION OF NOVEL GLYCOSYLATED L-ASPARAGINASE AS AN ALTERNATIVE AGAINST ACUTE LYMPHOBLASTIC LEUKEMIA.
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L-asparaginase (L-ASNase) is an important bacterial enzyme used as biopharmaceutical to treat acute lymphoblastic leukemia (ALL). Its use as medicine has important side effects such as pancreatitis, abnormalities in coagulation, hepatosplenomegaly, immunogenicity, among others. It has been counteracted by PEGylation; however, immunogenicity has been observed in PEG. Here we explore the production of recombinant L-ASNase from D. chrysamthemi glycosylated like-mammals in a Glycoswitch® Pichia pastoris strain as an alternative to PEGylation. In our results, the recombinant Erwinase occurred in three extracellular, glycosylated and biologically active variants; two of them tetramerics (Erw240) and (Erw160) with specific activity of 15.71 and 302.02 U mg-1 respectively; and one new monomeric version (Erw40) with 48.45 U mg-1. The lightweight tetramer and the monomer showed catalytic efficiency of 7.7 x 105 and 1.05 x 106 respectively. Mass spectrometry analysis of the more active tetrameric and monomeric versions showed mainly an oligosaccharide GlcNAc2Man7, bound to Asn170, which is part of a predicted immunogenic T-cell epitope. ELISA assay in vitro showed a significant reduction of antibody recognition in the Erw160, suggesting the oligosaccharide bound to L-ASNasa had a cloaking effect against antibodies. The new L-ASNase versions reported here could provide an alternative for the treatment of ALL.

Pharmacology area: Tecnología farmacológica (Pharmaceutical Technology)
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MINISYMPOSIA

1. CARDIOVASCULAR AGING

PREVENTING PREMATURE ENDOTHELIAL CELL SENESCENCE: THE ROLE OF ANGIOTENSIN-(1-7).
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Vascular aging is a complex multifaceted process displaying functional and structural alterations that ultimately favour vascular disease and atherosclerosis. Endothelial cell senescence, which may be induced by a wide variety of extracellular stressors, is one of the major mechanisms contributing to vascular aging. The senescent endothelial cell acquire a phenotype characterized by growth arrest and the acquisition of a senescence-associated secretory phenotype (SASP), that promotes the release of pro-inflammatory mediators and the onset of sterile age-related inflammation. In this context, the identification of pharmacological tools to interfere with endothelial senescence may help retarding vascular aging and its complications. Angiotensin (Ang)-(1-7) is a heptapeptide belonging to the so-called protective arm of the renin-angiontesin system (RAS). Ang-(1-7) is a ligand for the G-protein-coupled receptor Mas. In the vascular system, Ang-(1-7) has been acknowledged as a physiological antagonist for angiotensin II (Ang II), since it displays vasorelaxant, anti-proliferative and anti-inflammatory actions, among other. Here, we tested the capacity of Ang-(1-7) to act as an anti-senescence molecule. In human cultured endothelial cells, Ang-(1-7) was capable to attenuate the pro-senescence actions driven by Ang II in terms of DNA damage, senescence-associated beta-galactosidase (SA-beta-gal) activity and SASP-related cytokine release. Importantly, Ang-(1-7) also attenuated the endothelial cell senescence induced by non-RAS stressors, such as the pro-inflammatory cytokine interleukin (IL)-1beta. These protective actions of Ang-(1-7) were mediated by Mas receptors since they were blunted by the Mas antagonist drug A779. Furthermore, we demonstrated that Ang-(1-7) exerted its anti-senescence actions by activating two cytoprotective systems, i.e., the Nrf2/heme-oxygenase axis and the anti-ageing protein klotho. Overall, the Ang-(1-7)/Mas receptor axis may a valuable pharmacological target to attenuate endothelial senescence and to delay vascular aging induced by a variety of stressors.

Pharmacology area: Farmacología cardiovascular (Cardiovascular Pharmacology)
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ROLE OF NLRP3 INFLAMMASOME IN VASCULAR DAMAGE INDUCED BY ADIPOKINES.
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We have demonstrated that these adipokines promotes vascular inflammation and endothelial dysfunction. Moreover, our data suggest that vascular deleterious effects evoked by visfatin/eNampt or sDPP4 may involve the activation of the NLRP3 (nucleotide-binding domain, leucine-rich-containing family, pyring domain-cotainin-3) inflammasome. Indeed,

evidence from our laboratory demonstrates the activation of NLRP3 inflammasome by visfatin/eNampt, while the adipokine-evoked endothelial dysfunction is prevented by inhibiting its enzymatic activity with FK866, as well as by the antagonism of toll-like receptors-4 (TLR4) with CLI-095, the interference of NLRP3 inflammasome assembly with MCC950, or the IL-1 receptor blockade with anakinra. Therefore, we propose that visfatin/eNampt induces vascular damage by a TLR4-mediated pathway, leading to NLRP3-inflammasome activation and the paracrine production of IL-1beta. On the other hand, we have shown that sDPP4 induces vascular alterations by activating proteinase-activated receptors-2 (PAR-2) and upregulating thromboxane-A2 (TXA2) release. Moreover, the endothelial dysfunction evoked by sDPP4 is also dependent on its enzymatic activity, being attenuated by its inhibitors K579 and linagliptin, as well as by the specific PAR-2 antagonist GB83 and the TXA2 receptor blocker SQ-29,548. Interestingly, this pathway also leads to NLRP3 inflammasome activation, while the sDPP4-evoked endothelial damage is reduced by interfering NLRP3 inflammasome with MCC950. We conclude that vascular NLRP3 inflammasome activation can be a common pathway for different pro-inflammatory adipokines. Indeed, targeting NLRP3 inflammasome and some receptors linked to this pathway (TLR4, PAR-2, or IL-1) may represent therapeutic strategies to treat and/or prevent obesity-related vascular dysfunction.

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CARDIAC FIBROBLAST ROLE ON INFLAMMATORY PROCESS: INTERACTION WITH IMMUNE CELLS.

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The abundance and strategic location of cardiac fibroblasts and also macrophages in cardiac tissue damage, suggest the possibility of a highly coordinated interaction between both cell types, in order to orchestrate the different stages of cardiac remodeling. In particular macrophage is able to adapt their phenotype and activity according to the cytokine milieu present in the local cardiac environment. This phenomenon, known as macrophage polarization, contributes to the accumulation of pro-inflammatory M1 macrophages during the onset of cardiac remodelling, while also explaining the high levels of anti-inflammatory/profibrotic M2 macrophages found in the later stages of cardiac repair. While the effects of macrophages on cardiac fibroblast activity have been extensively studied, the ability of cardiac fibroblasts to modulate macrophage behavior is less understood. LPS, and Heparan sulfate as pro-inflammatory stimulus, triggers on cardiac fibroblast ICAM-1 and VCAM-1 expression levels, which allow spleen mononuclear cells and neutrophils adhesion. LPS triggers high TNF-α/IL-10 ratio, whereas, TGF-β1 a profibrotic stimulus triggers an increase on ICAM-1 and VCAM-1 expression levels, but low TNF-α/IL-10 ratio. Consequently, cardiac fibroblast under LPS-treatment promote monocytes-macrophages M1 polarization. By contrast, cardiac fibroblast under TGF-β1 promote monocytes-macrophages M2 polarization. Our results demonstrate that cardiac fibroblasts interact with immune cells and contribute to monocyte recruitment and induce their differentiation to M1 or M2 macrophages.

2. NOVEL MOLECULAR PATHWAYS FOR SCHIZOPHRENIA

DYSREGULATION OF THE AMYLOID PRECURSOR PROTEIN AND IRON IN SCHIZOPHRENIA.

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Schizophrenia (Sz) is a debilitating mental illness that disrupts the functioning of the mind. Impairments in certain cognitive functions are core features of Sz, which are not addressed for existing drug targets and remain a crucial unmet therapeutic need. Our hypothesis is that schizophrenia is a complex disease resulting from a loss-of-function of key pathways that govern neurodevelopment, neurotransmission and synaptic connectivity. The Amyloid Precursor Protein (APP), which we have extensively investigated in relation to Alzheimer's disease, is a key regulator of brain structure and function. Our data indicate that iron is elevated in autopsy orbitofrontal cortex from individuals with Sz relative to age- and sex-matched controls. We hypothesize that these changes are mediated by the downregulation of APP, which also occurs in prefrontal cortex region of individuals with Sz. Our group have characterized the age-dependent accumulation of iron in the brain of global APP knockout mice. Remarkably, global APP KO display features of Sz, including agenesis of corpus callosum and increased seizure activity. Therefore, we propose that down regulation of APP function may represent a common lesion that leads to inappropriate neurotransmission, synaptic pruning and synaptic function that are involved in the clinical manifestation of Sz.

THE UBIQUITIN PROTEASOME SYSTEM IN SCHIZOPHRENIA.

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The aetiology of schizophrenia remains unknow. It has been linked to abnormalities in dopaminergic, glutamatergic, GABAergic and serotoninergic neurotransmission as well as signalling pathways critical for brain growth and maturation. A common consequence of these pathway abnormalities in schizophrenia is a loss in proteostasis of the key components of these extra/intracellular pathways. Proteostasis requires the control of protein synthesis, folding, conformational maintenance, protein-protein interaction, trafficking and degradation. The ubiquitin-proteasome system (UPS) is central to proteostasis, suggesting it likely plays a pivotal role in the onset and progression of schizophrenia. UPS is a master regulator of neural development and the maintenance of brain

structure and function. It influences neurogenesis, synaptogenesis and neurotransmission by determining the localization, interaction and turnover of scaffolding, presynaptic and postsynaptic proteins. Although links between UPS dysfunction and neurodegenerative disorders have been known for some time, only recently have similar links emerged for neurodevelopmental disorders, such as schizophrenia. In this presentation, we will review the components of the UPS that are reported as dysregulated in schizophrenia by our group and others, and we will discuss specific molecular changes to these components that may explain the complex aetiology of this mental disorder as a syndrome.

DR. JORGE MARDONES RESTAT AWARD

1. USE OF NPSI-BCD COMPOSITE MICROPARTICLES FOR THE CONTROLLED RELEASE OF CAFFEIC ACID AND PINOCEMBRIN, TWO MAIN POLYPHENOLIC COMPOUNDS FOUND IN A CHILEAN PROPOLIS.

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Propolis is widely recognized for its various therapeutic properties, which are attributed to its rich composition in polyphenols. Polyphenols exhibit multiple biological properties, such as antioxidant, anti-inflammatory, anti-angiogenic, and others. Despite of its multiple benefits, oral administration of polyphenols results in bioavailability at the site of action. An alternative to face this problem is the use of biomaterials at nano-micro scale due to its high versatility as carriers and delivery systems of various drugs and biomolecules. In that sense, the aim of this work is to determine if microparticles of nanoporous silicon conjugated with a beta cyclodextrin polymer to form the nPSi-βCD composite are available material for the controlled release of the two main polyphenols of Chilean propolis, caffeic acid and pinocembrin. Moreover, it was studied their cytocompatibility with HUVECs. Using different physicochemical techniques, it was demonstrated that nPSi-βCD microparticles successfully retained and controlled release caffeic acid and pinocembrin. Furthermore, nPSi-βCD microparticles presented cytocompatibility with HUVECs culture at concentrations of 0.25 mg/ml. These results suggest that nPSi-βCD microparticles can be safely used to improve the bioavailability of caffeic acid or pinocembrin –and eventually other polyphenols– in the target site, thus enhancing its therapeutic effect for the treatment of different diseases.

Pharmacology area: Tecnología farmacológica (Pharmaceutical Technology)

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2. INDOMETHACIN IMPAIRS POLYAMINE METABOLISM IN LUNG CANCER CELLS: A KRAS MUTATION-ASSOCIATED FEATURE?

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Non-small cell lung cancer (NSCLC) is the most lethal and prevalent type of lung cancer. NSCLC patients carrying mutations in the Kirsten rat sarcoma viral oncogene homolog gene (KRAS) still lack targeted therapies. Also, the levels of polyamines (putrescine, spermidine, and spermine) are increased in cancer, playing a pivotal role in tumor proliferation. Indomethacin increases the levels of the polyamine-catabolic enzyme spermidine/spermine-N1-acetyltransferase (SSAT). Consequently, the aim of this study was to compare the effect of indomethacin in the polyamine metabolism of two NSCLC cell lines, with different KRAS mutation status. A549 and H1299 NSCLC cells (KRAS-mutated and wild-type, respectively) were exposed to indomethacin. Evaluations included SSAT expression and protein levels, and metabolic analysis of cells by CG-MS metabolomics. Moreover, the difference in polyamine synthesis enzymes among cell lines and the synergistic effect of indomethacin combined with inhibitors of these enzymes were investigated. Indomethacin increased the expression and levels of SSAT in both cell lines. In A549 cells, indomethacin significantly impairs polyamine metabolism. However, in H1299 cells, the impact of treatment on the polyamine pathway was non-significant. Evaluation of the levels of the polyamine synthesis enzymes showed that ornithine decarboxylase (ODC) is increased in A549 cells, whereas S-adenosylmethionine-decarboxylase (AMD1) and polyamine oxidase (PAOX), are increased in H1299 cells. Finally, indomethacin demonstrated a synergistic effect with the PAOX inhibitor MDL72527 in A549 cells, whereas in H1299 had a synergistic effect with the AMD1 inhibitors SAM486. Collectively, these results indicate that indomethacin alters polyamine metabolism in NSCLC cells and enhances the effect of polyamine synthesis inhibitors such as MDL72527 or SAM486. However, this effect varies depending on the basal metabolic fingerprint of each type of NSCLC cell. FONDECYT-1160807.

Pharmacology area: Otros (Others)

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INCORPORATIONS

1. AMPK ACTIVATION ON CARDIAC FIBROBLASTS: ROLE IN AUTOPHAGY AND CELL PROLIFERATION INDUCED BY CATECHOLAMINES.

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Activation of the adrenergic system is commonly associated with cardiac fibrosis and remodeling, and cardiac fibroblasts are key players in these processes. Interestingly, adrenergic stimulation activates both, cardiac fibroblasts autophagy and cell proliferation, however, the underlying mechanisms have not been elucidated. In the present study, we assessed the effects of adrenergic stimulation on autophagy and cell proliferation in cultured adult rat cardiac fibroblasts, which were treated with beta-adrenergic agonists and antagonists. Autophagy was determined by electron microscopy, subcellular distribution and protein levels of LC3-II, and the signaling pathways involved in its activation after stimulation with catecholamines were evaluated by western blot. Our results suggest that AMPK plays a key role in the induction of autophagy, through the inhibition of mTOR activity. Indeed, the AMPK pharmacological inhibitor, compound c, prevents the autophagy induced by adrenergic agonists, acting downstream of AKT in the beta2-adrenergic receptor/AKT/mTOR pathway. AMPK activation was also necessary for ERK1/2 phosphorylation and cell proliferation. In addition, the increase in autophagy correlates with intracellular collagen degradation. In summary, here we show that beta2-adrenergic stimulation activates AMPK and this protein governs both processes, autophagy and proliferation in cardiac fibroblasts, therefore, a pharmacological modulation in this pathway could contribute to reducing the harmful effects of adrenergic stimulation in cardiac fibrosis.

Pharmacology area: Farmacología cardiovascular (Cardiovascular Pharmacology)
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2. THE EFFECT OF THE ALLOSTERIC INHIBITOR OF RIPK1 (NEC-1) ON OVARY FUNCTION: IMPORTANCE OF NECROPTOSIS IN FOLICULAR DEVELOPMENT.

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The ovarian follicle develops between proliferation and cell death process. Three types of cell death have been reported: apoptosis, phagocytosis and necrosis. A fourth type of cell death, necroptosis, has been recently associated to ovarian function. However, the physiological relevance of necroptosis or its involvement in follicular development it is not yet well understood. In the present work we will use pharmacological tools (allosteric inhibitor necrostatin-1), to study the role that Necroptosis would have in follicular development and ovarian function in vivo. We used two groups of animals: Sham and NEC-1. Adults rats were hemiovariectomized and implanted with a miniosmotic pump with NEC-1 (20 µM), for 28 days, or remains without drug administration (sham). At the end of the procedure, rats were euthanized and the ovaries and plasma were collected. The ovaries were fixed for morphometric analyses. Plasma levels of steroid hormones were measured by EIA. We found that necroptosis inhibition did not affect the number of secondary follicles, but increased total antral follicles by accumulating atretic antral follicles. The number of type III precystic follicles was increased while the cyst number did not change. Corpus luteum didn't change in number but decreased the new (bigger size) CL. An increase in testosterone plasma levels was found. In conclusion, NEC-1 treatment by blocking necroptosis in vivo, favored cyst formation and the permanence of old corpus luteum thus necroptosis could be involved in luteolysis and in the transition to follicular cyst in the ovary. In vivo models help to describe new pharmacological targets to regulate follicular development and hence fertility.

Pharmacology area: Farmacología endocrina-reproductiva (Endocrine/Reproductive Pharmacology)
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3. TLR4, BUT NEITHER DECTIN-1 NOR DECTIN-2, PARTICIPATES IN THE MOLLUSK HEMOCYANIN-INDUCED PROINFLAMMATORY EFFECTS IN ANTIGEN-PRESENTING CELLS FROM MAMMALS.

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Mollusk hemocyanins have biomedical uses as carriers/adjuvants and nonspecific immunostimulants with beneficial clinical outcomes. Hemocyanins have a multivalent nature as highly mannosylated antigens. We have shown that hemocyanins are internalized by antigenpresenting cells (APCs) through receptor-mediated endocytosis by innate immune receptors, such as mannose receptor (MR). However, the contribution of other pattern recognition receptors to the proinflammatory signaling pathway triggered by hemocyanins is unknown. Thus, we studied the roles of Dectin-1, Dectin-2, and Toll-like receptor 4 (TLR4) in the hemocyanin activation of murine APCs, both in dendritic cells (DCs) and macrophages, using hemocyanins from Megathura crenulata (KLH), Concholepas concholepas (CCH) and Fissurella latimarginata (FLH). The results showed that these hemocyanins bound to chimeric Dectin-1 and Dectin-2 receptors in vitro. However, hemocyanin-induced proinflammatory effects in APCs from Dectin-1 knock-out (KO) and Dectin-2 KO mice were independent of both receptors. Moreover, the phosphorylation of Syk kinase was not detected after

hemocyanin stimulation. On the other hand, we confirmed a glycan-dependent binding of hemocyanins to chimeric TLR4 in vitro. Moreover, DCs from mice deficient for MyD88-adapter-like (Mal), were partially activated by FLH, suggesting a role of the TLR pathway in hemocyanin recognition to activate APCs. TLR4 role was confirmed through a decrease in IL-12p40 and IL-6 secretion induced by FLH when a TLR4 blocking antibody was used; a reduction was also observed in DCs from C3H/HeJ mice. Additionally, IL-6 secretion induced by FLH was abolished in macrophages deficient for TLR4. We further showed that KLH and FLH induced ERK1/2 phosphorylation. Our data showed the involvement of TLR4 in the hemocyaninmediated proinflammatory response in APCs, which could cooperate with MR in innate immune recognition of these glycoproteins.

Pharmacology area: Otros (Others)
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4. DISCOVERY OF NOVEL TASK-3 POTASSIUM CHANNEL BLOCKERS.

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TASK-3 is a two-pore domain potassium (K2P) channel highly expressed in hippocampus, cerebellum, and cortex. TASK-3 has been identified as an oncogenic potassium channel and it is overexpressed in different cancer types. For this reason, the development of new TASK-3 blockers could influence the pharmacological treatment of cancer and several neurological conditions. In the present work, we search for novel TASK-3 blockers by using a virtual screening (VS) protocol that includes pharmacophore modeling, molecular docking, and free energy calculations (MM/GBSA). With this protocol, 19 potential TASK-3 blockers were identified. These molecules were tested in TASK-3 using patch clamp, and one blocker (DR16) was identified with an IC50 = 56.8 ± 3.9 µM. Using DR16 as scaffold we designed DR16.1, a novel TASK-3 inhibitor with an IC50 = 14.2 ± 3.4 µM. Our finding takes on greater relevance considering that not many inhibitory TASK-3 modulators have been reported in the scientific literature until today. These two novels TASK-3 channel inhibitors (DR16 and DR16.1) are the first found using a pharmacophore-based virtual screening and rational drug design protocol.

Pharmacology area: Otros (Others)
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5. IMMUNOLOGICAL BASIS OF AUTISM: COGNITIVE EFFECTS OF AUTOANTIBODIES FROM AUTISTIC CHILDREN IN MEMORY AND LEARNING PROCESSES.

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Autism spectrum disorders (ASD) involve a range of complex neurodevelopmental disorders, characterized by social impairments, communication difficulties, and restricted, repetitive and stereotyped patterns of behavior. ASD exerts a significant physiological, emotional and financial burden on the families of the individual and society as a whole. Recently, beside the knowledge about genetic factors involved in this pathology, there is new evidence related to immunological causes of ASD. Therefore, it is of outmost importance to elucidate the molecular and physiological mechanisms of ASD pathology. Taking this into account, we hypothesized that ASD autoantibodies generates autoimmune-related cognitive impairment characteristic of ASD pathology. To achieve this aim, we used ex vivo experiments using hippocampal slices and a rat model where mothers were injected with ASD autoantibodies during pregnancy and/or breast milk period and the breeding was tested after that period using learning and memory test together with electrophysiological and immunohistochemical studies. Our results have shown that normal young rat hippocampal slices incubated with purified IgA autoantibodies from ASD patients and breeding rats from pregnant mothers injected with the same antibodies, impairs LTP as well as disrupts learning and memory. We also found that both LTP and learning and memory were significantly impaired in female but not male breeding rats and this alteration are correlated with the presence of ASD autoantibodies in hippocampal slices. These results demonstrate that ASD autoantibodies cross the transplacental barrier and also are ingested through breeding milk, crossing both intestinal and blood-brain barrier and impairs learning and memory in a sex-preference fashion. The pharmacological implicances of this research involve new mechanisms and possible therapeutical targets for ASD pathology

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6. ANTI-STEROIDOGENIC EFFECT OF THE RFRP-3 NEUROPEPTIDE AND ITS PARTICIPATION IN THE FOLLICULAR DYNAMICS IN THE RAT.

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Ovarian function is highly regulated, either by hormones, autonomic nerves and paracrine signals produced by the same ovarian cells. Changes of these signals modified the normal functioning of the ovary. Gonadotrophin inhibitor hormone (GnIH) is a neuropeptide that block the GnRH secretion at hypothalamic level and therefore the gonadotropic axis regulating the ovary. The recently described receptor (GPR147

or NPFF1, homologous in mammals) and RFRP-3 (mammalian homologous peptide of GnIH) in the ovary open the possibility to suggest that the local presence of the peptide and its receptor could participates as regulator of ovarian function. We studied whether RFRP-3 and NPFF1 receptor are present in the rat's ovary and the local effect of this neuropeptide on hormone production and follicular dynamics. We determine the presence of RFRP-3 in the rat's ovary. RFRP-3 was mainly in the granulose cells of antral follicles and corpora lutea. Then, we studied the effect of 10 ng/mL RFRP-3 on the production of ovarian steroids ex vivo. RFRP-3 inhibited the hCG-induced ovarian progesterone and testosterone secretion. In order to know if the chronic presence peptide in the ovary modified the follicular development and its function, we designed a local chronic treatment in vivo with RFRP-3. After 4 weeks of treatment there was a decrease in serum testosterone and an increase in size and number of corpora lutea suggesting the appearance of new corpora lutea and hence increased ovulation. No changes appeared in secondary, antral, cyst or atretic follicles. Data indicate a local effect of RFRP-3 that positively affect ovarian steroidogenesis and follicular dynamics. This study opens new pharmacological targets, such as neuropeptides, to treat disorders in ovarian function.

Pharmacology area: Farmacología endocrina-reproductiva (Endocrine/Reproductive Pharmacology)
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ORAL COMMUNICATIONS

1. THE N-ACETYLCYSTEINE-INDUCED REDUCTION OF CHRONIC ALCOHOL COMSUMPTION IS ASSOCIATED TO THE ACTIVATION OF THE NRF2/ARE PATHWAY IN HIG-ALCOHOL-DRINKING UCHB RATS.

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Current evidence suggests that neuroinflammation and oxidative stress are associated to chronic alcohol consumption and relapse, suggesting that the modulation of oxidative stress induced by chronic alcohol drinking can be a therapeutic target in alcoholism. There is evidence that oxidative stress activates the Nrf2 (Nuclear factor erythroid 2-like), that translocates into nucleus where it promotes the transcription of antioxidant genes containing the Antioxidant- Response-Element (ARE) including hemoxigenase 1 (HO-1) and NAD(P)H dehydrogenase quinone 1 (NQO1). Previously we have demonstrated that N-acetylcysteine (NAC), a cysteine precursor, with antioxidant action, inhibits alcohol consumption, neuroinflammation and alcohol-induced oxidative stress in chronic drinking rats (UChB). However, the mechanism of the antioxidant action of N-acetylcysteine it is not clear. The present study determinates whether the NAC-reduction of chronic alcohol consumption is associated to the activation of the Nrf2 /ARE pathway in high-alcohol-drinking rats. Chronic alcohol drinking (61days) female UChB rats were administered for nine consecutive days either (i) NAC (100mg/kg/day, per os); (ii) NAC + all-trans-retinoic acid (ATRA, a Nrf2 pharmacological inhibitor (10 mg/kg/day ip); (iii) ATRA+ saline); (iv) Saline. After determining the rates of alcohol consumption, all groups were euthanized for hippocampal histological and Western blot analyses. It was found that (i) N-acetylcysteine inhibits chronic alcohol intake (ii) N-acetylcysteine induced Nrf2 nuclear translocation (ii) N-acetylcysteine-induced inhibition of chronic alcohol intake was prevented by ATRA a Nrf2 pharmacological inhibitor. In conclusion these results support the idea that Nrf2 activation is the mechanism by which NAC inhibited chronic alcohol consumption and relapse.

Pharmacology area: Neuropsicofarmacología (Neuropsycopharmacology)
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2. D-LACTATE INDUCES NEUTROPHIL EXTRACELLULAR TRAPS (NET) RELEASE BY DISTURBING CELLULAR METABOLISM.

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D-lactate is produced during acute ruminal acidosis (ARA), a well-known fermentative disorder in cattle. Recently, we demonstrated that heifers with ARA show aseptic neutrophilic synovitis, characterized by the presence of D-lactate, abundant neutrophils, NET, and metabolic disturbances in synovial fluid. It has been described that D-lactate entry is required to induce NET-release. Since D-lactate is slowly metabolized by mammalian cells, we hypothesized that D-lactate induces metabolic disturbances in neutrophils, and so could induce NET-release. Blood neutrophils isolated from 5 healthy heifers were treated with 5 mM D-lactate in vitro. First, we performed a GC-MS untargeted metabolomic analysis. D-lactate altered galactose metabolism, starch and sucrose metabolism, nucleotide sugars metabolism and glycolysis. Using JC-1 probe we observed by flow cytometry that D-lactate reduced $\Delta\psi m$. In addition, D-lactate favored the glycogen degradation, and increased glucose-1-P and glucose-6-P intracellular levels. Also, D-lactate increased the AKT and GSK-3β phosphorylation. The inhibition of theses pathways with LY294002 and CHIR99021, respectively, interfered the decrease of glycogen and NET release. Our results suggest that D-lactate induces NET release by disturbing cellular metabolic pathways, involved in glycogen degradation.

Pharmacology area: Otros (Others)
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3. SYNTHESIS, CHARACTERIZATION, THEORETICAL STUDY AND IN VITRO EVALUATION OF BETA-LACTAMIC COMPOUNDS AND IMINES WITH POTENTIAL ANTIBACTERIAL ACTIVITY.

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One of the most serious problems worldwide is the resistance of the main pathogenic bacteria to the antibiotics used today. The evolution of the resistance seen in the light of the Darwinian and Lamarckian theories of adaptation gives rise to the understanding of the causes of resistance and if the causes are known, solutions can be proposed, otherwise, what will be achieved is to amplify the problem to such an extent that hospitals will become the repertoire of microbial infections resistant to any chemotherapeutic treatment. With the current biochemical knowledge, a rational design of antibiotics with in

silico experiments of chloromonobactams was proposed from imines p-substituted with stereochemistry (E), which demonstrated that both sets of molecules comply with the Lipinski rule of 5, which offers a viable pharmacokinetics towards the organism. The synthesis of chloromonobactams was carried out in two phases. The first is the synthesis of p-substituted imines with (E) configuration; the second is a [2+2] Staudinger cycloaddition to obtain chloromonobactams. The characterization of all the synthesized compounds was performed by physical tests (determination of Rf, melting point, and solubility tests), and spectroscopy (UV-visible and IR spectrophotometry, 1H and 13C NMR spectroscopy, and high-resolution mass spectrometry). The evaluation of the in vitro antibacterial activity was carried out by the disk diffusion method.The study strains were S. aureus sensitive to dicloxacillin, E.coli and P. aeruginosa sensitive to aztreonam. The results obtained so far show that the imines have antibacterial activity against the bacteria under study, with the p-iodo imine and the beta-lactam without substituents showing an activity similar to aztreonam on P. aeruginosa.

Pharmacology area: Otros (Others)
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4. ROLE OF MITOCHONDRIAL METABOLISM IN OXIDATIVE RESPONSE AND NETS RELEASE INDUCED BY PAF IN BOVINE NEUTROPHILS.

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Neutrophils (PMN) constitute the main line of cellular defense in the innate immune response. Since they obtain energy primarily through glycolysis, it is assumed that they do not produce ATP by oxidative phosphorylation. However, mitochondrion of PMN maintains a transmembrane potential, which is normally associated with respiratory chain and oxidative phosphorylation for ATP synthesis. PMN were isolated from healthy heifers and stimulated in vitro with platelet activating factor (PAF), a key biochemical mediator in various inflammatory conditions. Incubation with PAF 100 nM increased mitochondrial transmembrane potential and mitochondrial reactive oxygen species (mtROS) production. While mtROS levels were reduced using rotenone 10 uM (mitochondrial complex I inhibitor), these were increased by oligomycin 10 uM (mitochondrial complex V inhibitor). PAF 100 nM also stimulated respiratory burst in PMN, which was reduced not only with 2-deoxy-D-glucose 2 mM (2-DG, glycolysis inhibitor), but also with rotenone 10 uM, oligomycin 10 uM and carbonylcyanide-3-chlorophenylhydrazone 5 nM (CCCP, oxidative phosphorylation uncoupler). Finally, PAF 1 uM induced neutrophils extracellular traps (NETs) release, which was reduced by 2-DG and CCCP, but increased by oligomycin. These results suggest that PAF triggers respiratory burst and NETs release through glycolysis and mitochondrial metabolism-dependent mechanisms.

Pharmacology area: Farmacología molecular (Molecular Pharmacology)
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5. THE CONSUMPTION OF A CALAFATE EXTRACT MODULATES THE ENERGY EXPENDITURE, FUNCTION AND MITOCHONDRIAL DYNAMICS OF BROWN ADIPOSE TISSUE OF OBESE MICE.

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Obesity is a public health problem of global concern. In its pathogenesis, the White Adipose Tissue has a crucial role. There is a mitochondrial dysfunction and lower oxidative capacity in adipocytes of obese individuals, with modifications in their morphology. In contrast, Brown Adipose Tissue (BAT) has a thermogenic function through UCP-1. A new approach proposes to increase energy expenditure through diet. Our objective in this work was to evaluate the effect of a calafate extract rich in polyphenols on mitochondrial energy, function and dynamics (fusion / fission) of obese mice. The analyzes were performed on adult C57BL / 6J male mice, which were subdivided (n = 10 each) into 4 dietary regimens / treatments: Control Diet (C), Control / Calafate (extract: 50 mg total polyphenols / kg weight; CC), High Fat Diet (HF) and High Fat Diet / Calafate (HFC). The mice were subjected to indirect calorimetry. Post-euthanasia was evaluated: gene and protein expression of UCP-1, PGC-1alpha, OPA1 (fusion), DRP1 (fission) (qPCR, western blot or immunofluorescence), mitochondrial Oxygen Consumption Rate (OCR) (XF24 Seahorse), HSP70 (amount of mitochondria) and mitochondrial activity (with MTO). The consumption of calafate extract produced an increase in energy expenditure and a decrease in respiratory quotient. The treatment presented differences at the level of mitochondrial function, with an increase in thermogenesis (UCP-1) a recovery of OCR, and a significant effect on the MTO / HSP70 ratio. It did not substantially modify the mitochondrial morphology. The consumption of a calafate extract rich in polyphenols increases energy expenditure and improves mitochondrial function in obese mice. Additional studies on mitochondrial dynamics are required to complement these hypothesis.

Pharmacology area: Fisiología (Physiology)
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6. FROM HOMO SAPIENS TO HOMO TECHNOLOGICUS, BIOETHICAL CHALLENGE OF TRANSHUMANISM.

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Transhumanism, an empiricist thesis whose anthropology dispenses with metaphysics. It explains the human dynamism from functionalist neurobiologicism, which underlines the human to the functioning, of its neural connections, and that seeks its sustenance in scientific perfection. In the bioethical field it is founded is liberal utilitarianism. There are authors who argue that we are in the last stage of the development of homo sapiens, and in the era of homo technologicus, it has the possibility of continuing the evolution of the human species towards a superior, better and happier, using technology to its scope. Transhumanism raises many questions, among others. Has neurobiological physicalism been proven? Who tells me that the more perfect I am physically and psychically, that the more capacities I have, I will be happier? What is happiness? What does it mean to be better or more perfect, who determines it? We try a response in the moral and ontological field. Then there are issues of a practical nature when implementing the transhumanist plan: embryonic selection and eugenic elimination of embryos and fetuses with defects, problems derived from nanotechnology applied to the brain and neuroethics, cryopreservation problems, use of drugs that change personality, resource distribution problems, etc.
This study aims to address the ethical and anthropological challenge that underlies transhumanism.

Pharmacology area: Aspectos regulatorios (Regulatory aspects in Pharmacology)
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7. SYNTHESIS AND EVALUATION OF INDOLYL-BENZAMIDO-PIPERAZINES AS POTENTIAL MULTI-TARGET-DIRECTED LIGANDS IN ALZHEIMER´S DISEASE.
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Alzheimer's disease (AD) is a chronic, progressive and fatal neurodegenerative disorder affecting cognition, behavior, and function, being one of the most common causes of mental deterioration in elderly people: Around 50-60% of the overall dementias correspond to AD. World Health Organization estimates that about 46.8 million of people worldwide currently suffer from AD, thus becoming a major public health concern as the world's population ages. (World Alzheimer's report, 2018). Development of Multi-Target Directed Ligands (MTDLs) has emerged as a promising approach for targeting complex etiology of Alzheimer's disease (AD). Following these approach, and given our interest in the search and development of novel drugs displaying affinity acting as promiscuous ligands. In the present work, a novel series of indolylpropylpiperazinyl piperazinebenzamides were synthesized and biologically evaluated as multifunctional ligands in the following targets: acetylcholinesterase, SERT, and beta-amyloid peptides The synthesis involved connection between Piperazine benzamides with N-Boc substituted piperazine derivative. Boc cleavage and further coupling with indolylpropyl tosylates was achieved in two step-one pot reaction, obtaining the final compounds with good to excellents overall yields. Finally, the obtained compounds were evaluated in its capabilities for AChE inhibition, SERT- affinity, β-amyloid inhibition, and cell toxicity (viability) with very promising results.

Pharmacology area: Farmacodinamia (Pharmacodynamics)
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8. DEVELOPMENT OF A RECOMBINANT VACCINE CANDIDATE AGAINST HANTAVIRUS.
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Andes virus is the main causative agent of Hantavirus cardiopulmonary syndrome (HCPS) in South America. There are currently no vaccines or treatments against Andes virus. However, there are several evidences suggesting that antibodies against Andes virus envelope glycoproteins may be enough to confer full protection against HCPS. The main goal of the present work was to develop a vaccine candidate against Hantavirus, based on the surface glycoproteins Gn and Gc. With this purpose, the sequence encoding the extracellular domains of both antigens was introduced into the methylotrophic yeast Pichia pastoris. After induction with methanol, the recombinant antigens accumulated intracellularly as insoluble aggregates. After cell disruption, the recombinant antigens were solubilized and purified by metal-ion affinity chromatography. The immunogenicity of both antigens was determined in immunization assays in both mice and Syrian hamsters. In both species it was possible to detect the presence of specific antibodies against Gn and Gc. Part of these antibodies showed neutralizing activity. The results obtained to date suggest that the Gn and Gc antigens from Andes virus, produced in P. pastoris, have the potential to become the first commercial vaccine against HCPS.

Pharmacology area: Otros (Others)
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9. ADHERENCE TO ANTIHYPERTENSIVE PHARMACOLOGICAL TREATMENT IN ELDERLY PEOPLE FROM HUALPEN SUBMITTED TO A TRANSMEDIAL PSYCHOEDUCATIONAL PROGRAM.
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In Chile, the number of elderly people has steadily increased, with 20% of the population projected by 2025. This change associated with a sedentary lifestyle is linked with an increased prevalence in chronic pathologies such as Hypertension. The national prevalence is 27.6%. The lack of pharmacological adherence constitutes one of the main problems in the control of the disease, considering that only approximately 50% of the patients adhere properly. Given this problem, a Transmedial Psychoeducational Program (PST) was developed to support primary care treatment, focused on knowledge of the disease, as well as promoting the benefits of pharmacological treatment and a healthy lifestyle.

The PST was evaluated in two CESFAM in the commune of Hualpén with three levels of intervention: Group A (n = 104) through a mobile application "AFAM-Health", Group B (n = 97) using video capsules and Group C (n = 98) as control.

The average age of the 299 elderly was 72 ± 7.6 years, 83.7% have as their source of income the retirement salary, 88.6% lives, accompanied, 74.3% attend their health checks alone and 68.6% of them walk. After one year of intervention, group A was significantly more adherents (Morisky-Green test) over time with values of 52, 73, 64 and 64% of adherents at the beginning, third, sixth and twelfth month, respectively. Group A had 4.5 ± 1.8 medications / day, 43.4% corresponded to antihypertensives, Losartan is the main one.

From these results, it is determined that older adults who use the "AFAM-Health" App as a support increase pharmacological adherence unlike those who only receive the traditional primary care treatment.

Pharmacology area: Farmacología cardiovascular (Cardiovascular Pharmacology)

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POSTERS

1. SEARCH FOR ANALOGS OF M554 AND M890 FOR INTERACTION WITH THE GBΓ DIMER IN REGIONS INVOLVED IN THE REGULATION OF THE GLYCINE RECEPTOR.

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Ethanol is the drug with highest consumption levels, with effects at different levels of the Central Nervous System. Accute consumption at high levels can induce coma and death. This molecule modulats the activity of Glycine receptor (GlyR), a ligand gated ion channel that belong to the cys-loop subfamily of ion channels. Recently it has been identified that Gbg protein as a modulator of the channel interacting with the cytoplasmic domain and potentiating the activity of the channel. With determined structure of Gbg and GlyR cytoplasmic domain, it has been able to identify chemical entities to inhibit the etanol effects. Initially, peptides were designed and then peptidomimetic small molecules were developed, like M554 and M890. These molecules interact with Gbg and inhibit etanol effects in vitro and in vivo. After that new molecules were designed applying bioisosteric changes in the original molecules M554 and M890. Through bioinformatic technics like docking , molecular dynamics and free energy calculations (MM-GBSA), it has been identified the derivatives (R,S)-M554_3, (S)-M554_13, (R)-M554_13, M890_4 y M890_5 which interact in the Gbg hotspot surface with conserved aminoacids. Finally, cytotoxicity assays were performed in HEK cells determining that the molecules were not toxic for cells.

Pharmacology area: Farmacología molecular (Molecular Pharmacology)
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Acknowledgments: Jin Chunyang of Research Triangle Institute RTI National Fund of Scientific and Technological Development FONDECYT Physiology department Biological Sciences Faculty

2. BIOGUIDED ISOLATION OF SECONDARY METABOLITES PRESENT IN THE MEDICINAL SPECIES CALDCLUVIA PANICULATA (CUNONIACEAE) WITH INHIBITORY ACTIVITY IN VITRO ON THE ENZYME A-GLUCOSIDASE.

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Diabetes mellitus (DM) is a metabolic disease characterized by an increase in blood sugar levels. According to the World Health Organization, 422 million adults worldwide had DM by 2014. There are studies based on ethnobotanical knowledge that support the use of medicinal plants with hypoglycemic activity, ascribing their activity to the presence of phenolic compounds. Mapuche medicine suggests the consumption of "Tiaca" (Caldcluvia paniculata) as a hypoglycemic treatment. The objective of the research was to evaluate in vitro the inhibitory activity of secondary metabolites present in C. paniculata on a-glucosidase. For this purpose, the plant material was defatted and macerated in a hydroalcoholic solution for 7 days. The hydroalcoholic extracts were dried, resuspended (MeOH:H2O - 70:30) and partitioned by liquid extraction, with solvents of increasing polarity. The activity of the partitions was determined by inhibition on a-glucosidase: Aqueous solutions of the dry extract were prepared at different concentrations (1-100 ug/mL), using p-nitrophenyl-(1,4)-a-D-glucopyranoside as a substrate and acarbose as positive inhibition control. The positive control of inhibition on the enzyme (acarbose) presented an IC50 of 1288 ug/mL. Ethyl acetate partition presented the lowest IC50, reaching values of 13.6 and 14.5 ug/mL for leaf and stem respectively. The most active partition was fractionated by column chromatography using silica gel and eluted with solvents of increasing polarity. The 15 groups obtained were evaluated for their inhibitory capacity on a-glucosidase. Group G. 9 presented better inhibition with an IC50 of 20.2 ug/mL. The chromatographic profiles of leaves and stems were analyzed by HPLC, observing similar profiles and the presumptive presence of phenolic compounds. The results obtained are conclusive regarding the hypoglycemic property of C. paniculata.

Pharmacology area: Farmacología de Productos Naturales (Natural Products Pharmacology)
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3. DETECTION AND IDENTIFICATION OF ANTIBACTERIAL COMPOUNDS IN LIQUID FERMENTATIONS OF FUNGUS STEREUM SP. BY HPTLC-BIOASSAY-MS.

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Basidiomycetes belonging to higher fungi, offer an exciting field to obtain new structures with high potential for medical applications. Higher fungi have an important advantage as producers of bioactive secondary metabolites: they release them to liquid media. Then, the objective of this work was to detect and identify compounds with antibacterial activity in liquid fermentations of fungus Stereum sp. Pure mycelial cultures were produced from impressions of spores of fruiting bodies, which were then cultured in YMG medium (glucose, malt extract, yeast extract and agar) previously sterilized. For liquid fermentation, the following was performed: small sections (15-10) of 5 mm diameter plug were cut under sterile

conditions and transferred to an Erlenmeyer flask containing liquid YMG medium. The flasks were incubated at 20-22 °C on a Shaker orbital shaker with constant shaking. The cultures were stopped when abundant mycelia were observed, the glucose source was emptied, and the pH was about 7. The liquid culture was filtered to separate the broth and the mycelium. Bioactive compounds were extracted with ethyl acetate from culture media. The total extract was concentrated to dryness in a rotary evaporator (45 °C), weighed and stored at 4 °C. The extract dissolved in methanol was seeded on HPTLC plates silica gel 60 F254. Separation was performed using the mixture of toluene-ethyl acetate (3.15 : 1.85 v/v) as a mobile phase. The extract was seeded in triplicate by dividing the HPLTC plate into three sections: the first section was used for the bioassay (direct bioautography), the second section for the chemical derivatization and the third section for the mass spectrometry analysis (MS). After chromatography, the first section was dried and a buffer solution was atomized. The plate was immersed in Bacillus subtilis bacteria suspension and incubated at 37 °C for 2 hours. Subsequently, the plate was atomized with a solution of methylthiazolidiphenyl-tetrazolium bromide (MTT), incubated at 37 °C for 30 min and finally dried completely on a heating plate at 50 °C for 5 min. A zone of inhibition was detected on the HPTLC plate as a colorless zone/band on a purple background. Using the third section of the plate (dried previously), this bioactive/inhibitory zone was directly eluted by means of the TLC-MS interface coupled to the electrospray ionization source (ESI) of a triple quadrupole mass spectrometer. Full scan mass spectra (m/z 100-1000) were recorded in positive (ESI+) ionization mode. The bioactive compound tentatively corresponds to Himanimide C.

Pharmacology area: Farmacología de Productos Naturales (Natural Products Pharmacology)
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4. EFFECTS OF ARSENIC (AS) EXPOSURE ON BLOOD-BRAIN BARRIER AND COLONIC PERMEABILITY IN HEALTHY YOUNG RATS.

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Arsenic (As) is a toxic metalloid, which has become a health burden worldwide. Growing evidence indicates that As has harmful effects on the central nervous system (CNS), as this metalloid crosses the blood-brain barrier (BBB). On the other hand, there is little evidence suggesting that loss of intestinal permeability might affect BBB permeability. The aim of this study is to evaluate if oral exposure to As affects intestinal and BBB permeability, as this pollutant might have an impact on what now is known as the brain-gut axis. Methods: Female Sprague–Dawley rats (PND35) where given 10 ppm of NaAsO2 in the drinking water for 24h (n=5), and compared to control rats (n=6). At 24h the following samples were collected: brain, colon, lung, stool and liver tissues. Each sample was lyophilized and then microwave digested in order to determinate total As concentration by HPLC-HG-AFS. Additionally, colonic permeability to FITC-dextran 4.4kDa (FD4) was evaluated ex vivo for 120 and 180 min by everted gut sac technique. Results: Gut permeability to FD4 is increased in animals exposed for 24h to 10 ppm of NaAsO2 in comparison to controls. In addition, the metalloid concentration was higher in every studied tissue of exposed rats, in comparison to controls. In the brain, As was found in hypothalamus and cerebral cortex. This data suggest that As is able to cross the BBB and increases gut permeability in the rat, an effect that might lead to alterations in BBB. In conclusion, a toxic pollutant such as As might cause alterations in the brain-gut axis, effects which gives a novel approach in the study of As toxicity.

Pharmacology area: Toxicología (Toxicology)
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5. TWO CONSERVED ALPHA-HELICES IN CORTICOTROPHIN RELEASING FACTOR BINDING PROTEIN CONTAINING A HYDROPHOBIC PATCH DETERMINES ITS SORTING TO THE REGULATED SECRETORY PATHWAY.

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Corticotrophin releasing factor binding protein, (CRF-BP) is a 37 kDa glycoprotein that binds CRF with high affinity. CRF-BP in the periphery controls CRF levels in plasma during pregnancy. In the central nervous system, CRF-BP facilitates the traffic of CRFR2alfa acting as an escort protein. Previously, it has been shown that CRF-BP enters the regulated secretory pathway (VSR). However, the sorting signal(s) are presently unknown. We decided to determine the sorting signal(s) of CRF-BP to VSR. We used NPS @, an in silico secondary structure prediction tool and PEPWHEEL to draw predicted alpha helixes and the in silico modeling of CRF-BP protein structure. Additionally, we did studies of sorting of chimeras containing the putative sorting signals in PC12 cells over-expressing the selected chimeras. In silico analysis and modeling of CRF-BP protein structure showed the presence of three alpha-helix domains, (50-74), (128-149), (229-251). The alpha-helixes domain (50-74) and (229-251) in CRF-BP is highly conserved among different mammalian species and has a hydrophobic patch characteristic of other sorting domains to the VSR. The results show that the alpha-helix domain (50-74)-CRFBP is capable of restore the sorting of a chimeric variant of proCART precursor, without its sorting domain to the VSR. Furthermore, the presence of the alpha-helix domain (50-74)-CRF-BP in the chimeric variant of proCART allowed its secretion triggered by a depolarizing stimulus. Our results show that the preserved alpha-helix domain (50-74)-CRF-BP, present in the amino terminal of CRF-

BP, is responsible for its destination to the VSR. Further studies are needed to evaluate if the other alpha-helix domains also play a role in the sorting of CRF-BP to the VSR.

Pharmacology area: Farmacología molecular (Molecular Pharmacology)
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Acknowledgments: Funded by FONDECYT N| 1150244 and N| 1191274

6. TTAGP 1.0: A COMPUTATIONAL TOOL FOR THE SPECIFIC PREDICTION OF TUMOR T CELL PEPTIDES.

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Nowadays, cancer is considered a global pandemic and millions of people die every year because this disease remains a challenge for the world scientific community. Even with the efforts made to combat it, there is a growing need to discover and design new drugs and vaccines. Among these alternatives, antitumor peptides are a promising therapeutic solution to reduce the incidence of deaths caused by cancer. In the present study, we developed TTAgP, an accurate bioinformatic tool that uses the random forest algorithm for antitumor peptide predictions, which are presented in the context of MHC class I. The predictive model of TTAgP was trained and validated based on several features of 922 peptides. During the model validation we achieved sensitivity = 0.89, specificity = 0.92, accuracy = 0.90 and the Matthews correlation coefficient = 0.79 performance measures, which are indicative of a robust model. TTAgP is a fast, accurate and intuitive software focused on the prediction of tumor T cell antigens.

Pharmacology area: Otros (Others)
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Acknowledgments: FONDECYT number: 1180387 and DIUFRO Projects (DI12-PEO1 and DIE14-0001) of the Universidad de La Frontera.

7. NGF INCREASED CHOLINE ACETYL TRANSFERASE AND THE VESICULAR ACETYLCHOLINE TRANSPORTER EXPRESSION IN RAT OVARY EX VIVO.

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The ovary is an endocrine organ which is regulated by hormonal and neural signals. It is well known that noradrenaline controls ovarian steroidogenesis and folliculogenesis. Its source comes from sympathetic neurons that innervate the ovary. In addition, evidence suggests that acetylcholine enhances follicular development and its intraovarian production would be in granulosa cells which express choline acetyltransferase (ChAT), vesicular acetylcholine transporter (VAChT) for storage and acetylcholinesterase. Besides, cholinergic muscarinic receptors (M1, M3, M5) are expressed in ovarian follicles. However, it is not known how this intraovarian cholinergic system is regulated. In vitro studies had shown that human granulose cells incubated with neuronal growth factor (NGF), a neurotrophin produced by ovary, increased ChAT. The objective of the present work was to determine if NGF enhances acetylcholine production in the rat ovary. 26 days old rats ovaries were incubated with 100 ng/mL NGF during 3 and 24 hrs. 7 days old rats were treated with 50 mg/Kg guanethidine during 3 weeks to induce a chronic endogenous NGF increment and, after 3 months, ovaries were obtained. We measured mRNA levels by qRT-PCR, acetylcholine levels by fluorometric assay, NGF and noradrenaline by ELISA kit, and NGF by western blot. After 3 hrs, NGF produced an increment in ChAT and VAChT mRNA levels, but a decrease in acetylcholine in medium. After 24 hrs, we found a modest but constant increase in acetylcholine production. Guanethidine treatment didn't induce an endogenous NGF increment despite that noradrenaline levels decreased. Altogether these data suggest that NGF regulates intraovarian acetylcholine production and storage ex vivo. Further research is needed to elucidate if a longer time of NGF stimulation is needed to better visualize the neurotransmitter.

Pharmacology area: Farmacología endocrina-reproductiva (Endocrine/Reproductive Pharmacology)
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8. AMPHETAMINE AND TEMPOL MODULATE EXTRACELLULAR CONCENTRATION OF DOPAMINE AND THE PHOSPHORYLATION LEVEL OF THE DOPAMINE TRANSPORTER.

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Amphetamine (AMPH) is a highly reinforcing, widely abused stimulant drug that increases dopamine extracellular levels in the mesocorticolimbic system, in neurons projecting from the Ventral Tegmental Area to Nucleus Accumbens and Prefrontal Cortex. AMPH-stimulated efflux of dopamine through the dopamine transporter (DAT) only happens if DAT is previously phosphorylated. Some kinases that act on DAT are PKC, ERK and PKA and their activity is modulated by different signaling pathways. The increase in the production of reactive oxygen species (ROS) after the intake of AMPH, besides producing brain damage, can modulate the action of these kinases. Scavenging ROS may be a way to avoid the toxic effects of oxidative stress induced by AMPH. Tempol is an antioxidant that has neuroprotective activity, diminishing the presence of oxidative markers in the brain. It has been shown that Tempol interferes with the development of behavioral sensitization induced by cocaine. We evaluated the effect of Tempol and AMPH in the phosphorylation level of DAT using rat Nucleus Accumbens synaptosomes that were stimulated with AMPH, Tempol and Tempol before AMPH. We also measured the concentration of dopamine in the medium where the synaptosomes were incubated by microdialysis and electrochemical detection. Western Blot analysis showed that AMPH increased the phosphorylation of DAT and that the presence of Tempol avoided this increase. The extracellular

concentration of dopamine in the presence of AMPH alone was significantly increased. No changes were observed in the presence of Tempol alone. Interestingly, AMPH in the presence of Tempol induced a lower increase in dopamine concentration. Taken together, these findings suggest a role of ROS in the mechanism by which AMPH increases dopamine extracellular levels in Nucleus Accumbens.

Pharmacology area: Farmacodinamia (Pharmacodynamics)
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9. COLCHICINE COMPETITIVELY ANTAGONIZES THE ALPHA 3 SUBUNIT OF THE GLYCINE RECEPTORS.
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Glycine receptors (GlyRs) are anion-selective neurotransmitter-gated ion channels, member of the pentameric Ligand Gated Ion Channels (pLGICs) family. GlyRs are essential players in the physiology of the central nervous system and impairment of its function underlie many neurological diseases, including epilepsy, autism, chronic pain, anxiety and schizophrenia among others. The function of GlyRs containing the alpha1 or alpha2 subunits, can be competitively inhibited by colchicine independently of microtubule depolymerization. Interestingly, a recent report showed that colchicine binds directly to the GlyRs containing the alpha3 subunit, suggesting that the alpha3 GlyRs mediated the suppression of the inflammatory pain exerted by colchicine. However, the functional effects on the alpha3 GlyRs function elicited by colchicine are still undefined. Using electrophysiological techniques and molecular docking simulations, here we show that colchicine is an inhibitor of the alpha3 GlyR function. Colchicine, elicited concentration-dependent inhibitory effects on alpha3 GlyRs at micromolar range. Single-channel recordings show that the colchicine inhibition is associated with a decrease in the open probability of the ion channel. Molecular docking assays suggest that colchicine preferentially bound to the orthosteric site in an agonist-free, closed state of the ion channel. Our results thus define the pharmacological modulation of colchicine on alpha3 GlyRs.
Pharmacology area: Farmacología molecular (Molecular Pharmacology)
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10. CHARACTERIZATION OF THE NEUTROPHIL/LYMPHOCYTE RATIO IN A SAMPLE OF PATIENTS WITH RHEUMATOID ARTHRITIS.
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Introduction: Rheumatoid arthritis (RA) is a systemic disease, still unknown etiology and autoimmune character characterized by chronic inflammation in the synovial membrane. The activity of the disease in RA is determined with DAS28 index, which allows evaluating the efficacy of the drug therapy administered. Monitoring the evolution of the disease activity is essential to avoid disability in long term. The increase in the neutrophil/lymphocyte ratio (NLR) has been described as a parameter of inflammation associated with a predominance of neutrophils and a decrease in lymphocytes. This NLR relationship has proven to be a good predictor of inflammation in chronic diseases such as diabetes and cancer. Objective: To characterize the NLR relationship in patients with RA. Methods: 11 AR patients and 11 controls of similar ages and same sex were recruited and signed an informed consent approved by Ethical Scientific Committee. A blood sample was performed on a Sysmex XS-1000i device. The absolute values of neutrophils were divided by lymphocytes. The values of DAS28 and erythrocyte sedimentation rate (ESR) were analyzed. Statistical tests were used for variables. Results: The NLR was higher in RA patients, 2.72±0.68 versus controls with 1.53±0.38 p<0,0001. The classification of disease activity according to DAS28 shown that NLR was 3.69 ± 0.25 for high activity, 2.49 ± 0.29 for moderate and 2.23 ± 0.32 for low and the coefficient of HSV-NLR correlation was 0.34 and DAS28-NLR was 0.815. Conclusion: The NLR was higher in AR patients than in the control group. It is shown that NLR correlates positively with DAS28 and HSV. In addition, the NLR was higher for patients with high disease activity, compared to moderate and low.

Pharmacology area: Otros (Others)
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11. GPER ANTAGONISM OF THE POTENT ANTHOCYANIN-INDUCED VASODILATION AND NO PRODUCTION IN A VASCULAR BED AND ISOLATED ENDOTHELIAL CELLS.
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Anthocyanins are colored water-soluble flavonoids present in red-bluish berries, fruits and vegetables. Flavonoids conserve structural similarities with other molecules such as steroids. Genistein is recognized as a natural phytoestrogen with potent estrogenic activity. Considering the potent vasodilation elicited by the glycosylated or anthocyanin aglycones is strictly endothelium-dependent, we proposed that the anthocyanin-induced vasodilation is mediated by the estrogen receptor coupled to a trimeric G protein (GPER) or estrogen receptor alpha (ERa), both associated to a rapid, non-genomic mechanism. We evaluated the vascular response induced by the anthocyanin delphinidin and its 3-O-glucoside derivative (D3G) in pre-contracted mesenteric vascular beds in the presence or absence of 1μM-G36, a purported GPER receptor antagonist, or 1μM fulvestrant a recognized ER α/β antagonist. Also, we quantified the NO production elicited by

anthocyanidins by chemiluminescence. Both and D3G elicit concentration-dependent vasodilation as potent as acetylcholine used as a standard control and 10 to 100 times more potent than 17-beta-estradiol (E2) and genistein respectively. The anthocyanins activity is dependent on the endothelium and eNOS enzymatic activity (97-98%, p <0.001). G36 significantly reduced the anthocyanins vasodilation (p <0.01) such as G-1, GPER agonist (p <0.001). However, the anthocyanins response was not blocked by fulvestrant, which decreased E2 activity by 28%. The perfusion of the mesenteric vascular bed with 100 nM or 1 µM of delphinidin and D3G, or its application to endothelial cells culture, increased NO production compared to the controls. We conclude that anthocyanins, glycosylated or not, induce a potent vasodilator response mediated by NO production that may be linked to GPER activation at the vascular level. In addition, the anthocyanin mechanism is rapid and non-genomic in nature.

Pharmacology area: Farmacología cardiovascular (Cardiovascular Pharmacology)
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12. MOLECULAR CHARACTERIZATION OF HEAD AND NECK SQUAMOUS CELL CARCINOMA (HNSCC) 3D MODEL AND METASTATIC CAPACITY EFFECTS OF OLD DRUGS WITH NEW ANTITUMOR ACTIVITY.

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Head and neck squamous cell carcinoma (HNSCC) has an incidence in worldwide of more than 350000 people and the mortality associated with this cancer is 50%. Although the treatment for these patients generally consists of surgical, pharmacological and radiation therapies, survival at 5 years is only 53%. One of the reasons for high mortality and low survival is due to the presence within tumor mass a subset of cells called cancer stem cells (CSCs), which represent the most tumorigenic subpopulation. This population grows like spheroids and in its center has hypoxic cells that are highly resistant to chemotherapies. Here, the goal was to obtain in vitro spheroid CSC from HNSCC (HNCSCs) and determine expression protein level involving on metabolism, hypoxia, autophagy, and antitumor target, in addition to assess the effects of Itraconazole and hydroxychloroquine on invasion capacity on spheroid cultures. Spheroid formed from Cal27 and HEp-2 cell lines using culture selecting conditions and protein expression levels was determine by immunoblotting. Spheroid was treated with Itraconazole and hydroxychloroquine and then seeded over matrigel to Boyden chamber 3D invasion assays. Successful formation of spheroid from the Cal27 and Hep-2 cell lines, where needed 4500 and 3500 cells respectively to obtain sizes greater than 300 µm. Spheroids were also subjected to hypoxic conditions with oxygen concentrations below 5%, VDAC, PDK1, Hexokinase II, Hif-1 and LC3B protein expression levels were different between cell line and monolayer or spheroid cultures. HNCSCs exposure to Itraconazole and hydroxychloroquine modulate invasive capacities, compared with control conditions. Here we showed that protein expression patterns are different between monolayer and spheroid cultures and the effects of two different drugs on metastatic capacity of HNCSCs.

Pharmacology area: Farmacología molecular (Molecular Pharmacology)
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13. MESENCHYMAL STEM CELL SECRETOMES (MSCSS) ADMINISTRATION IMPROVES BEHAVIOURAL DEVELOPMENT, MOTOR AND COGNITIVE IMPAIRMENTS INDUCED BY PERINATAL ASPHYXIA.

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Perinatal asphyxia (PA) induces deficits in neurological reflexes, development, motor coordination, emotional behaviour and cognition. At present, no treatment significantly attenuates or prevents these sequelae. Mesenchymal stem cells (MSCs) have been proposed as a therapeutic tool for several CNS diseases, since MSCs display remarkable antioxidant, anti-inflammatory and repairing features (neurogenesis, synaptogenesis, myelination). MSCs exert paracrine effects by secreting a combination of nano-vesicles and soluble factors (referred to as secretomes). Preconditioning MSCs with pro-inflammatory cytokines (TNFalfa plus INFgamma) or hypoxia-like environment (deferoxamine) improves their effectiveness. The aim of this study was to determine whether intranasal administration of secretome derived from preconditioned human MSCs prevents the (i) behavioural development, (ii) motor and (iii) cognitive disabilities resulting from PA. PA was induced by immersing foetuses-containing uterine horns into a water bath at 37 °C for 21 min. Two hours after birth and at postnatal day (P)7 MSCSs (6 ug/16 ul, obtained from 1x10^6 preconditioned-MSCs) or 16 ul of vehicle were administered intranasally to asphyxia-exposed or control rats. Neurobehavioral development was evaluated by monitoring the righting reflex (at P1, P4, P7 and P14); negative geotaxis (P7, P14 and P21), and cliff aversion (P7, P14 and P21). Locomotor activity (P7) and motor coordination (P60) were evaluated by open field and rotarod, respectively. Anxiety (P30), by open field and novel object recognition memory (P30). All the PA induced effects were positively affected by MSCSs treatment, including improvements in: (i) the remarkable developmental delay in the performance of righting and cliff aversion reflexes; (ii) the decrease in locomotor activity and deficits in motor coordination and balance; (iii) an increase in anxiety and novel object recognition memory deficits.

Pharmacology area: Neuropsicofarmacología (Neuropsycopharmacology)
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14. INHIBITORY EFFECT OF COUMARINS DERIVATIVES ON THE VIABILITY OF HELICOBACTER PYLORI ATCC 43504 AND RECOMBINANT CARBONIC ANHYDRASE ACTIVITY.

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Helicobacter pylori (Hp) is a Gram negative pathogen that affects more than 50% of the world population, and it is responsible for different gastric pathologies. Since increasing antibiotics resistance cause serious failure in Hp eradication therapy, new pharmacological targets need to be identified to affect the viability of this bacteria. Carbonic anhydrase (CA, EC 4.2.1.1) is an interesting new Hp target since it is involved in neutralizing the acid pH of the human stomach cooperatively with urease. It has been described that specific coumarin derivatives can inhibit CA. Besides, several coumarins exhibit a strong antibacterial activity. Objective: to clone and characterize carbonic anhydrase from the highly aggressive strain Hp ATCC 43504 and to determine the coumarins derivatives effects on both, the recombinant protein and Hp viability. Methodology: a-CA was cloned, expressed in cell line HEK 293 and purified. Recombinant α-CA was characterized by esterase activity and protonography. We determined the inhibitory effect of coumarins derivatives against recombinant α-CA by esterase activity and on Hp to determine its MIC and MBC. Results: A 50 kDa functional recombinant Hp a-CA was cloned that forms a dimer structure in solution. The recombinant Hp a-CA it is closely related to F78 strain, with a 99.1% of identity. Esterase activity was determined, complemented by protonography analysis, and kinetic parameters. Overall, six coumarins showed inhibitory activity against Hp, with MIC ranging from 125 ug/mL to 15 ug/mL, and two coumarins were inhibitors of the recombinant protein. Conclusion: The characterization of this highly aggressive Hp CA strain and the determination of inhibitory effects of coumarins, makes them a potential candidate for Hp therapy and eradication.

Pharmacology area: Farmacología de Productos Naturales (Natural Products Pharmacology)
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15. IDENTIFICATION OF POTENTIAL CANDIDATE GENES RELATED TO HYPOXIA INDUCIBLE FACTOR 1 ALPHA (HIF-1 ALPHA) INVOLVED IN PERINATAL ASPHYXIA IN RATS.

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Perinatal asphyxia (PA) is characterized by interruption of oxygen bioavailability at birth. Hypoxia implies HIF-1 alpha (HIF-1a) activation, a key sentinel protein, which, upon translocation to the nucleus, binds to response elements (HREs), promoting transcription of several genes. Potential HIF-1a activated genes were identified in the rat genome following hypoxic conditions, by extracting promoter sequences of Rattus Norvegicus from the UCSC database Genome Browser. 8762 genes with the HIF-1a binding sequence (5`-RCGTG-3`) were identified using the "R" software. These genes were introduced to the Gen Ontology platform for performing an enrichment analysis, selecting the following processes linked to PA: (i) Hypoxia (865 genes); (ii) Glucose Metabolism (330 genes); (iii) Neurogenesis (1243 genes); (iv) Apoptosis (814 genes); (v) Angiogenesis (165 genes), and (vi) Regulation of Gene Expression (2076 genes). 865 hypoxia-associated genes were further selected and compared with experimental data by ChIP-Seq, with 772 and 98 genes from human and zebrafish genomes, respectively, identifying 79 genes for the three species. The 8762 genes were then analysed by the Kyoto Encyclopedia of Genes and Genomes (KEGG) platform, selecting the HIF-1 pathway, identifying 47 genes. The 79 genes filtered for human, zebrafish and rat were compared with the 47 genes obtained by the KEGG platform, yielding 12 genes. Finally, 12 genes were compared with 47 genes referred by the literature to be associated to PA, identifying 5 genes: (i) Bcl2; (ii) Hif-1a; (iii) Ldha; (iv) Pdk1, and (v) Vegfa. The pharmacological inhibition of HIF-1a to establish the gene expression levels of candidate genes in an in vivo model of PA is studied, providing a proof-of-principle for the participation of HIF 1a on the regulation of gene expression following PA.

Pharmacology area: Farmacología molecular (Molecular Pharmacology)
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16. RESOLVIN D1 INCREASES COLLAGEN-1 SYNTHESIS ON RAT CARDIAC MYOFIBROBLAST THROUGH ALX/FPR2 RECEPTOR ACTIVATION.

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Background: Cardiac fibroblast (CF) to cardiac myofibroblast (CMF) differentiation is mainly mediated by TGF-b1 released from immune cells by angiotensin II (Ang II) effect, or in vitro conditions due to mechanical stress. CMF are able to produce extracellular matrix proteins, mostly collagen, in the scar formation proccess. Also, in the CF-to-CMF process it has been demonstrated the upregulation of Kinin-B1 and AT1R receptor, among others. However, the presence of Resolvin D1 (RvD1) receptor, ALX/FPR2, has not been elucidated. RvD1 is an anti-inflammatory lipidic mediator that regulates matrix proteins like a-SMA and collagen in various cell types, yet there is not evidence of this effect on CMF. Purpose: To demonstrate that the CF-to-CMF differentiation increases ALX/FPR2 protein levels, and consequently, ALX/FPR2 activation by RvD1 enhances collagen-1 synthesis. Methods: Secondary culture of adult rat CF was starved for 24 hours, stimulated with TGF-b1 (10 ng/mL) for 72 hours on fetal bovine serum (FBS) 1% to induce CMF differentiation and then treated with RvD1 at different intervals in presence and absence of PD98059, LY294002 (ERK ½ and AKT inhibitors) and WRW4 (ALX/FPR2 antagonist). The proteins levels of p-EKR ½, p-AKT and collagen-1 were measured by western blot. Results: After CMF differentiation, it was found an increase of ALX expression. On CMF, RvD1 activated the ERK ½-AKT pathways and enhanced the expression of collagen-1. These effects were blocked by PD98059, LY294002 and WRW4. On the other hand, RvD1 did not decrease α-SMA protein levels, which suggests that it does not reverse CMF differentiation. Conclusions: Our results suggest that the CF-to-CMF differentiation increases the protein levels of ALX/FPR2, and that RvD1 enhances collagen-1 synthesis through ERK ½ and AKT pathways.

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17. PROCYANIDINS-RICH EXTRACT NANOEMULSIONS O/W AS A POTENTIAL ANTITUMORAL TOOL: EVALUATION IN MELANOMA CELL LINE.

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During the last 10 years, the incidence of melanoma in Chile has incremented 27.2%. Approximately 80% of all skin cancer-related deaths are attributed to melanoma and long-term survival of patients is only 5%. This type of cancer is considered a multifactorial disease related to environmental interaction and genetic susceptibility that trigger a constitutive activation of several signaling pathways, promoting proliferation and survival of tumoral cells. Current therapies cause a considerable number of side effects, still representing a global human health issue. Recently, the use of polyphenols has been reported for both prevention and treatment of melanoma. Some evidence demonstrates that different extracts of polyphenols (grape, pomegranate, among others) are high potential candidates to treat various types of cancer. Of note, the main limitations of these molecules are the low bioavailability and the necessity of a high concentration dose to cause a biological effect. The aim of this study was to utilize the nanotechnology to encapsulate procyanidins and to evaluate its antitumoral activity in B16F10 melanoma cell line. To achieve the proposed objective, an avocado peel procyanidin-rich extract was used and cellular viability, proliferation and migration in B16F10 cells were evaluated. Nanoemulsions were elaborated by solvent evaporation and were in a nanometric range of 170 ± 2 nm and showed low polydispersity (between 0.1 and 0.2). The nanoformulations showed negative zeta potential (−44 ± 4 mV), realizing a stable system. The administration of procyanidin-rich extract nanovehicles was more efficient in reducing cellular viability, proliferation and migration compared to free extract. Altogether, our results suggest that encapsulation of procyanidins significantly improves its effect on melanoma therapy.

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18. MATERNAL HIGH-FAT DIET CONSUMPTION DURING PREGNANCY AND LACTATION IMPAIRS THE INHIBITORY SYNAPTIC TRANSMISSION OF CA1 REGION OF HIPPOCAMPUS OF THE YOUNG OFFSPRING.

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Before birth and early in life the brain development is acutely sensitive to its environment. Experimental and clinical data indicate that maternal obesity can predispose the offspring to suffer metabolic and neuronal alterations. Most studies have focused on the functional relationship between maternal obesity and hypothalamus alterations. However, the impact of maternal nutrition on other brain areas remains elusive. Recently, it has suggested that fetal exposure to maternal obesity causes decreased neurogenesis and impaired hippocampal learning. The hippocampus is important for learning and memory, and its development is sensitive to the metabolic environment in utero. In a model of maternal obesity induced by a high-fat diet (HFD, 60Kcal in fat) consumption we

study whether this adverse prenatal environment impairs the hippocampal synaptic transmission. In offspring mice, during adolescence, using electrophysiological recordings in the CA1 area of mice hippocampus, we observe that maternal HFD consumption increases the frequency and amplitude of Inhibitory postsynaptic currents. This increase in inhibition level onto pyramidal neurons could have important consequences in the excitation/inhibition balance, being able to modify the hippocampal cognitive function in juvenile offspring mice.

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19. PHARMACOKINETIC VARIABILITY IN PATIENTS WITH KIDNEY TRANSPLANTATION, TREATED WITH CYCLOSPORINE.
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Introduction: Cyclosporine (CsA) is an immunosuppressive drug, used to prevent organ rejection in transplant patients. However, this drug is characterized by having a narrow therapeutic range and high inter and intra-individual pharmacokinetic variation. There are some genetics variants involved in pharmacokinetic variability, both at the level of cyclosporine absorption and metabolization. However, genetic variants of relevant impact are not yet identified. Objective: to evaluate the genetic variants involved in the pharmacokinetics of cyclosporine and to establish an association between the pharmacogenetic profile, and the safety and efficacy of the treatment during the first three months after transplantation. Methodology: One hundred and seven patients (107) with kidney transplants from San Juan de Dios Hospital (Project No. 028-13) were retrospectively included and were genotyped for the genetic variants CYP3A4 *1B rs2740574, CYP3A4*22 rs25599367, CYP3A5*3 rs776746, POR*28 rs1057868, MDR1 3435 rs1045642, MDR1 2677 rs2032582 and MDR1 1236 rs1128503. Genotypic frequency were associated with blood concentrations of cyclosporine (CsA), creatinine value and blood pressure within the first three months post-transplant. Results: Heterozygous patients for CYP3A4*1B had a lower creatinine value from the second post-transplant week. In relation to cyclosporine levels, an increase was observed, in patients with at least one altered allele for CYP3A5*3 from the second post-transplant week. Conclusions: The results show that genetic variants can account for variations in the pharmacokinetic parameters of cyclosporine, which affect the efficacy and safety of cyclosporine treatment. It is expected that, based on the results found, a genetic predictive panel of response to CsA will be setted-up to be used before transplantation.

Pharmacology area: Farmacología molecular (Molecular Pharmacology)
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20. ANTIFUNGAL AND ANTIBIOFILM EFFECT OF OREGANUM VULGARE ESSENTIAL OIL AGAINST CANDIDA ALBICANS AND NON-ALBICANS.
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Oral candidiasis is the most common fungal infection in humans. The most frequent causative agent isolated is Candida albicans (C. albicans), but the number of resistant strains such as Candida krusei (C. krusei), Candida tropicalis (C.tropicalis) and Candida Glabrata (C.glabrata) has increased. It is treated locally with miconazole and nystatin; however, patients present high recurrence rates due to the formation of biofilms. Biofilms are biological communities adhered to a surface (oral mucosa, dental prostheses) with high resistance to antifungals, which has driven the search for natural alternative therapies, such as essential oils. Oregano essential oil (OE-O) is effective and a therapeutic option on bacterial biofilms, but its activity on fungal biofilms is unknown. Methods. The minimum inhibitory concentration (MIC) was determined on reference strains for C.Albicans (fluconazole sensitive and resistant) and non-albicans strains (C.tropicalis, C.krusei and C.glabrata). The antibiofilm effect was evaluated by: a) morphogenesis inhibition assay: an inoculum was incubated for 5 hours at 37°C in the presence of the OE-O MIC, then the percentage of filamentous cells was counted using a Neubauer chamber; and b) inhibition of biofilm adhesion: an inoculum was incubated in a 96-well plate in the presence of the CIM of OE-O for 4 hours at 37°C, the adhered biofilm was stained with crystal-violet and absorbance was measured in microplate reader. Fluconazole and nystatin were used as controls. Results. AE-O significantly inhibited both biofilm adhesion and morphogenesis of the strains studied compared to controls. Conclusion. These results indicate that OE-O has significant antibiofilm activity in both C. albicans and non-albicans strains.

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21. CHRONIC EXPOSURE TO MODAFINIL IN JUVENILE RATS MODIFIES DOPAMINE, GLUTAMATE AND GABA EXTRACELLULAR LEVELS IN NUCLEUS ACCUMBENS
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Modafinil (MOD) is a psychostimulant that enhances wakefulness and vigilance. The proposed mechanism of action of MOD is the blockage of dopamine (DA) transporter. Moreover, some studies showed that MOD affects glutamate and GABA release in striatum, ventral pallidum and nigro-striatal nuclei. Since clinical trials are testing MOD for the treatment of attentional deficit disorder (ADD), evaluating the effects of MOD in healthy young individuals is important, considering a high rate of overdiagnosis. Herein, we evaluate the effects of MOD on glutamate, GABA and DA extracellular levels in nucleus accumbens (ACb) of juvenile rats.
Eleven male juvenile *Sprague-Dawley* rats were treated from PND22 with MOD (75 mg/kg i.p.) or vehicle for 14 days. Micro-dialysis in the ACb were performed 24 hours after the last injection of MOD to measure DA by HPLC coupled to electrochemical detection and glutamate and GABA by HPLC coupled to fluorometric detection.
We observed that both groups increase their DA release after K^+ 70 mM stimulus, however being significant only for control rats. Regarding glutamate and GABA, animals treated with MOD had higher basal extracellular levels in ACb than control group. Interestingly, our previous data showed no differences in DA, GABA and glutamate tissue content in ACb or VTA between treatments. Although, there is a tendency for lower tissue levels for DA, glutamate and GABA in ACb in the treated group.
These results show that MOD affect the reward circuitry in young animals which could impact on socialization, as we previously demonstrated. More studies are needed to unravel the effects of stimulants, specially on young population, over important social skills like playing, social interactions and memory.

Pharmacology area: Neuropsycopharmacology
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22. HIGH-FAT DIET EXPOSURE INCREASES THE EXPRESSION OF KEY PROTEINS IN DOPAMINERGIC NEUROTRANSMISSION OF RAT LATERAL SEPTUM.

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Obesity is a global pandemic that must be studied from many points of view, such as social, preclinical, clinical, economic, etc. At the level of the central nervous system, there are several structures involved in the control of food intake, being one of the most important to promote the feeding the lateral hypothalamus (LH). The main brain area that controls the neural activity of LH is the lateral septum (LS), which it sends GABAergic projections towards LH, controlling feeding behavior. In addition, LH projects glutamatergic/orexinergic neurons to the ventral tegmental area (VTA), which it sends dopaminergic projections to the nucleus accumbens and LS. This circuit is very important for the intake of rewarding foods, but it is not known the effects of chronic exposure to high-fat diet (HFD) on LS neurotransmission. For this work we used 2 groups of male Sprague-dawley rats exposed from weaning to postnatal day 60 (PND 60) to chow diet (control) and HFD. At PND 60 the animals were euthanized and the LS was microdissected to measure by western blot key proteins involved in LS dopaminergic neurotransmission. Our results demonstrate that exposure to HFD results in a significant weight gain at the end of the experimental period together with an increase in retroperitoneal fat levels. In LS the chronic exposure to HFD resulted in an increase in the expression of the type 2 dopamine receptor (D2) and the dopamine transporter (DAT) compared to control rats. These results suggest that these proteins functionally reduce the dopaminergic tone in LS, which would affect their inhibitory control over LH activity. However, the implication of these results will be evaluated in subsequent experiments.

Pharmacology area: Neuropsicofarmacología (Neuropsycopharmacology)
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23. BOLDINE PREVENTS TGF-B1-INDUCED DIFFERENTIATION OF CARDIAC FIBROBLASTS BY INHIBITING FOXO1.

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Diabetes and myocardial infarction promote the development of cardiac fibrosis. Normally, cardiac fibroblasts (CF) are responsible for the synthesis and maintenance of extracellular matrix components (ECM), whereas in pathological conditions CFs differentiate into cardiac myofibroblasts, generating an imbalance in the secretion of the ECM proteins. TGF-beta1 plays a crucial role in the development of cardiac fibrosis by regulating the expression of FoxO1, a transcription factor that is involved in functions such as apoptosis, oxidative stress and cell differentiation. On the other hand, boldine, a natural alkaloid, has been shown to exert antifibrotic effects in experimental models of diabetes. Therefore, this work attempted to demonstrate the antifibrotic effect of boldine in a model of cardiac fibrosis in vitro. To respond to this

hypothesis, the differentiation of adult Sprague-Dawley rats CF by TGF-beta1 was used as in vitro model of cardiac fibrosis. The differentiation of CF was determined by the expression of alpha-SMA and CTGF, through western blot (WB) and immunocytochemistry (ICQ). On the other hand, the activation of FoxO1 was evaluated by analyzing phospho-FoxO1 (WB) and nuclear location of FoxO1 (ICQ). AS1842856 a FoxO1 activity inhibitor was used to evaluate the role of FoxO1. TGF-beta1 10ng/ml increased the expression of alpha-SMA and CTGF at 48h, which was corroborated by an ICQ against alpha-SMA. In addition, TGF-beta1 increased the activity of FoxO1, which was determined by decreased phosphorylation of FoxO1 and increased nuclear localization, whereas inhibition of FoxO1 prevented the differentiation of CF induced by TGF-beta1. Finally, boldine 50uM and 100uM abolished the differentiation of CF and the activation of FoxO1 induced by TGF-beta1. Collectively ours results suggest that boldine prevents TGF-beta1-induced CF differentiation by inhibiting FoxO1.

Pharmacology area: Farmacología cardiovascular (Cardiovascular Pharmacology)
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24. CHEMICAL-BIOLOGICAL CHARACTERIZATION OF ANTIOXIDANT PIGMENTS PURIFIED FROM EXTRACTS AND FRACTIONS OF PENICILLIUM MURCIANUM AND ITS POTENTIAL COSMETIC APPLICATIONS.

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In order to find new sources of active metabolites as functional ingredients for the cosmetic industry, the idea of studying filamentous fungi that produce natural pigments arises. These compounds meet the requirements of having low toxicity, adverse effects, and generation of polluting waste or expensive raw material. Therefore, due to their nature and structural diversity they could have great potential for cosmetic use, for example, as hair dyes. To characterize the fungal pigments and evaluate their antioxidant effect, Penicillium murcianum species was selected. This fungus is an eco-type found in Chilean native forests, which was cultivated under conditions previously optimized for the production of brown-yellow pigments. To facilitate the chemical-biological analysis, the extract obtained from the culture broth was fractionated by Centrifugal Partition Chromatography (CPC) with a phase system of ethyl acetate: butanol: water. The above allowed to purify several fractions, which were evaluated for their antioxidant activity in the DPPH assay. These fractions recorded an inhibition close to 90% and an IC50 value of 4.64 mg / mL for the crude extract. In the case of the purified fractions, two were selected with the highest antioxidant activity corresponding to an IC50 of 1.17 mg / mL and 0.708 mg / mL, which were subsequently analyzed by HPLC / PAD / MS. Our preliminary results revealed the presence of azafilones such as monashexenone, monankarin and monaphilol and the anthraquinoids sterigmatocystin, endocrocin and flavokermesic acid, which would be responsible for the yellow pigmentation of the extract. These compounds have bibliographic antecedents related to antioxidant and antimicrobial activities, among others, which highlights a great opportunity for future research and applications for these metabolites of P. murcianum.

Pharmacology area: Farmacología de Productos Naturales (Natural Products Pharmacology)
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27. IN VITRO PROPAGATION OF RODOPHIALA PRATENSIS AND ITS TOXICITY IN VITRO ON EPITHELIAL CELLS OF GASTRIC ADENOCARCINOMA (AGS).

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Amaryllidaceae is a family of bulbous plants, producers of alkaloids which are biogenetically related and exhibit high pharmacological activity. Lycorine and homolycorine alkaloids have been studied as potent antitumor agents. However, the study of these molecules is difficult due to the low availability and production in the plant in the wild, so the objective of this study is by in vitro propagation to obtain biomass of R. pratensis in an efficient and sustainable way, to identify the type of Alkaloids that are produced and assess their cytotoxic potential on tumor cells. Methodology: Rhodophiala prantesis bulbs, were sterilized and cut into twin-scales, to sow them in Murashige-Skoog growth medium, supplemented with sucrose and different combinations of naphthalenacetic acid and 6-benzylaminopurine, the alkaloid analysis was performed by CG-MS. AGS cells were cultured in DMEM medium supplemented with SBF (10%), antibiotic (1%). The assay was performed in 96-well plate using resazurin at 6 and 24 hours after exposure of the alkaloid extract. R. pranthesis callus were obtained in in vitro culture in semi-solid medium. In addition, 25 alkaloids were identified in the bulb's alkaloid extract which decreased the viability of AGS cells at 6 and 24 hours of exposure. Conclusion: Hormonal combinations were evaluated for the production of callus of R. pratensis, in addition the alkaloids have cytotoxic activity on AGS as a function of exposure time.

Pharmacology area: Farmacología de Productos Naturales (Natural Products Pharmacology)
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26. SYNERGISTIC EFFECT OF GENTISIC AND GALLATE DERIVATIVES WITH STANDARD THERAPY FOR COLORECTAL CANCER CELLS.

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Colorectal cancer (CRC) is the third leading cause of cancer death in the world. The standard drugs currently used for the treatment of CRC are 5-fluouracil (5-FU), oxaliplatin and irinotecan. These chemotherapeutic agents are effective in the early stages of the disease, presenting high toxicity and many side effects. Therefore, new therapies directed to the metastatic cells of the CRC are urgently needed, with high pharmacological efficacy, reducing the side effects and treatment costs. In recent years, neoplastic mitochondria are an attractive pharmacological target for cancer therapy, since they have higher mitochondria potential. In our laboratory, gallic acid derivatives linked to an aliphatic chain of ten carbons associated with triphenylphosphonium (TPP+C10), a lipophilic cationic molecule that induces the uncoupling of the electron transport chain was synthesized and evaluated in CRC cells as well as gentysic derivative (GA-TPP+C10).The objective of this study is to evaluate the synergistic effect of the compounds, GA- TPP+C10 and TPP+C10 in combination with conventional drugs for the treatment of CRC, 5-FU and oxaliplatin, using the COLO 205 metastatic human CRC cell line. Through the MTT assay, the cytotoxicity of the combinations was evaluated after 48 h of treatment and by flow cytometry the synergy of the combinations inducing apoptosis was evaluated. The observed results showed that bothTPP+C10 and GA-TPP+C10 are synergistic with low concentrations of 5-FU and Oxaliplatin, inducing greater apoptosis of the colorectal cells. In conclusion, the results suggest that these new compounds could synergize the effects of conventional therapy against colorectal cancer.

Pharmacology area: Farmacología molecular (Molecular Pharmacology)
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27. CHANGES IN DOPAMINE RECEPTOR TYPE 2 EXPRESSION IN PREFRONTAL CORTEX INDUCED BY EARLY-LIFE INTESTINAL DYSBIOSIS.

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Intestinal microbiota has been shown to modulate central nervous system function. For instance, a reduction in the gut's microbiota richness and diversity through the use of antibiotics, sensitizes mice to drug seeking behaviors. Furthermore, this behavior is driven by changes in dopamine receptor expression within the mesocorticolimbic system, which strongly supports the relevance of the microbiota-gut-brain axis in the development of addictive behaviors. In most animals, early-life gut colonization by microbial symbionts occur begins at birth, with bacteria coming from the mother, and is a process that happens in parallel with early stages of brain development. Therefore, changes in maternal gut microbiota richness and diversity would impact on the development of neuropsychiatric diseases later in life, including addiction. To test this, we administered pregnant Spraque-Dawley dams a cocktail of oral wide-spectrum antibiotics (neomicyn, bacitracin, vancomycin and pimaricin) from embryonic day 18 till post-natal day (PND) 7, and then evaluated dopamine receptor 2 (D2) expression in the prefrontal cortex (Pfx) of female and male offspring at PND60, and compared with the offspring of pregnant dams given saline. The results show that Pfx D2 expression in female offspring of antibiotic exposed dams is reduced (although not significant) when compared to age matched controls, but no differences were observed in the male offspring. These results suggest that a reduction in the diversity and richness of gut microbes during early-life provokes changes in PFx D2 expression in females, but not males, and furthermore, this changes might impact the female's drugs seeking behaviors.

Pharmacology area: Neuropsicofarmacología (Neuropsycopharmacology)
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28. EARLY-LIFE EXPOSURE TO ORAL WIDE-SPECTRUM NON-ABSORVABLE ANTIBIOTICS AND THEIR EFFECTS ON DOPAMINE RECEPTOR 2 EXPRESSION IN THE SUBSTANTIA NIGRA OF SPRAGUE-DAWLEY RATS.

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Microbial gut colonization begins at birth, where the transfer of intestinal microbes from mother to infant is key in early-life development of gut, immune and brain physiology. We have previously shown that exposing pregnant Sprague-Dawley

dams to an oral cocktail of wide-spectrum non absorbable antibiotics (neomycin, bacitracin, vancomycin all three at a dose of 100 mg/kg and pimaricin at 5microg/kg) from embryonic day 18 until post-natal day (PND) 7, lowers intestinal microbial richness and diversity in the male offspring at PND35. Additionally, early-life exposure to antibiotics lowers dopamine receptor 1 (D1) expression in key areas of the mesocorticolimbic circuit of male offspring when compared to age matched controls that were not exposed to antibiotics perinatally. This suggest that early-life exposure to antibiotics, which affects gut microbial ecology, impacts on dopaminergic circuits related to reward. However, another question was raised: what happens in substantia nigra (SN), another major source of dopamine that is also involved in reward and motor function. Thus, we evaluated D2 expression in the SN of males (PND35) from Sprague-Dawley dams exposed to the aforementioned cocktail of antibiotics, and compared it with control rats. Immunohistochemical analysis revealed that early-life exposure to antibiotics does not affect D2 expression in comparison to control rats. This result suggest that reduction of microbial diversity and richness in early life, affects specifically the mesocorticolimbic circuit, with no effects on the D2 expression in the SN. Such specific effect further suggests that within the microbiota-gut-brain communication, there are very specific pathways that may in part underlie the basis of neuropsychiatric disorders, such as addiction.

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29. MECHANICAL STIMULATION INCREASES EXTRACELULLAR ATP AND NO SECRETION TO THE MEDIA OF CELL CULTURES; PHYSIOLOGICAL IMPLICATIONS.

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As a requirement of many protocols it is common to add drugs to cell cultures to investigate cell processes in the presence of a determined pharmacological agent. However, if this procedure is not well controlled by appropriate standards, it may cause additional experimental variability. Our working hypothesis proposed that pipetting/agitation of the culture medium causes extracellular ATP secretion, which in the case of endothelial cells is also related to NO secretion. Endothelial cells of the rat mesentery, fibroblasts, and oocytes of Xenopus Laevis were used. In endothelial cell cultures, 100 uL of Tyrode buffer is gently applied; extracellular fluid sample is collected to measure ATP and NO production. ATP and metabolites are quantified as fluorescent ethene purines; separated and quantified by HPLC procedures. NO was determined by a fluorescent probe as the [DAF-NO] complex. Buffer application increases medium ATP from 117 ± 75.7 to 317 ± 25 pmoles ATP/mg protein ($p < 0.001$); the signal peaked by one minute, thereafter, ATP decays. This stimulus also rapidly and significantly increases NO production ($p < 0.0001$). This increase is blunted by 150 µM L-NAME, an eNOS inhibitor, and by apyrase (4 U/mL), suggesting the participation of extracellular ATP. The agitation of fibroblast culture medium in culture increases 4-fold ATP secretion, a transient effect that decreased in minutes. Likewise, Xenopus oocytes agitation by a variable inclination agitator increased 3.8 times extracellular ATP ($p < 0.01$). In conclusion, different mechanical stimuli secrete extracellular ATP in different cell types, suggesting that cells respond to chemical and sensory stimuli by increasing nucleotide secretion. The nucleotide surge may cause unexpected variations in the final cellular response due to indirect or direct purinoceptor activity.

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30. VAS DEFERENS EPITHELIUM REGULATES THE TISSUE MOTOR ACTIVITY INDUCED BY ELECTRICAL NERVE STIMULATION

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Respiratory, intestinal and / or vascular epithelial cells modify the activity of its adjacent smooth muscle layer. We studied whether the prostatic vas deferens epithelium modulate the motor response induced by electrical stimulation by releasing an epithelial messenger. To this aim, the isometric muscular contraction of the prostatic segment of the rat vas deferens intact or mechanically denuded of its epithelium was recorded using a force displacement transducer. The tissues were placed in a super fusion bath with Tyrode buffer at 37°C, 95% O2-5% CO2. Muscle tension was recorded by a Grass polygraph coupled to a transducer; contractions were induced by electrical field depolarization or with chemical stimuli. The muscular contraction induced by the exogenous application of 100 uM norepinephrine decreased following epithelium removal (1.5±0.2g versus 0.8±0.1g, n =10, $p<0.0038$), but not those elicited by ATP or KCl. Exogenous ATP, at concentrations that do not induce contractions (1-100 uM), reduced the motor effect induced by electric trains of 0.15 Hz. Epithelial removal reduced this ATP effect ($p < 0.05$). ATP reduces muscle contraction induced by 4 Hz trains, in the phasic component, epithelial removal causes a greater inhibitory effect of ATP ($p < 0.05$). The inhibition of NO synthesis with 100 uM N-omega-nitro-l-arginine does not modify the inhibitory effect of ATP on the electrical stimulus. In contrast, indomethacin increases the inhibitory effect of ATP on 0.15 Hz in the absence of epithelium, and on 4 Hz, both in the presence and absence of epithelium. Altogether, present results suggest that a non-identified arachidonic acid derivative, but not NO, sensitizes the motor response of the duct, demonstrating that the epithelium participates and modulates its motor activity.

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31. NEONATAL EXPOSURE TO ESTRADIOL VALERATE REDUCES THE NEUROCHEMICAL EFFECTS OF METHYLPHENIDATE IN THE NUCLEUS ACCUMBENS OF ADULT FEMALE RATS.

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Sex hormones produce several effects in reproductive and non-reproductive tissues. In this sense, brain expresses sex hormones receptors in cortical and limbic areas. Nigrostriatal and mesocorticolimbic pathways are modulated by sex hormones, affecting the expression of key dopaminergic proteins in adult animals. However, few studies have been focused in long-term effects produced by early exposure to sex hormones. Last time, our lab has been shown some neurochemical and behavioral changes produced by neonatal administration of sex hormones in brain dopamine areas such as higher levels of dopamine (DA), expression of tyrosine hydroxylase and addictive-like behaviors induced by morphine. Last time, we published that adult rats exposed during first hours of postnatal life to sex hormones had a lower locomotor activity induced by methylphenidate (MPD) than control rats and this effect was associated with a lower expression of the dopamine transporter (DAT) in nucleus accumbens (NAcc). Therefore, the aim of this work was studied the basal and stimulate (MPD: 5 mg/kg) extracellular levels of DA, Glutamate and GABA in NAcc of adult rats exposed during the first hours of postnatal life to estradiol valerate (EV: 0.1 mg/50µL). Our results showed a lower NAcc DA release induced by MPD in EV rats compared to control rats. In control rats we observed in NAcc a reduction in extracellular levels of GABA after MPD administration, however this effect was not observed in EV rats. In addition, basal and stimulate glutamate levels in NAcc were not different between experimental groups. These results suggest that neonatal exposure to EV affect the neurochemical response to psychostimulants in adulthood, which could be a vulnerability factor to increase the doses of abuse drugs.

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32. MITOCHONDRIAL EXPRESSION OF SVCT2 IN HIPPOCAMPAL NEURONS TREATED WITH OLIGOMERIC AB.

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Introduction: Alzheimer's disease (AD) is a neurodegenerative disorder characterized by progressive memory impairment and cognitive dysfunction. The most distinctive phenomenon correlating with AD is the presence of aggregates form of the β-amyloid (Ab) peptide. Experimental evidence has shown that the oligomeric form is able to induce reactive oxidative species (ROS). Ascorbic acid (AA) is an essential micronutrient with a preponderant role in oxidative stress and its main transport system in the brain is SVCT2, that incorporates the reduced form of vitamin C (ascorbic acid, AA), which is the prevalent form in vivo. Recent works show the relevance of AA in AD's progression by the inhibition of SVCT2, nevertheless, these studies didn't analyze how vitamin C is acquired by neurons and compartmentalized within organelles. In this work, we studied the subcellular localization of the SVCT2 transporter in primary cultures of mouse hippocampal neurons, and the changes in localization and expression levels associated to oligomeric Ab treatment. Material y methods: 18 days embryos hippocampus of C57BL/6 mice were dissociated and plated. Were cultured in vitro by 9 days until its treatment with oligomeric Ab. At 24 and 48 hours of treatment the subcellular localization of SVCT2 was evaluated, though immunofluorescence. Results: It was observed that hippocampal neurons show expression of SVCT2 which is located mainly at the mitochondria. Treatment with Ab oligomers increases the colocalization of SVCT2 with this organelle. Discussion: Our results suggest that mitochondrial AA might be relevant for neuronal survival in response to ab damage and suggest that this transporter would be a new therapeutic target to treat this disease.

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33. NOVEL CAFFEIC ACID DERIVATIVES AGAINST ORAL CANCER CELLS.

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The cancer is a cellular process characteristic by uncontrolled growth and dissemination, being the second cause of death worldwide. Likewise, the oral cancer is one of the ten most common neoplasm in the world, mainly concern the tongue. The gold standard therapies are surgery, which can be complemented with radiotherapy and/or chemotherapy. The latter, represent the first-line therapies, involving decreasing tumor size and abolishing microscopic disease. However, this cancer has small average of survival, several side effects and drug resistances. Therefore, new drugs are currently being studied, such as novel caffeic acid derivatives reporter before with antitumoral effect in breast cancer. We evaluated these new compounds by measurements of cytotoxic effect by MTT assay, mitochondrial effects by evaluation of mitochondrial-transmembrane potential by flow cytometry and ATP levels through luminescence assay. We showed that the derivates have cytotoxic effect in human cancer cell lines Cal27 and Hep-

2. We found that the compounds generated the decrease in cellular ATP levels and decrease the measuring ATP levels on human cancer cell line Cal27 and Hep of the mitochondrial-transmembrane potential. In addition, we evaluated the selectivity of these compounds by exposing normal epithelium cells to their action by evaluation of cytotoxicity. Our results demonstrated the novel caffeic acid derivates exert a selective cytotoxic effect by mitochondrial mechanism.

Pharmacology area: Farmacología Odontológica (Dental Pharmacology)
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34. CARDIOMYOCYTES HYPERTROPHY INDUCED BY CHRONIC STIMULATION WITH FRUCTOSE: BENEFICIAL EFFECT OF METFORMIN.

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Fructose intake has been increased in recent decades. Studies have indicated that high fructose consumption would be associated with an increase in the incidence of cardiovascular diseases (CVD) such as cardiac hypertrophy. Cardiac hypertrophy is an adaptive response to chronic work overload imposed on the heart, where cardiomyocytes undergo numerous morphological and functional changes. According to literature, fructose is able to decrease cell redox defenses and reduce adenosine monophosphate-activated protein kinase (AMPK) activation. AMPK activation is related to cardioprotective effects, and during hypertrophy allows the heart to change its metabolism. The objective of this work was to study the effect of fructose on cardiomyocyte hypertrophy and AMPK activation in the presence of metformin, a cardioprotective drug widely used in the treatment of type 2 diabetes mellitus. Cultured neonatal rat cardiomyocytes (1-3 days) were treated with 25 mM fructose at different times. The mRNA levels of hypertrophic markers (Beta-MHC, ANP, BNP and RCAN1.4) were determined by qRT-PCR. Phosphorylated -AMPK, total- AMPK and Beta-MHC protein levels were determined by inmunowestern blot (WB). The results showed that fructose increased mRNA levels of the hypertrophic markers and Beta-MHC protein levels. Further, it was observed that metformin was able to increase AMPK activation even in the presence of fructose which could prevent the progression of cardiomyocyte hypertrophy induced by this carbohydrate.

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35. INDUCTION OF CELLULAR SENESCENCE IN PRIMARY ADULT MOUSE CARDIAC FIBROBLASTS.

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Introduction: Cellular senescence – a hallmark of aging – is related with the biomarkers appearance and up-regulation, such as senescence-associated beta-galactosidase activity (SABG), activation of tumor suppressor proteins (p53, p21CIP1) and increase of DNA damage associated proteins (gammaH2A.X). Recent studies suggested that interleukin-1beta (IL-1b) and doxorubicin (Dox) promote cardiac aging through an inflammatory process. Cardiac fibroblast (CF) keeps the extracellular matrix homeostasis and actively participates on damage-associated inflammatory and scaring processes that are altered in cardiac aging. Few studies have evaluated the increase of these biomarkers on CF by pro-inflammatory molecules such as IL-1b and Dox as potential inducers of cardiovascular damage. Objective: The objective is to evaluate IL-1b and Dox effects upon SABG, levels of tumor suppressor- and DNA damage-associated proteins as biomarkers of cellular senescence. Methods: CF were isolated from 8-10-week-old C57BL/6 male mice. CF were serum-starved by 24 hours prior to stimulation with IL-1b (2,5 ng/mL) and Dox (10 nM) for 24 hours. SABG was measured by microscopy with a commercial kit (Cell Signaling TECHNOLOGY®) and protein levels of p53, p21CIP1, and gammaH2A.X by immune-blot. Results: IL-1b and Dox increase the percentage of SABG positive cells as long as the protein levels of p53, p21CIP1, and gammaH2A.X. Conclusions: IL-1b and Dox show an inductor effect of cellular senescence – featured by increased SABG and p53, p21CIP1 and gammaH2A.X levels – on CF.

Pharmacology area: Farmacología cardiovascular (Cardiovascular Pharmacology)
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36. NEUROPHYSIOLOGIC MODULATION OF NUCLEUS ACCUMBENS BY THE ACTIVATION OF SEROTONIN RECEPTOR 5-HT$_{2A}$

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Serotonin is a neurotransmitter implicated in most processes related to mood, sleep regulation, sexual behavior and cognitive modulation, being important in synaptic transmission as well. While, serotonin transmission is not completely, new evidences showed an important role for serotonin receptor activation in prefrontal cortex (PFC), dorsal raphe nucleus (DRN) and ventral tegmental area (VTA), specifically the activation of serotonin receptor 5HT2A. Electrophysiological studies in PFC showed that serotonin has effects on excitability profiles of pyramidal cells of layer V through the activation of 5HT2AR where its expression pattern is very high. These cells

project to nucleus accumbens (Nacc; another nucleus with high expression of 5HT2AR), where the specific role of 5HT2AR activation is not known regarding synaptic transmission. To address this issue, we used electrophysiological whole cell patch clamp and current clamp techniques to record in acute coronal slices from Nacc of adult Sprague Dawley rats, MSNs in presence of serotonin and TCB-2 (agonist of 5-HT2AR). The experiments were made in presence of picrotoxin and tethrodotoxin to determine and characterize the specific activation of 5-HT2AR in MSN only of the excitatory inputs. Our results show a decrease in the amplitude of mEPSCs (miniature excitatory postsynaptic currents), which is related with a postsynaptic event, and no significant changes in the frequencies of mEPSCs. Also, we observed changes in firing rate and action potential threshold parameters in the presence of TCB-2, augmenting the firing rate and diminishing the action potential threshold. Taken together, the activation of this receptor within MSNs of the Nacc has effects on excitability parameters of these cells and also a possible modulation of serotonin and dopamine transmission within this nucleus.

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37. MESENCHYMAL STEM CELL SECRETOME (MSCSS) ADMINISTRATION REDUCES OXIDATIVE STRESS AND NEUROINFLAMMATION INDUCED BY PERINATAL ASPHYXIA IN RAT HIPPOCAMPUS.

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Perinatal asphyxia (PA) is an important obstetric risk occurring at the time of delivery. Surviving children develop long-lasting motor and cognitive deficits. PA implies a primary energy crisis resulting from oxygen interruption, followed by a secondary insult linked to the required re-oxygenation, leading to oxidative stress, neuroinflammation and cell death, affecting basal ganglia and hippocampus. Neuroinflammation activates NF-kappa b signalling, which implies p65 nuclear translocation and pro-inflammatory gene expression, resulting in glial activation and apoptosis. Oxidative stress activates Nrf2, the antioxidant defense master regulator, promoting antioxidant gene transcription, including hemoxigenase 1 (HO-1) and NAD(P)H dehydrogenase quinone 1 (NQO1). MSCs have been proposed as potent agents to treat several conditions associated with neuroinflammation and oxidative stress. Preconditioning of MSCs with pro-inflammatory cytokines or hypoxic conditions improve their effectiveness. The aim of this study was to determine whether a single intranasal administration of secretome derived from preconditioned human MSCs to asphyxia-exposed rats activate (i) the Nrf2 pathway, (ii) reducing oxidative stress, (iii) neuroinflammation and (iv) cell death in rat hippocampus. Two hours after birth MSCSs (6 ug/16 ul, obtained from 1x10^6 preconditioned-MSCs) or 16 ul of vehicle were administered intranasally to asphyxia-exposed or control rats. Animals were euthanized at day P7. Oxidative stress was monitored by the GSSG/GSH ratio, Nrf-2 nuclear translocation and HO-1 and NQO1 protein levels by Western blots (WB) and immunofluorescence (IFI). Neuroinflammation by microglial reactivity (anti-Iba-1, IFI) and p65 nuclear translocation (WB). Cell death by cleaved caspase-3 protein level (WB). The administration of MSCSs: (i) lowered the PA increased GSSG/GSH ratio, and (ii) increased both Nrf2 nuclear translocation and HO-1, NQO1 levels, (iii) reduced the microglial reactivity and nuclear P65 and (iv) reduced cleaved caspase levels.

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38. A COMPUTATIONALLY GUIDED ANTIBODY AFFINITY OPTIMIZATION METHOD BASED ON MACHINE LEARNING SINGLE-POINT MUTANTS SELECTION.

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Antibodies are the biomolecules with therapeutic applications (TAbs) with highest growth and great effectivity. Currently, we know 78 monoclonal antibodies approved by the FDA, and many others are in last clinical stages. The applicability of antibodies depends on two properties: specificity, and affinity. Antibody affinity maturation is a natural process and can be experimentally emulated from diversity libraries and epitope selection, process called optimization. This selection is affected by external factors and unexplored conformational states, the discovered sequences have variable affinities with values we can improve. On the other hand, the next generation sequencing, the molecular three-dimensional modelling, and molecular docking have enabled perform rational antibody design without protein crystal complex. This process is assisted by computers and complex algorithm based on biophysical properties of the intermolecular forces that drive the binding energy, the interface between the epitope on the antigen, and the complementary determinants region on the antibody are dependent of the residues type and position in this no continuous loop. To improve and optimize the affinity we need explored the residues substitution and evaluate the energy free changes. The combinatory is about 10^9 unique sequences, while the free energy methods are computational requirement extensive. We develop a Abpred, an algorithm based in protein interfaces features and machine learning selection to propose a point-mutations combinatory to optimize the affinity. We train Abpred using an artificially-balanced dataset derived from SKEMPI-2.0; 1392 single-point mutations on 50 Ab-antigen

complexes. Evaluation on blind-test (20% of dataset) achieved an RMSE of 1.66 kcal/mol, and 0.593 correlation. Abpred enable the affinity optimization in silico to accelerate the design of news TAbs.

Pharmacology area: Farmacología molecular (Molecular Pharmacology)
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39. SELECTION OF MOLECULES FOR THE FUNCTIONALIZATION OF GENE-CHARGED-GOLD-NANOPARTICLES THAT ACHIEVE TARGETING OF PROSTATE CANCER CELLS.
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Metal nanoparticles (NPms) have attracted the interest of biomedical researchers due to their potential application in the diagnostic and treatment of diseases like cancer. These NPs can enter the cell depending on their size, charge, and surface functionalization. NPs between 10 to 100 nm have a large surface area that can be functionalized with different molecules for targeting (antibodies, ligands of cell surface proteins), detection (fluorescent probes) and treatment (drugs, proteins, nucleic acids) of affected cells. In this way, NPs could allow detection of target cells and be a therapy, at the same time. Such NPs has been described as ¨theranostic NPs¨. Among molecules used to successfully target cancer cells are folic acid, RGD peptides, and EGF‑receptor antibodies. In this work, we show the formulation and characterization of gold nanoparticles designed for gene delivery treatment of prostate cancer cells. Selectivity for prostate cancer cells of NPms functionalized with folic acid or anti-LOX-1 antibody were compared. These NPms were tested in four prostate cancer cell lines: LnCap, Du-145, C4-2B, and PC3 while RWPE-1 was used as a control cell line. Molecules that mediate cell targeting of NPs are determinant in the success of theranostic nanoparticles. These NPs could favor cancer early detection, improving treatment response and/or achieving a complete recovery.

Pharmacology area: Tecnología farmacológica (Pharmaceutical Technology)
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40. CYTOTOXICITY OF NITROFURANS AND C-5 SUBSTITUTED FURANS. EVIDENCE OF NITROREDUCTION-INDEPENDENT CYTOTOXIC EFFECTS.
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Nitrofurans constitute a family of drugs that have antibacterial and antiparasitic effects. The consumption of these causes various adverse effects which are mainly attributed to the monoelectronic reduction of the 5-nitro group mediated by host enzymes. This triggers the generation of nitroanion radical which, upon entering redox cycling with molecular oxygen, results in the formation of superoxide anion radical and the regeneration of the nitro derivative. The generated oxidative stress allows to explain, at least in part, the adverse effects attributed to nitrofurans. However, not all side effects are attributable to that mechanism. In order to demonstrate that the cytotoxic effects of nitrofurans are not solely due to the reduction of the 5-nitro group, derivatives of 3 commercial nitrofurans (nitrofurazone, nitrofurantoin and nifuroxazide) were synthesized in which the 5-nitro group was substituted by Methyl, Bromo or Hydrogen, maintaining the parental skeleton. The synthesis was carried out in aqueous solution through ultrasound, mixing the aldehydes with the corresponding hydrazines. Subsequently, the effect of these derivatives on cell viability and their ability to generate intracellular ROS was evaluated. The results show that the cytotoxic effect of furan derivatives is dependent on the presence of electron withdrawing groups in position 5 of the furan ring; while the generation of intracellular ROS does not depend exclusively on the presence of the 5-nitro group. This suggests new toxicity mechanisms independent of oxidative stress induced by redox cycling of nitrofurans.

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41. ELEPHANT BLACK GARLIC EXTRACT PREVENTS MITOCHONDRIAL DYSFUNCTION INDUCED BY BETA-AMYLOID PEPTIDE IN MOUSE HIPPOCAMPAL SLICES.
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Soluble oligomers of the beta-amyloid peptide (SO-Aβ) are central elements in the pathogenesis of Alzheimer's Disease (AD). It has been demonstrated that SO-Aβ forms pores in the plasma membrane which leads to Ca2+ influx and leakage of large molecules, such as ATP. Cytosolic Ca2+ overload induces mitochondrial dysfunction and synaptic failure resulting in cell death. Some studies suggest that common black garlic (Allium sativum) prevents the toxicity of SO-Aβ due to its enriched composition of sulfur metabolites. However, there are no studies on the biological activity of elephant black garlic (Allium ampeloprasum), and recently in our laboratory we identified new sulfur compounds that are not present in common garlic. The objective of this work was to evaluate neuroprotective properties of elephant black garlic extract (BG) against SO-Aβ toxicity. In mouse hippocampal slices, BG (20 µg/mL) prevented the decrease in cellular viability induced by SO-Aβ (2.5 µM) by

60±6%. In parallel, BG kept the mitochondrial membrane potential stable compared with SO-Aβ (SO-Aβ: 67±3%; SO-Aβ+BG: 96±5%), suggesting a direct effects on mitochondrial function; additionally, the intracellular (SO-Aβ: 70±6%; SO-Aβ+BG: 110±9%) and extracellular ATP levels (SO-Aβ: 180±23%; SO-Aβ+BG: 125±16%), were recovery when BG was present. We also observed that BG normalized the levels of the mitochondrial fusion protein MFN1 (SO-Aβ: 49± 10%; SO-Aβ+BG: 91±13%). On the other hand, our results showed that BG preserved the synaptic structure and prevented the decrease in SV2 (SO-Aβ: 64±4%; BG: 97±9%) and PSD95 proteins (SO-Aβ: 45±8%; SO-Aβ+BG: 76±7%) and also the decrease on transient intracellular calcium induced by SO-Aβ (54±5%). Finally, our results suggest that the bioactive compounds present in BG could be new pharmacological tools to treat the SO-Aβ toxicity.

Pharmacology area: Farmacología de Productos Naturales (Natural Products Pharmacology)
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42. ROLE OF ROCK-1 AND ROCK-2 IN ADHESION AND MIGRATION OF TRYPANOSOMA CRUZI- ACTIVATED MACROPHAGES TREATED WITH ATORVASTATIN.

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Chagas Disease (CD) is caused by protozoan Trypanosoma cruzi (T. cruzi). The most severe CD clinical manifestation is Chronic Chagas cardiomyopathy (CCC). Currently treatment is benznidazole, a trypanocidal therapy, ineffective against CCC. Therefore, is interesting to develop new therapeutic pharmacologic strategy. It has been observed in autopsies of patients with CCC that present an important leukocytes infiltration in cardiac tissue, and more of 50% are macrophages. The macrophage infiltration needs the macrophages adhesion and migration from the vascular endothelium to damage site. Rho-associated protein kinase (ROCK) 1 and 2 are serine-threonine kinases activated by small GTPase RhoA. ROCK phosphorylate myosin light chain (MLC), promoting cellular contraction and generating focal adhesion. On the other hand, ROCK phosphorylate cofilin, increasing actin polymerization. Therefore, ROCK activation affects cellular migration and adhesion. In our laboratory we have observed, T. cruzi activates ROCK in human macrophages, leading to proinflammatory phenotype and atorvastatin inhibited ROCK, changing the phenotype of this cells. However, it has not been studied ROCK-1 and ROCK-2 role in T. cruzi effect and atorvastin over macrophages adhesion and migration. Human macrophages U937 were used, with a knockdown for ROCK-1 and ROCK-2 and with both proteins constitutively active by nucleofection. T. cruzi-activated macrophages were treated with atorvastin and adhesion process to endothelial cells (HUVEC) were determined by microscopy and western blot. Also, migration was studied by microscopy time lapse.

It was observed that both isoforms are involved in adhesion and migration increase promoted by T. cruzi and is inhibit by atorvastin. These results allow us to propose atorvastin as a therapy to decrease macrophages infiltration in CCC.

Pharmacology area: Farmacología molecular (Molecular Pharmacology)
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43. IMPAIRMENT OF SPIKE TIMING-DEPENDENT PLASTICITY IN PREFRONTAL CORTEX IN A KETAMINE TREATED MICE.

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The medial prefrontal cortex (mPFC) is a key structure involved in cognitive functions like working memory and decision-making. Long-term changes in synaptic plasticity, i.e: long-term potentiation (LTP) and long-term depression (LTD) have been proposed as the cellular substrate of these cognitive processes. During the postnatal development, the maturation of mPFC circuits depends largely on the network of inhibitory interneurons, which modulate the pyramidal neurons (PYN) function. The mPFC interneurons are especially vulnerable to injury during adolescence and the impairment of GABAergic interneurons function is involved in several neuropsychiatric diseases, including schizophrenia (SZ). However, it is still unknown how the disruption of GABAergic interneurons development during adolescence can affect the long-term synaptic plasticity in the adult brain. Using electrophysiological and pharmacological approaches, we evaluate the efficacy of synaptic transmission and spike-timing-dependent plasticity in mPFC in an SZ mice model based in the adolescence treated with non-competitive NMDAR antagonist ketamine (Ket). Through recording in PYN of the layer II / III of mPFC slices in adulthood, we found that the frequency of spontaneous and miniature inhibitory post-synaptic currents (sIPSC and mIPSC) was lower in Ket treated animals than control. Also, we observe that the paired-pulse ratio of eIPSC was higher in Ket mPFC slice than control. Using the STDP protocol we found that while the protocol in t-LTP induced a similar potentiation that in control, the t-LTD protocol was unable to induce depression, conversely it can induce LTP. These data suggest that hypofunction of NMDAR that impair the GABAergic interneurons maturation and function, decrease the GABA release, which can reverse the temporal dependence of STDP-LTD modifying the synaptic plasticity and function of mPFC network in adulthood mice.

Pharmacology area: Fisiología (Physiology)
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44. CHARACTERIZATION OF THE ADIPOGENIC PROTEIN E4ORF1 FROM ADENOVIRUS 36 THROUGH AN IN-SILICO APPROACH.

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Adenovirus 36 (Ad-36) is related to human obesity due to its adipogenic activity mediated by the Early 4 Open Reading Frame 1 (E4orf1) protein. Mechanisms underlying adipogenic effect of E4orf1 are not completely understood; however, it has been characterized the increased proliferation and differentiation of fat cells through the activation of the Phosphatidyl Inositol 3 Kinase pathway by binding proteins containing PDZ-domains. We aimed to characterize E4orf1 structure and analyze its interactions with PDZ-domain containing proteins in order to recognize important residues with pharmacological purpose. In-silico approaches such as homology modeling, molecular dynamics and molecular docking between E4orf1 and five PDZ-domains from different proteins (PDZ-1 and 2 from Disk Large Homolog 1; PDZ-3 from Membrane Associated Guanylate Kinase 1; and PDZ-7 and 10 from Multy PDZ-Domain Protein 1) were performed. Mutagenesis of selected residues was performed to evaluate its importance in the stabilization of E4orf1:PDZ complexes. We predicted the first 3D model of E4orf1, which suggests a key role of residues at c-termini region (114 to 125), demonstrating its importance in initial stabilization. The complex formed by predicted E4orf1 and PDZ10 was more stable than others. Moreover, residues at “core” region (residues 80 to 85) in E4orf1:PDZ10 complex were important in stabilization as demonstrated by its electrostatic interactions. Mutagenesis highlighted residues 80-85, demonstrating its importance in complex stabilization. In conclusion, E4orf1 forms a stable complex with PDZ10 domain, being residues 80-85 of particular importance. Characterization of E4orf1 interactions provides a first approach in discovering druggable targets for Ad-36 induced obesity.

Pharmacology area: Farmacología molecular (Molecular Pharmacology)

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45. RECOMBINANT INTERFERON AS THERAPEUTIC CANDIDATE FOR CANINE VIRAL DISEASES.

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Chilean dog population is estimated in 3,2 million, with a continue growing in pet market associated with care products. In health expenditure, vaccines reach 25% of total cost in pet's care. Among pathologies that affects pets, viruses are the main source of infectious diseases, including distemper, parvovirus and tracheobronchitis. Vaccines against parvovirus are currently available, however still is considered one of the most important disease worldwide, with a high prevalence level in our country. Nowadays, there is not a large spectrum and specifics antiviral drugs, having to use human antivirals or from another species. Specifically, cytokines used as therapeutics activates immune system and helps to defense against virus, preventing replication and infection. In this work we developed a prototype drug for canine specifically antiviral treatment, based in a recombinant dog-derived interferon. The molecule was produced in a E. coli expression system. Besides, specific interferon activity was measured by OAS-2 and PKR mRNA quantification, in Madin Darby Canine Kidney (MDCK) cell line and dog lymphocytes primary cultures stimulated with Canine recombinant interferon was obtained with purity around 88% and the antiviral markers were enhanced in canine cells, hence the cytokine could be an option for antiviral therapy in canine population.

Pharmacology area: Otros (Others)

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46. THE GLUCOSE TRANSPORTER (SGLT-2) AS A POSSIBLE URINARY MARKER OF EARLY DIABETIC NEPHROPATHY.

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Hyperglycemia is characterized by high blood glucose concentrations (≥126 mg / dL) and over time these glucose levels damage renal microvasculature. Diabetic nephropathy is the final complication of this pathology, where well-characterized morphological and functional alterations occur. At present there is no specific and early urinary marker that allows determining the risk of developing diabetic nephropathy once diabetes is established. Therefore, the objective of this work was to evaluate SGLT-2 as a specific urinary marker of renal damage in a murine model with early diabetic nephropathy. Type 1 diabetes was induced in Wistar rats by administration of streptozotocin (60 mg / kg, ip). Diabetic rats were sacrificed 21 days after induction. Proteinuria and creatinine were determined as renal function tests. Western blotting analyzed the expression of SGLT-2 and β2-microglobulin. An increase in the urinary excretion of SGLT-2 was found in the third week of damage with diabetic nephropathy and the presence of β2-microglobulin in the urine as a marker confirmed the presence of damage. These results suggest SGLT-2 as a new urinary marker of diabetic nephropathy that allows predicting the risk of developing diabetic nephropathy. Keywords: Diabetic nephropathy, streptozotocin, β2-microglobulin, SGLT-2

Pharmacology area: Fisiología (Physiology)

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47. TMA-6 (2,4,6-TRIMETHOXYAMPHETAMINE) MODULATES NEUROPLASTICITY IN THE PREFRONTAL CORTEX AND ENHANCES HEAD-SHAKES BEHAVIOR IN SPRAGUE-DAWLEY RATS.

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The search for new analogs of the entactogen MDMA (3,4-methylenedioxy-methamphetamine, "ecstasy") is of great relevance for the development of new drugs useful in the treatment of prevalent neuropsychiatric diseases. While the central effects described for MDMA clearly differ from other psychotropic compounds, it has been proposed that the presumed low potency hallucinogen TMA-6 (2,4,6-trimethoxyamphetamine) might induce MDMA-like effects at low doses in humans. Since both hallucinogens and MDMA might exert different effects on cognitive processes, one may anticipate differential effects with respect to neuroplasticity at key sites of the central nervous system. Here we compared the acute effects of TMA-6 with MDMA on (i) in vivo induction of long-term potentiation (LTP) in the prefrontal cortex of the rat, and (ii) induction of paroxysmal head rotations termed “head-shakes”, which is considered a behavioral proxy in rodents for human hallucinogenic-like effects. The results obtained showed that 20 mg/kg TMA-6 not only prevented the induction of LTP in the prefrontal cortex but turns it into a long-term depression-like event. In addition, TMA-6 significantly increased the number of head-shakes, verifying the hallucinogenic nature of this compound. In contrast, 10 mg/kg MDMA significantly increased the prefrontal cortical LTP but fully abolished the number of head shakes. Taken together, and unlike the presumption based on the subjective interpretation of its effects in humans, the inverse electrophysiological/behavioral profile of TMA-6 referred to MDMA suggests that the former seems to lack entactogenic-like effects, rather supporting the hallucinogenic essence of this drug.

Pharmacology area: Neuropsicofarmacología (Neuropsycopharmacology)
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48. SITE-SPECIFIC PEGYLATION OF L-ASPARAGINASE: AN ALTERNATIVE FOR THE TREATMENT OF ACUTE LYMPHOBLASTIC LEUKEMIA.

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L-asparaginase (ASNase) is a therapeutic enzyme considered a cornerstone in the treatment of Acute Lymphoblastic Leukemia (ALL), the most common cancer in children worldwide. Four formulations of bacterial origin are available in the market for the treatment of ALL. However, despite their effectiveness, they generate immunological reactions, decreasing the action of the drug and damaging patient safety. PEGylation is one of the strategies adopted to reduce the immunogenicity of asparaginase, which consists of the covalent binding of polyethylene glycol (PEG) chains to the enzyme, at specific or random sites. This allows reducing the recognition of the enzyme by the immune system and its early elimination from the bloodstream. In this work, we performed the N-terminal PEGylation of E.coli ASNase, with methoxy polyethylene glycol-carboxymethyl N hydroxysuccinimidyl ester (mPEG-NHS 10kDa) in 100 mM PBS at pH 7.5 and PEG: ASNase ratio of 25:1. As a result, we obtained the monoPEGylated ASNase with an activity of 134 ± 11.5 IU/mg and acceptable kinetic parameters. MonoPEGylated ASNase also showed more stability at different pH, temperatures and against human serum proteases than the native enzyme, demonstrating its potential as a less immunogenic biopharmaceutical in the treatment of ALL.

Pharmacology area: Tecnología farmacológica (Pharmaceutical Technology)
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49. CHARACTERIZATION OF CA125 ANTIGEN EXPRESSED IN HUMAN CERVICAL CARCINOMA CELLS.

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Epithelial ovarian cancer is the seventh death cause related to cancer in women worldwide. Due to the absence of early stage clinical symptoms, patients are usually diagnosed when decease had spread further than ovary with an unfavorable prognostic. Cancer antigen 125 (CA125) is a serum marker extensively used in gynecology for monitoring epithelial ovary cancer patients. It’s a repetitive peptide antigen of the membrane glycoprotein MUC16, whose over-expression in ovarian cancer has been linked with both pro-metastatic and pro-tumorigenic properties. In the present study, we characterized the N-oligosaccharides bonded to the C-terminal region of MUC16, expressed in cervical carcinoma cell culture

by adenoviral transduction. Enzymatic deglycosylation, HPLC, and MADI-TOF analysis showed mainly complex type of oligosaccharide N-linked, with bi-antennary, mono-sialylated and mono-focusylated core structures, predominantly. It has been previously reported that N-glycan profiles from cancer patients shows an increase of these structures, compared to healthy groups. Furthermore, these N-glycan structures have been described as part of innate and adaptive immune response recognition by CA125 antigen. Concluding, the glycosylation status of the CA125 may provide specific biomarkers and therapeutic target for gynecologic cancer.

Pharmacology area: Otros (Others)
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50. DESIGN, SYNTHESIS AND BINDING AFFINITIES OF CYCLOALKYLAMINES AND PIPERIDINES ESTERS DERIVATIVES ON ALPHA4BETA2 NICOTINIC RECEPTOR AND MONOAMINE TRANSPORTERS (HSERT AND HDAT).

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The monoaminergic and cholinergic neurotransmitter systems exhibit, in the central nervous system (CNS), a wide range of functional interactions and mutual regulations. Furthermore, acetylcholine (ACh) actions mediated by nicotinic receptors (nAChRs), as well as monoamines such as serotonin (5-HT) and dopamine (DA), are involved in the modulation of several brain functions, including (but not limited to) cognition, voluntary movement, motivation and reward, mood, attention and learning, as well as in the physiopathology of a variety of diseases. In addition, most of the drugs currently used for the treatment of neurological and neuropsychiatric disorders such as Parkinson`s and Alzheimer`s diseases, depression, drug addiction, schizophrenia, etc., have mechanisms of action associated to the regulation of one or more of these systems. Indeed, there are some examples of therapeutically useful drugs, which act through simultaneous interactions with SERT/DAT and nAChRs. Therefore, it seems attractive to search/formulate ligands that show such a promiscuous profile fusing structural aspect of nicotinic ligands and antidepressants. Here, we design and synthesize cycloalkylamines and piperidines esters derivatives. Binding experiments were assessed for alpha4beta2nAChR on brain synaptosomes and hSERT and hDAT from specific cell lines. The ester moieties were acetyl, propionyl and benzoyl derivatives in order to study steric effect into the binding site. Our results indicate that, some compounds are able to displace radioligands from nicotinic receptor and MAT, showing a promiscuous behavior. However, the ranges of affinities were in the micromolar order. In addition, docking experiments were performing in order to rationalize the binding mode and the similar interaction between nAChR, SERT and DAT with our compounds.

Pharmacology area: Farmacodinamia (Pharmacodynamics)
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51. RATIONAL DESIGN AND BIOLOGICAL EVALUATION OF TRIAZOLOPYRIDINES AGAINST T. CRUZI.

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Since a while ago, Azole-containing compounds have been recognized for their antifungal features and with well-known applications in current clinical field. Some of features highlighting azole heterocycle come from its specific metabolism, those related to its high binding affinity to heme-containg proteins such as cytochrome p450. The p450 complex has the function of metabolizing drugs, exogenous and endogenous molecules that could render in cell toxicity. Lanosterol 14α demethilase (CYP51) is the key enzyme in sterol biosynthesis in Trypanosoma cruzi (Tc), agent that causes Chagas disease. The inhibition of this enzyme will induce accumulation of toxic metabolites that cause the death of the parasite. Recently, we have synthesized and characterized derivatives of triazolo pyridines, based on a rational study (structure-activity), where we found that compound 1 has antiproliferative effect against the replicative form. From this structure, we obtained 24 molecules with variations in positions 3 and 7 in the ring [1,2,3] triazolo [1,5-a] pyridine with different electrophiles such as pyridines, thiophenes, benzenes and pyrazines. Studies (structure/activity) of this new series have shown that the compound 2 increased the trypanocidal potency. On the other hand, preliminary docking studies showed that the association energy of the compound to CYP51 are similar to the binding energy values of fluconazole to CYP51, our approximations have shown that both the azole fraction and pyridine are potentially related to the coordination with hemo. This has led to design of a new proposal of antichagasic drugs with a possible action on CYP51.

Pharmacology area: Otros (Others)
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52. EXPLORING NOVEL ALLOSTERIC MODULATORS OF ALPHA3 GLYCINE RECEPTORS: STRUCTURE-ACTIVITY RELATIONSHIP OF 2,6-DI-TERT-BUTYLPHENOL DERIVATES.

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Glycine Receptors (GlyRs) are pentameric anion-permeable ligand-gated ion channels highly expressed in spinal cord and brain stem, where mediate processes such as motor coordination and sensorial processing. The modulation of glycine receptors composed by alpha3 subunit (a3GlyRs) by positive allosteric modulators (PAMs) has been associated to generation of analgesia in chronic pain models. Previously, we shown that the non-sedative propofol analog 2,6-di-tert-butylphenol (2,6-DTBP) is a PAM of a3GlyRs. Here, we analyzed series of 2,6-DTBP analogs using bioinformatics and electrophysiological methods. Whole-cell recordings shown that 2,6-DTBP (0,1 mM) potentiate a3GlyRs-evoked currents in a 171±21%. The first set of experiments evaluated the impact of the tert-butyl group positions around the phenolic core. These studies concluded that the presence of two tert-butyl groups at the position 2 and 4 around the phenolic core (i.e. 2,4-DTBP) were sufficient to generate an a3GlyR PAM with a significantly higher efficacy. A second set of molecules evaluated the impact of additional chemical groups on the 2,4-DTBP scaffold (i.e. methyl, ethyl, oxime, amine, carboxylic, sulfonamide groups). Whole-cell recordings shown that ≈30% of these compounds displayed improved efficacy in comparison to 2,6-DTBP (≈3-4 fold of a3GlyR potentiation). Bioinformatics analysis shown that all the compounds evaluated have an ADME profile compatible with drugs-like molecules. Pharmacophore modeling shown that the localization of polar, hydrophobic and charged groups within the 2,4-DTBP is pivotal for the a3GlyR modulation. Through a systematic analysis of 26 analogs of 2,6-DTBP, the present work allowed the identification of novel a3GlyRs PAMs with improved efficacy. In addition, the generation of a pharmacophore based on these findings expand the possibilities for further design and development of new GlyRs PAMs.

Pharmacology area: Farmacología molecular (Molecular Pharmacology)
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53. ATORVASTATIN AND ROSUVASTATIN TREATMENT CONTRIBUTES TO THE DECREASE OF PROLIFERATION IN HUMAN UMBILICAL ARTERY SMOOTH MUSCLE.

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The treatment of choice for hypercholesterolemia is the use of statins. Enzymes that inhibit the enzyme 3-hydroxy-methylglutaryl coenzyme A reductase, which is a limiting factor in the biosynthesis of liver cholesterol. Despite this, clinical studies have revealed that statins can exert atheroprotective effects, beyond the decrease of serum cholesterol. These effects have been called pleiotropic effects and include anti-proliferative and anti-migratory properties in vascular smooth muscle cells, which are a key cell type in atherosclerotic plaque development. For this reason, Human Umbilical Artery Smooth Muscle cultures were stimulated with 10 ng/ml of platelet-derived growth factor and treated with 20 µM atorvastatin and 20 µM rosuvastatin for 24 hours. The proliferative effect was subsequently evaluated for 48 hours by spectrophotometry using the CellTiter 96® AQueous One Solution Reagent colorimetric assay. The migratory effect was also evaluated for 4 hours by optical microscopy using transwell with 8 µm pores. Migrated cells were counted by means of ImageJ software with automated macros function commands. It was observed that 20 µM statins treatment reduces cell proliferation in a 48-hour period (p=0,05). However, there is no decrease in migratory cell capacity after being treated with atorvastatin (p=0,05) and rosuvastatin (p=0,05). For this reason, atorvastatin and rosuvastatin treatments might contribute to the proliferation reduction in smooth muscle cells, which are involved in atherosclerotic plaque formation. Financial support: FONDECYT 1171765 & DIUFRO DI19-2018

Pharmacology area: Farmacología cardiovascular (Cardiovascular Pharmacology)
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54. EXPRESSION AND CHARACTERIZATION OF A NOVEL SINGLE-CHAIN ANTIBODY AGAINST VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) IN GOAT MILK.

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Tumor angiogenesis is a hallmark of cancer and plays a significant role in establishing a vascular supply within the tumor which is essential for tumor growth and metastasis. The vascular endothelial growth factor (VEGF) plays an important role in angiogenesis process promoting endothelial cell proliferation, migration, and invasion. VEGF is overexpressed in many solid cancers including lung cancer, one of the most common cancers in the world. According to this, different approaches have been developed to suppress tumor angiogenesis, including anti-VEGF monoclonal antibodies as part of the cancer immunotherapy strategy. However, high production costs limit the widespread access to this treatment. In this study, we designed a novel single-chain monoclonal antibody (anti-VEGF) that can bind to VEGF. This antibody can be efficiently expressed in the mammary gland of goats by adenoviral transduction and purified from their milk. Results showed that anti-VEGF was able to avoid cells migration in a wound healing test and suppressed VEGF-induced microvessel sprouting in rat aortic ring assay. Furthermore, in vivo efficacy was evaluated on a xenograft lung tumor model where anti-VEGF treatment had an inhibitory effect on tumor growth. Our findings suggest the therapeutic potential of anti-VEGF as an anti-tumor agent correlated with suppression of angiogenesis.

Pharmacology area: Otros (Others)
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55. DIVERSITY LIBRARY SEQUENTIAL SCREENING TO IDENTIFY COMMON DESCRIPTORS OF THE SGLTS INHIBITORS.

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Type II Diabetes Mellitus (T2DM) have an estimated 8.5% of the world adult population affected in 2014 and a projection of reaching 17.9% in 2060. Many different approaches have been developed for tackling the no-insullinic management of hyperglycemia in the diabetic patient, exploring the diverse variety of targets that the multifactorial nature of T2DM offers, which includes classicals insulin sensitizers like metformin, and also new pharmacological developments. One new approach to control T2DM is the use of sodium-glucose transporter 2 (SGLT2) inhibitors. While SGLT1 is involved in intestinal glucose intake, SGLT2 is located in the proximal tubules of kidneys, and is responsible for most of the glucose reabsorption (>90%). Inhibition of SGLT2 in combination with traditional treatments have shown improvements in the control of glycemia and a concomitant reduction in cardiovascular risk. Challenges in the development and extension of the chemical space associated with the inhibitory activity of SGLT2 are the high costs in time and money of the in vitro research and the lack of the human transporter atomic coordinates, due to the difficulties to crystallizing membrane proteins. These two problems can be addressed through a methodology that includes an exploration of molecular dynamics from the protein transporter and the structure-based virtual screening. We have developed a robust pipeline perform a virtual screening through massive molecular docking against a diversity library of molecules for the exploration of the chemical space for the discovery of new inhibitors. We use SGLT2 and known inhibitors to perform a sequential refinement to optimize the identification from a not supervised methods and extend the methodology to the other potential candidates transporter with therapeutics applications.

Pharmacology area: Farmacología molecular (Molecular Pharmacology)
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56. AMYLOID BETA OLIGOMERS INTERRUPT NUCLEAR Ca2+ TRANSIENTS AND GENE EXPRESSION INDUCED BY GABAZINE IN HIPPOCAMPAL NEURONS

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Ca2+ signals are essential mechanisms that regulate neuronal plasticity. Nuclear Ca2+ transients generated by neuronal activity induce changes in gene expression and in dendritic spine remodeling, which are mediated by the rapid activation and expression of transcription factors. Among them, Npas4 is known for inducing distinct activity-dependent gene programs that regulate the expression of neurotrophic factors and antioxidant enzymes. Thus, Npas4 may provide a molecular link between neuronal activity and the activation of memory and neuroprotection signaling pathways. Amyloid-beta oligomers (A-beta Oligomers) are synaptotoxins that induce aberrant Ca2+ signals and promote Reactive Oxygen Species (ROS) generation,leading to synaptic plasticity disruption. In this work, we studied the effects of AβOs in nuclear Ca2+ signals production and gene expression induced by Gabazine, a GABA(A) receptor blocker that functions as an inductor of synaptic activity. To this aim, we transfected primary hippocampal neuronal cultures with a genetically encoded Ca2+ indicator with nuclear destination (GCaMP3-NLS). We pre-incubated these cultures with AβOs for 6 h and applied Gabazine at the microscope stage, to record nuclear Ca2+ signals by live-imaging. We also performed immunocytochemistry to evaluate CREB phosphorylation and RT-qPCR to evaluate the mRNA expression of Npas4, BDNF and of the antioxidant enzymes Glutamate-Cysteine-Ligase (GCL) and NADPH-Quinone-Oxidoreductase (Nqo1) in these conditions. Our results indicate that neurons treated with A-beta Oligomers showed reduced nuclear Ca2+signals and diminished Npas4, GCL and Nqo1 mRNA expression levels in response to GBZ. In summary, the present results indicate that A-beta Oligomers altered the activation of signaling pathways induced by gabazine, leading to a disruption of neuroprotective gene expression pathways essential to memory and learning processes, which are affected in neurodegenerative diseases.

Pharmacology area: Neuropsicofarmacología (Neuropsycopharmacology)
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57. EARLY EFFECT OF A HIGH-FAT DIET ON PERIGONADAL AND HEPATIC FAT IN RATS.

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Obesity is a multifactorial disease of great impact in Chile and worldwide. Its causes are heterogeneous and there are no totally effective therapeutic interventions; therefore the study and advances in this subject are of great relevance. The dramatic increase in the levels of obesity in the population is related to a progressive change to sedentary lifestyles and

excessive consumption of highly caloric foods, such as high-fat diets (HF). These factors lead to excessive accumulation of adipose tissue, and in the long term may result in ectopic fat storage. One way to study this disease and its comorbidities is the use of rodents fed with HF diet, as a model of diet-induced obesity. The aim of this work was to study the early effects of a HF diet on the accumulation of adipose tissue. For this, male Sprague-Dawley rats were fed a HF diet (62% of calories from fat) from postnatal day 30 for either 15, 30 or 60 days, and were compared to age-matched rats fed with a control diet (14% calories from fat). At the end of each treatment, perigonadal fat was weighed and total hepatic lipids were extracted. HF treated rats showed a significant body weight gain only until the end of the treatment compared to control diet rats. Regarding fat tissue, perigonadal fat was 77% higher in rat fed an HF diet and the percentage level of lipids in the liver increasing up to 8%. These results together suggest that a HF diet generates important physiological changes in the animal, producing in a short period a state of adiposity consistent with pre-obesity in rats.

Pharmacology area: Farmacología gastrointestinal (Gastrointestinal Pharmacology)
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58. STUDY OF A MOLECULE THAT INTERFERES IN Gβγ BINDING WITH THE CYTOPLASMIC DOMAIN OF GLYCINE RECEPTOR A1.
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Ethanol is the most widely used drug of abuse in the world. Its effects go from desinhibition, headaches, nausea, vomiting, even respiratory depression and death. Recently, the glycine receptor (GlyR) has been identified as one of the targets in which this drug acts, enhancing its inhibitory activity. This mechanism involves the interaction of the cytoplasmic domain of GlyR (GlyR-DC) with the βγ dimer of the G protein (Gβγ). Through bioinformatic studies, molecule M554 was selected, which binds to Gβγ at the same site of interaction for GlyR-DC inhibiting the effects of ethanol in vitro and in vivo. In this project it was studied whether this molecule inhibited the interaction between these 2 proteins. For this objetive, a fusion protein of GlyR-DC and Glutathione S-transferase (GlyR-DC-GST) was expressed and purified. Comparative studies of GST pull-down showed that GlyR-DC-GST retained its ability to interact with Gβγ. At the same time GlyR-DC was incubated with cell extracts, and the affinity of GlyR-DC with Gβγ in the absence and in the presence of 200 µM M554 was compared. Densitometric analysis allowed to determine that the interaction between both proteins effectively decreased in the presence of this molecule. Therefore, these results show that this molecule decreases the binding capacity of Gβγ with GlyR-DC, leaving clear that this is the basal mechanism for the inhibition of ethanol effects and supporting the projections that M554 could have a pharmacological potential to treat acute ethanol intoxication.

Pharmacology area: Farmacología molecular (Molecular Pharmacology)
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59. COMPLEX INHIBITION OF OXPHOS AND A-KETOGLUTARATE DEHYDROGENASE COMPLEX BY GENTISIC ACID-TPP+ INDUCES CELL DEATH IN BREAST AND LUNG CANCER CELL LINES.
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Cancer cells have a more hyperpolarized mitochondrial membrane potential than normal cells, which allows selectively guide towards mitochondria small cationic molecules such as triphenylphosphonium (TPP+), which participate as chemical chaperones for pharmacophores moieties. This property was used to synthesize from the natural product, gentisic acid, derivatives bound to TPP+ (GA-TPP+C10). This mitocondriotropic compound is capable of produce a time-dependent complex inhibition of mitochondrial bioenergetics characterized by 1) initial phase of mitochondrial uptake with uncoupling effect of oxidative phosphorylation, 2) inhibition of complex I-dependent respiration and, 3) a late phase of mitochondrial accumulation with inhibition of alfa-ketoglutarate dehydrogenase complex activity. This complex is part of the tricarboxylic acid cycle composed of three subunits that oxidizes and decarboxylates alfa-ketoglutarate which is necessary for the synthesis of aspartate, an essential amino acid to proliferation and cell survival. The above was verified using human breast and lung cancer cell lines, by the addition of exogenous permeable metabolites: alfa-ketoglutarate (dm-alfaKG), aspartate (m-Asp) and pyruvate (pyr). It was shown that dm-alfaKG and m-Asp, but not pyr, produce a decrease in cell death caused by GA-TPP+C10. Moreover, the bioenergetic crisis induced triggers a drastic mitochondrial membrane potential drop, G1-phase cell cycle arrest with a significant increase in ROS. In addition, this blockade of mitochondrial functions triggers a metabolic remodeling toward glycolysis and pro-survival AMPK activation. Our results describe an anti-cancer mechanism of GA-TPP+C10 that induce a complex inhibition of mitochondrial bioenergetics in a time-dependent manner in breast and lung cancer cells that may have relevance at therapeutic level.

Pharmacology area: Otros (Others)
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60. ELECTROPHYSIOLOGICAL RECORDINGS OF 3-(PYRIDIN-3-YLMETHOXY)QUINUCLIDINE (Q-01), A NOVEL SELECTIVE COMPOUND FOR THE A7B2 NICOTINIC ACETYLCHOLINE RECEPTOR.
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A novel heteromeric alpha7beta2 nicotinic acetylcholine receptor (nAChR) with functional properties different from those of alpha7 and alpha4beta2 nAChRs, was recently identified. Although its functions are not known, it appears this nAChR may be involved with Alzheimer's disease. To date there are no synthesis reports of ligands for alpha7beta2 nAChR. Our work was based on the design and synthesis of new ligands, as well as on establishing their possible effects with electrophysiological records in interneurons from the stratum radiatum of the rat hippocampal CA1 region. Thus, of eleven synthesized ligands, only Q-01 and EQ-01 inhibited the Choline-induced ionic current: 51 and 100%, respectively; that is to say, these acted as antagonists of alpha7 and alpha7beta2. However, Q-01 presented an inhibition similar to dihydro-beta-eritroidine (selective antagonist of nAChR containing the subunit beta2); suggesting that Q-01 might be more selective for alpha7beta2 nAChR than EQ-01. To understand the electrophysiological results, molecular docking studies were performed for the compound Q-01 at the nAChRs α7 and alph7beta2. These studies suggest that Q-01 would be more selective by subtype alpha7beta2.

Pharmacology area: Otros (Others)
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61. STRUCTURE-BASED VIRTUAL SCREENING STUDIES TO IDENTIFY NOVEL POTENTIAL AGONISTS FOR SALMO SALAR GHSR1A-LR.

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Ghrelin is a growth hormone (GH) secretagogue and functions primarily as a GH-releasing hormone and as an orexigen. It has also been documented to be involved in the immune system, stress response, energy metabolism and growth in fish. Its receptor, Growth hormone secretagogue receptor (GHS-R), is a class A G protein-coupled receptor (GPCR) mostly expressed in the hypothalamus. In Atlantic salmon (Salmo salar) and other salmonids, a ghrelin receptor isoform called GHSR1a-LR has been identified. This receptor has an unique characteristic: the second extracellular loop (ECL2) is notably longer than in others GHS-R. In particular, GHSR1a-LRs have the characteristic that ghrelin or GH secretagogues treatment either does not increase intracellular Ca2+ or requires pharmacological doses to activate the receptor. Given the high conservation in folding and topology of class A GPCR receptors, a comparative model of GHSR1a-LR receptor from Salmo salar was generated. This model was based on the crystallographic structure of Neurotensin-1 receptor in active-like conformation (PDB ID: 4XES). Subsequently, a conformational exploration was carried out through accelerated molecular dynamics simulations (aMD). These simulations allowed us to obtain diverse conformational variants (inactive, intermediate and active), favoring the selection of small molecules compatible with the receptor active state. Finally, we searched for potential agonists through virtual screening, applying an ensemble docking based strategy using multiple snapshots of GHSR1a-LR receptor extracted from aMD trajectories. A small molecule library, containing 4997 positive or neutral charged compounds obtained from ZINC12 database, was filtered under parameters of drug-like properties and possible central nervous system activity. From this campaign four agonist-potential molecules, based on predicted affinity in different receptor structural samples, were identified.

Pharmacology area: Otros (Others)
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62. SYNAPTIC EFFECTS OF THE ALKALOID GELSEMINE ON CORTICAL NEURONS.

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Several behavior studies have suggested that the natural alkaloid gelsemine has different biological effect, such as analgesia and anxiolytic. Nevertheless, until now there is few information about neurophisyological mechanism, and pharmacological target associated to this biological effect. Early studies from our lab have shown that gelsemine decreases the frequency of glycinergic and glutamatergic events in the spinal cord neurons culture that suggest important effect in the synaptic function. Despite these advances, it is unknown if gelsemine can modulate GABAARs and GABAergic synapsis, which is relevant in the pathological anxiety phenomena. Here, we examined the functional effects of gelsemine on native sistem using electrophysiological techniques. Studies performed on cultured neurons was realized to explore the potential effect of gelsemine in the synaptic activity of cortical neurons, which express the functional component of a GABAergic synaptic. Our electrophysiological result show that gelsemine 50 mM produced a significative reduction of the frequency, but not in the amplitud of the GABAergic and glutamatergic synaptic activity. I addittion, Effects of gelsemine on the agonist sensitivity and on the desensitization rates of GABAAR native, are evaluated. Analysis of concentration – response curves revealed that gelsemine significantly decreased the apparent affinity for GABAAR without changing the maximal current amplitudes. Analyses of the GABA-activated currents stimulated by saturating agonist concentrations indicated that gelsemine did notmodify the fraction of desensitized current or the decay time constant of receptors. Our results that gelsemine is able to negatively modulate the synaptic activity of cortical neurons. Future studies may contribute to shed light on the mechanisms underlying the beneficial effects of the Gelsemium alkaloids in the control of pathological anxiety through the modulation of inhibitory receptors.

Pharmacology area: Farmacología molecular (Molecular Pharmacology)
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63. CYTOTOXIC EFFECT OF HYDROXYCHLOROQUINE, ITRACONAZOLE AND CISPLATIN ON SPHEROID CULTURE OF ORAL SQUAMOUS CELL CARCINOMA.
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Oral Squamous Cell Carcinoma (OSCC) is the most common type of oral cancer and Cisplatin is the chemotherapeutic agent most commonly used, which induces cell death by apoptosis. Furthermore, tumor cells (TC) acquire resistance against cytotoxic effect of the drug. Therefore, it is necessary to develop more effective treatments based on the metabolic changes that occur in TC. Hydroxychloroquine and Itraconazole are two drugs traditionally used in traditional medicine, as immunomodulator and antifungal, respectively. Recently, it has been described that they may have antitumor effect, because Hydroxychloroquine may inhibit autophagy, a mechanism of adaptation to metabolic stress, and Itraconazole would disturb energy metabolism. Tumor spheroid cultures are an in vitro model suitable for antitumor activity evaluation because they can reproduce tumors in vivo main characteristics, such as hypoxia-related drug resistance and the presence of Cancer Stem Cell (CSC), which may be responsible of chemotherapy resistance and tumor recurrence. In this project the expression of the markers of CSC CD44, CD56 and ALDHA1 in spheroidal cultures of Cal-27 (COCE cells) was evaluated through flow cytometry. The results show greater expression of these markers in spheroidal cultures, compared to monolayer cultures. In addition, cytotoxicity for spheroidal cultures was determined by the MTT assay for Hydroxychloroquine, Itraconazole and Cisplatin. The IC50s were 249, 472 and 577 micromolar at 48 hours and 272, 298 and 261 micromolar at 72 hours, respectively. A viability decrease induced by these drugs was observed in a concentration-dependent manner. At present is being evaluated if the drug combinations have a synergy effect reducing the individually required concentration.

Pharmacology area: Farmacología Odontológica (Dental Pharmacology)
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64. NEONATAL EXPOSURE TO TESTOSTERONE PROPIONATE INDUCES AN INCREASED EXPRESSION OF RGS9-2 AND PKCB2 IN NACC AND VTA OF ADULT FEMALE RATS.
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Research in programming is focused on the study of stimuli that alters sensitive periods in development, such as prenatal and neonatal stages, that can produce long-term deleterious effects in various organs or tissues such as the brain, affecting brain circuits and related behaviors. Previously, we have demonstrated that neonatal programming with sex hormones affects the mesocorticolimbic circuitry, increasing the synthesis and release of dopamine (DA) in striatum and Nucleus accumbens (NAcc); also, we have observed a reduction of locomotor activity in response to methylphenidate in female rats treated with testosterone propionate (TP). However, is not clear if the alterations observed in our model are related to modifications in the signaling pathway or DA release/uptake. Interestingly, RGS9-2, which is expressed in dopaminergic neurons, can inhibit the signal transduction of dopamine receptor 2 (D2) and have been related to drug addiction and movement disorders. Also, PKCβ2 can increase the amphetamine-stimulated dopamine efflux regulating the Dopamine Transporter (DAT) activity. The objective of this work was to evaluate if the neonatal reprogramming with Estradiol Valerate (EV) or TP affects the expression of Rgs9-2 and Pkcβ2 in NAcc and Ventral Tegmental Area (VTA) in adult rats using qPCR. The expression of Rgs9-2 and Pkcβ2 was increased in NAcc and VTA of female rats treated with TP; no significant changes were observed in males under any condition. These results suggest that the neonatal exposure to TP modifies the expression of Rgs9-2 and Pkcβ2 in female rats, and this modification can account for the modifications in response to methylphenidate observed in our model. Further analysis using WB or IHC are needed to depict the functional alteration in this model.

Pharmacology area: Otros (Others)
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65. SIMVASTATIN AND 15-EPI-LIPOXIN A4 INDUCE CARDIAC REPAIR THROUGH NOTCH 1 ACTIVATION IN CHRONIC CHAGAS CARDIOMYOPATHY.
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Chagas Disease, caused by Trypanosoma cruzi, is endemic in Latin America and worldwide because of migration. Without appropriate treatment this disease progress to a chronic phase that could affects the heart. Chronic Chagas Cardiomyopathy (CCC), the most severe clinical manifestation, involves a progressive inflammatory myocarditis affecting ventricular wall causing cardiovascular complications due to diminished cardiac function and heart failure. Despite intense research no one drug can stop or reverse the progressive heart damage. Simvastatin, a drug that decreases blood cholesterol, has anti-inflammatory effects and inhibits platelet aggregation. Previously, we described that simvastatin reduces myocardial inflammation caused by T. cruzi through 15-epi-lipoxin A4 (15-epi-LXA4) production, a pro-resolutory inflammation molecule.

Several reports suggest that simvastatin activates Notch pathway after a stroke enhancing blood flow by promoting angiogenesis. CCC progress with myocardial inflammation, endothelial damage with micro focal ischemia and fibrosis. We propose that simvastatin reverts cardiac damage in the chronic T. cruzi infection by 15-epi-LXA4 production and Notch 1 pathway activation. BALB/c mice were chronically infected with T. cruzi Dm28c strain and treated with simvastatin 1 mg/Kg/day and 15-epi-lipoxin A4 25 µg/Kg/day for 20 days. At day 80 post-infection animals were euthanized to analyze the heart, Notch pathway, fibrosis, and angiogenesis process. In chagasic mice, the cardiac function was restored with simvastatin and 15-epi-lipoxin A4 treatment. The Notch signaling pathway was active in cardiac tissue, a finding that correlated with drug treatment, the fibrosis process was decreased, and angiogenesis was also evidenced in this model. Thus, we concluded that simvastatin and 15-epi-LXA4 improve cardiac architecture and function through Notch 1 activation by increasing blood flow and decreasing cardiac remodeling. Thus, it could be incorporated rapidly in CCC treatment.

Pharmacology area: Farmacología cardiovascular (Cardiovascular Pharmacology)
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66. INHIBITORY ACTIVITY OF ACETYLCHOLINESTERASE AND BUTYRYLCHOLINESTERASE FROM AMARYLLIS BELLADONNA ALKALOIDS.

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Alzheimer's disease, a neurodegenerative disorder characterized by an irreversible and progressive loss of memory. Traditionally the pharmacological treatment associated with the disease in its medium to moderate stages, or similar diseases related to a deficit of the neurotransmitter acetylcholine are mainly guided through inhibitors of the enzyme acetylcholinesterase. Only four compounds have been approved as treatment against alzheimer diseases: donepezil, rivastigmine, galantamine and memantine. In most cases these drugs are well tolerated, however, various side effects such as: nausea, vomiting, among others may occur. That is why it is necessary to search for new molecules with pharmacological potential for their treatment. A potential source of acetylcholinesterase inhibitors is Amaryllis belladonna, belonging to the Amaryllidaceae family, widely distributed worldwide. Several studies support the presence of alkaloids in this species with varied biological activities. Considering the variation in the production of metabolites reported in this species, depending on the geographical distribution, it is interesting to analyze the alkaloids present in the representative of this family introduced in our country and its pharmacological potential. The objective of this research was to evaluate the inhibitory activity of alkaloids isolated from Amaryllis belladonna on enzymes acetylcholinesterase and butyrylcholinesterase.The alkaloids present in the plant's bulbs were obtained by maceration with methanol and subsequently fractionated, for the isolation. The putative alkaloid composition of the fraction was analyzed by GC-MS, highlighting the presence of type licorin and crinamine. The inhibitory activity of the alkaloid extract and the isolated compounds was evaluated by the Ellman method, finding IC50 values (ug / mL) for hexane, chloroform and butanol extract of 17.12, 8.89 and 19.09 for acetylcholinesterase and 77.27, 55.44 and 200 for butyrylcholinesterase respectively.

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67. USE OF MEDICINES BY SOUTHERN BRAZIL FARMERS AND ITS RELATIONSHIP WITH EXPOSURE TO PESTICIDES FROM DIFFERENT CROPS.

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Agriculture in its development model has not been addressing issues such as the environment. Consequently, countless substances ended up being released into the environment, plenty of them with the ability to alter the behavior of several physiological systems, inducing numerous pathologies. Concomitantly, several studies have demonstrated a growing use of medicines. Therefore, the goal of this study is to investigate the relationship between the use of medicines by farmers and their exposure to pesticides. Upon approval by the Research Ethics Committee from the Federal University of the Southern Border, farmers were randomly selected in two cities: Mafra, Santa Catarina and Planalto, Paraná, both in Southern Brazil. The subjects were then asked to fill up a form for data collection. A total number of 251 farmers participated in the research, being 123 from Mafra and 128 from Planalto. The average age of the subjects in this study was 48,4 ± 14,4 and 114 were female while 137 were male. Out of these, 23,1% (58) are making use of neuropsychiatric drugs, 32,7% (82) of cardiovascular drugs, 17,5% (44) of metabolic disorder drugs, 0,8% (2) of respiratory disorder drugs, 1,6% (4) of gastrointestinal drugs and 2% (5) of musculoskeletal purposes drugs. When correlation tests were performed between the type of crops and the drugs used by the respective farmers, the results showed a greater use of medicines for metabolic ($p = 0.014$) and musculoskeletal disorders ($p = 0.025$) from wheat crops farmers in Mafra. Thus, the data suggests that farmers in wheat crops are more likely to make use of drugs for metabolism and musculoskeletal disorders, as it might be related to the specific pesticides used in this crop.

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68. SUCRALOSE INTAKE IMPAIR THE HIPPOCAMPAL POSTSYNAPTIC INHIBITORY CURRENTS.

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Non-caloric sweeteners (NCS) are widely used in foods with the aim of reducing sugar consumption and caloric intake. Sucralose is the most used NCS worldwide and in Chile, with the current "Labeling Law", its consumption has been increasing. Sucralose intake causes alterations in the composition of the intestinal microbiota, which is closely related to mental health through the crosstalk communication between gut and brain. Considering that microbiota can affect behavior and modulate GABA levels, brain plasticity and cognitive function, we wonder whether NCS affect the integration and synaptic function in hippocampal CA1 pyramidal neurons in adult Sprague Dawley rats treated with 0.5% sucralose in the drinking water for a period exceeding 17 days. Using patch-clamp recordings in whole configuration we observe that membrane potential pyramidal neurons NCS treated rats have a more depolarized value than control group and no effect on the trigger threshold of action potentials. Also, we observe that frequency of the spontaneous inhibitory synaptic currents in PYNs is lower than control slices. The paired pulse protocol did not show differences between animals treated with NCS and control, suggesting that NCS intake modify the presynaptic and postsynaptic excitability, no apparent effect on the release of GABA. These results show for the first time that permanent consumption of sucralose may have affect GABAergic synaptic efficacy in the central nervous system.

Pharmacology area: Fisiología (Physiology)
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69. ANTITUMOR PROTOTYPE BASED ON POLYMERIC NANOPARTICLES WITH APPLICATION IN GENE THERAPY.

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Gene therapy is a therapeutic strategy mainly focused on correcting altered or mutated genetic sequences, which can induce the development of hereditary or acquired pathologies, such as cancer. Nanoparticles based on biocompatible polymers have been used as carriers for therapeutic molecules, due to their ability to encapsulate labile molecules such as linear or plasmidial DNA by electrostatic interactions. Thus, it could prevent their degradation by nucleases present in the environment. Safety and effectiveness of the polymers make feasible in vivo tests and future commercial products as described and approved by regulatory agencies, such as the FDA. The aim of this work was to design and evaluate a formulation based on polymeric nanoparticles as gene therapy applied to prostate cancer. Nanoparticles were elaborated using a double emulsion method, with solvent evaporation, and PLGA as the main matrix agent, associated with a cationic polymer. A model plasmid, which transcribes a tumor progression blocker was encapsulated. Physicochemical characteristics of nanoparticles were analyzed by Zetasizer Nano, and their effect was evaluated in vitro, in a human tumor cell line. Nanoparticles were obtained in nanometric size range, with a polydispersion index (PdI) less than 0.2, and the surface charge was positive. Morphologically, nanoparticles were spherical according to Transmission Electron Microscope images. It was also observed an increase in the genetic transformation rate for human tumor cells, and an alteration in the characteristic tumor progression markers expression. These results are promising for the development of new therapeutic candidates based on nano delivery system for complementary treatment in different types of cancer.

Pharmacology area: Farmacología molecular (Molecular Pharmacology)
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70. RESOLVIN D1 PREVENTS CARDIAC HYPERTROPHY AND FIBROSIS IN ANGIOTENSIN II-INFUSED C57BL/6 MICE.

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Background: Resolvin D1 (RvD1) is an endogenous specialized lipid mediator enzymatically derived from docosahexaenoic acid and synthesized locally in acute inflammatory processes, where it exerts pro-resolving effects demonstrated in diverse pathological models. Angiotensin II (Ang II), in cardiac tissue, contributes to the development of cardiac hypertrophy and fibrosis. To date, there are not studies on the potential protection that RvD1 may provide at the structural and functional level in an Ang-II infusion model. Purpose: To evaluate RvD1 effects in Ang II-induced cardiac hypertrophy and fibrosis. Methods: Alzet® osmotic mini-pumps filled with Ang II (1.5 mg/kg/day) were implanted in C57BL/6 mice for 14

days, previous basal left ventricle (LV) functionality assessment. RvD1 (3 ug/day) was injected intraperitoneally. At the end of the infusion period, the animals were sacrificed, and functional and histological parameters were studied. Results: 14-day Ang II infusion increased heart weight/tibia length ratio, LV thickness, ejection fraction, shortening fraction and collagen deposition at the interstitial and perivascular area. Treatment with RvD1 significantly prevented LV dysfunction, hypertrophy and collagen deposition in both areas. Conclusions: RvD1 prevents Ang II-induced cardiac hypertrophy and fibrosis demonstrating cardioprotective properties. Further studies will be performed to elucidate the possible mechanisms of action of RvD1. Ethics approval: The Institutional Animal Care and Use Committee of the University of Chile (CICUA) approved the protocol (CBE2018-12).

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71. ANTIBIOTIC SUSCEPTIBILITY PROFILE OF HELICOBACTER PYLORI IN THE ARAUCANÍA REGION.
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Introduction: Antibiotic resistance is one of the main causes of therapeutic failure in eradication treatments of Helicobacter pylori (Hp), which currently and according to Maastricht consensus the standard consists of a triple scheme of a proton-pump inhibitors (PPI) +2 antibiotics. In recent years, high resistance rates to the main antibiotics used in these treatments have been reported in several countries. Even varying between geographical areas of the same country, which prevents generalizing efficient therapies in certain populations without previous susceptibility studies Aim: To evaluate the susceptibility profile of Hp, in the Araucania region, against antibiotics used in eradication therapy. Methods: A descriptive study on 37 Hp, isolates from gastric biopsy samples on dyspeptic patients was performed in main health centers of the Araucania. The susceptibility profile against amoxicillin, clarithromycin, levofloxacin, metronidazole and tetracycline was performed by agar dilution. Minimum inhibitory concentration values were evaluated according European Committee on Antimicrobial Susceptibility Testing, using Hp ATCC 43504 as a quality control strain. Results: All isolates reported resistance at least one antibiotic and 81.08% showed resistance to two or more antibiotics. 13.8% of the Hp isolates were resistant to amoxicillin, 45.94% to clarithromycin, 41.66% to levofloxacine, 81.08% to metronidazole and 16.66% to tetracycline. Conclusion: The resistance rates to metronidazole, clarothromycin and amoxicillin were higher to reported in Chile and there are not previous reports to LVZ. These results show the need of future studies of therapeutic efficacy in the Araucanía as well a new review of current eradication strategies.

Pharmacology area: Farmacología gastrointestinal (Gastrointestinal Pharmacology)
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72. ANALYSIS OF THE FUNCTIONAL SPECIFICITY IN THE SUGAR PORTER FAMILY TO IDENTIFY NEW INHIBITORS OF GLUT1.
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The movement of glucose and other sugars and polyols with essential functions in the energy metabolism of living beings through biological membranes is carried out by transporter proteins belonging to the family of sugar transporters (SP family, TC code 2.A.1.1), where we find the human GLUT transporters, the hexoses transporters in yeasts, among others. There are highly specific transporters for a physiological substrate, and there are others much more promiscuous. There is an increasing interest in pharmacology to design GLUTs inhibitors, whose development has risen since the determination of the crystallographic structure of human GLUT1. For glucose homeostasis diseases that occur with hyperglycemia and cancer, the therapeutic use of these molecules has been proposed. In the first case, its potential use is in the downregulation of the incorporation of glucose to the blood, while in cancer the aim is to avoid the dispensation of glucose to cells with active proliferation. Several inhibitors of transporters are designed from substrate/solute modifications. To advance in the understanding of the molecular bases that explain the varied selectivity of the SP family, we carried out the present work, which consists of the identification and characterization of sequence profiles related to the specificity of substrates for the SP family. To achieve this goal, we first characterized the functional diversity of the family by identifying functional subclasses using supervised methods that build the functional classification based on empirically obtained transport activities reported for the transporters from databases. From the analysis of the specificity determinant positions (SDPs), we build structures to find inhibitors in a pharmacophore-like mode to accelerate the identification of new inhibitors and predict the selectivity of other GLUT1 orthologues.

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73. INHIBITORY ACTIVITY ON GLYCOGEN PHOSPHORYLASE A OF PHENOLIC EXTRACTS FROM LEAVES AND FRUITS OF 8 UGNI MOLINAE TURCZ GENOTYPES.

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One of the strategies used for the discovery of natural products with hypoglycemic activity is the analysis of species used by traditional medicine, such as Ugni molinae, Myrtaceae, popularly known as murtilla. In this context, the analysis of the inhibition on the activity of Glycogen phosphorylase A (Gpa), an enzyme expressed in brain, muscle and liver, which has major role on post-prandial hyperglycemic peaks in diabetic patients, could account for potential candidates for the development of new treatments. Therefore, the aim of this work was to demonstrate and compare, through an in vitro spectrophotometric methodology, the Gpa inhibitory activity of phenolic-rich extracts obtained from leaves and fruits of 8 murtilla genotypes from the INIA-Carillanca germplasm bank, which were cultivated at the same edaphoclimatic conditions. Compared to caffeine (IC50 = 5,3 ug / mL), the leaves ethanolic extracts were more potent (EETs; IC50 between 1,03 – 3,52 ug / mL; p ≤0,05), while the fruit acetonic extracts were less potent (EACs; IC50 between 27,9 – 86,1 ug / mL; p ≤ 0,05) than the reference substance, as well as less potent than the leaves extracts. Based on our results, the leaves and fruits of U. molinae could be a potential source of bioactive phenolic compounds for the treatment of hyperglycemia, through the development of functional foods or phytopharmaceuticals. On the other hand, based on the results from this work, INIA-Carillanca will be able to classify the genotypes for their potential hypoglycemic effects, which will allow the future to promote the cultivation of murtilla for its agronomic and commercial value, as well as for its medicinal properties.

Pharmacology area: Farmacología de Productos Naturales (Natural Products Pharmacology)
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74. EVALUATION OF ANTICANCER POTENTIAL OF DHA + P1G10 IN CELL LINES DERIVED FROM CANCERS WITH HIGH INCIDENCE IN CHILE.
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Cancer is one of the most frequently diagnosed diseases worldwide, being the second most frequent cause of death. Previous studies have established that omega-3 fatty acids, mainly DHA (docosahexaenoic acid), have protective effects against various types of cancer, among these, gastric cancer, which is one of the most common cancers in the world, with one of the highest mortality rates in Chile. On the other hand, Carica papaya protein fraction P1G10 also has proven anti-cancer properties due to its proteinase activity. Our data indicate that gastric adenocarcinoma cells (AGS) are more sensitive to DHA than non-tumor gastric epithelium cells (GES-1), determining, through MTT, an IC50 of 40.47 µM in AGS. Through Hoechst/Annexin V/IP was found that DHA promotes apoptosis in AGS cells, but not in GES-1. It was also determined that DHA decrease procaspase-3 protein levels in AGS cells only. In vivo assays in BALB/c NOD/Scid mice conclude that DHA treatment for 6 weeks significantly decreases the volumes of tumors generated by AGS cells xenografts. To assess the effects of DHA+P1G10 in vitro, we determined cell proliferation through MTT, treating cell lines derived from the main types of cancer in our country: gastric, lung, gallbladder and breast. To observe cell apoptosis/necrosis visually, we will stain treated cells with Hoechst/Annexin V/PI solution, and to gain further insight into the mechanism of DHA+P1G10 -induced apoptosis, we will examine protein levels of procaspase-3 and caspase-3/7 activity using Western blot and luminescence assay, respectively. Thus, this research seeks to determine and validate the joint use of DHA and the protein fraction P1G10, on cell lines of the main types of cancer in Chile.

Pharmacology area: Farmacología de Productos Naturales (Natural Products Pharmacology)
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75. ANXIOGENIC EFFECT OF AMPHETAMINE ON ZEBRAFISH USING A NOVEL TANK DIVING TEST AND MONOAMINE TRANSPORTER GENES EXPRESSION.
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Monoamine Transporters regulate neurotransmission via the reuptake of dopamine, serotonin and norepinephrine in the brain and regulate the neurotransmitters homeostasis. This class of protein are target for a wide number of compounds including antidepressants, drugs for neuropsychiatric and neurodegenerative disorders, and substance of abuse, such as amphetamine. This drug of abuse has been described that produce anxiogenic effect on rodents and it is well known that interacts with monoamine transporters inducing monoaminergic release. In our group we have used zebrafish as models for behaviour using different drugs that acts over nicotinic receptors and monoamine transporters. The novel tank diving test has been used as a model for to test anxiolytic behaviour on zebrafish, the time spending on the bottom of the tank has been describe as anxiogenic-like behaviour in this model. This work shows the anxiogenic-like effects produced by amphetamine on the novel tank diving test. Additionally, we design the primers and detect the genes expression of Crebs, DAT, NET and SERTa, SERTb by PCR. Also we measure the expression changes using qPCR for this monoamine transporter using a chronic dose of amphetamine. Our results indicate that, amphetamine induce anxiogenics-like effects of diving

behaviour and increase genes expression of MAT´s at different level.

Pharmacology area: Farmacología molecular (Molecular Pharmacology)
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76. INHIBITION OF ADAPTIVE CELLULAR RESPONSES, BY DOXYCYCLINE, INDUCED BY MITOCHONDRIAL INHIBITION TRIGGERED BY GA-TPP+C10 EVOKES SYNERGISTIC CYTOTOXIC EFFECT ON HUMAN BREAST CANCER CELLS

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The metabolic plasticity of cancer cells is the main limiting factor in the research of effective pharmacologic treatments, for development of drug resistance, being one of the major obstacles in the clinical treatment, as a promising target for new anti-cancer drugs therapies. Mitochondria have been the main factor in the metabolic plasticity. This organelle also participates promoting metastasis, tumor-initiating cells and survival and propagation of cancer stem cells, which transforms it into an attractive therapeutic target. Previously reports have demonstrated that GA-TPP+C10 triggered a mitochondrial dysfunction, characterized by an inhibition of electron transport chain (ETC) and AKGDH complex inhibition which triggers cell death. In order to increase this effect, we have analyzed mitochondrial resistance mechanism generated by the action of this compound, highlighting an increased expression of PGC1α and ETC components-related genes encoded by mitochondrial DNA. In order to inhibit the resistance generated by this inhibitory mechanism of action, we have incorporated a second agent, doxycycline, which demonstrated inhibits the synthesis mitochondrial proteins by blockage of only mt-ribosome activity. This effect inhibits the adaptive survival response generated to the action of GA-TPP+C10 evidenced by inhibition of ETC-related protein. Interestingly, the combined therapy increments significatively the mRNA levels, both ETC-components and mitochondrial biogenesis signaling factors (PGC1a-TFAM-NRF1-NRF2), which suggests a greater mitochondrial damage, evidenced by a decreased mitochondrial mass with a consequent decreased of the maximal respiration. In addition, concomitant use of GA-TPP+C10 and doxycycline is able to generate a selective synergic cytotoxic effect on the activation of apoptotic processes in BC cells, which suggest that this combined strategy based on the blockage of mitochondrial bioenergetics inhibition-induced adaptive response may have therapeutic relevance in breast cancer.

Pharmacology area: Otros (Others)
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77. AMYLOID BETA OLIGOMERS INDUCE MITOCHONDRIAL DYSFUNCTION BY ITS DIRECT INTERACTION WITH MITOCHONDRIAL MEMBRANES ON HIPPOCAMPAL SLICES.

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Alzheimer's disease (AD) is a neurodegenerative disorder characterized by impaired learning and memory loss. Amyloid beta peptide (Aβ) plays a key role in the pathogenesis of AD, especially soluble oligomers (SO-Aβ) because can reproduce the major aspects of the disease. In vitro studies have associated mitochondrial dysfunction with an early role in the AD; however, the molecular events are not understood with precision. In this work, we have studied the intracellular effects of SO-Aβ treatments, on mitochondrial morphology and mitochondrial potential (ΔΨm). We found that the degree of colocalization between Aβ and TOM20 was increasing at 24 h of SO-Aβ treatments, with a Manders coefficient (0.640 ± 0.1). Furthermore, we evaluated the ΔΨm using the JC-1 probe, we observed that at chronic treatments (24h), SO-Aβ shown a decrease on ΔΨm near to 50% of the control conditions. Additionally, at the same times (SO-Aβ, 24h) strong changes were observed in the size of the mitochondrial network in primary cultures, displacing the equilibrium towards a more granular pattern in mitochondria that present a positive colocalization with Aβ. Secondly, the intracellular distribution of SO-Aβ (2.5uM) in a mouse hippocampal slices model was evaluated by immunohistochemistry and electron transmission microscopy (TEM), where we observed the presence Aβ targeted with gold nanoparticles in an intramitochondrial zone. On the other hand, it was observed that ΔΨm showed a progressive decrease in time manner on under SO-Aβ treatments (JC-1590/520 C: 1.01 ± 0.01; SO-Aβ 3h: 0.78 ± 0.04). This study suggest a new pathogenic mechanism in AD, where cytotoxic effects of SO-Aβ are related with their direct interaction with the mitochondria, and reveals a novel therapeutic strategies for neuroprotection.

Pharmacology area: Fisiología (Physiology)
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78. CYTOTOXIC EFFECT OF COMBINATIONS OF ITRACONAZOLE, HYDROXYCHLOROQUINE AND CISPLATIN IN HEAD AND NECK CARCINOMA IN LOW GLUCOSE CULTURES.

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Head and neck cancer (HNC) is the sixth most common malignancy in the world, corresponds to 6% of cancer cases and is responsible for 1-2% of deaths worldwide. The most prevalent HNC subtypes are laryngeal cancer and oral squamous cell carcinoma. These pathologies are very aggressive and have poor prognosis and recurrences, which can be caused by a possible resistance to chemotherapy. Cisplatin is the chemotherapeutic most used to treat these pathologies, however it has been high rates of resistance. This resistance may be caused partially by "Cancer stem cells", which are resistant to stress stimuli such as starvation and low oxygen levels. It has been described in some studies that there are drugs, such as Itraconazole and hydroxychloroquine, an antifungal drug that acts at the mitochondrial membrane of the tumor cell by inhibiting the VDAC1 receptor, and an antimalarial/immunosuppressive that has been described with antineoplastic potential by inhibiting autophagy, respectively. In this way it is proposed that the combination of these drugs sensitize the effect of Cisplatin. Cell viability tests were performed with the compounds at 24, 48 and 72 hours under normoxia conditions with low glucose medium (1,0 g/L) in two cell lines, laryngeal squamous cell carcinoma (HEp-2) and squamous tongue carcinoma (CAL-27), using as a control oral dysplastic cells (DOK). This will be carried out in order to obtain the IC50 of each compound and determine their cytotoxic effect. A cytotoxic effect has been observed for all compounds assessed on tumor cells, highlighting the efficacy of Hydroxychloroquine over Cisplatin and Itraconazole. The combination of hydroxychloroquine or itraconazole with cisplatin, improve the cytotoxic effects on tumor cells.

Pharmacology area: Farmacología Odontológica (Dental Pharmacology)
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Acknowledgments: Research funded by Fondecyt Iniciación 11180533.

79. ANTIMICROBIAL SUSCEPTIBILITY TESTS OF HELICOBACTER PYLORI ISOLATES FROM PATIENTS IN THE BIOBÍO REGION: COMPARISON OF AGAR DILUTION AND DISK DIFFUSION.

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Antimicrobial susceptibility testing for Helicobacter pylori is increasingly important due to resistance to the most commonly used antimicrobial agents. The Gold Standard proposed by the CLSI is the agar dilution method, but it is difficult to perform routinely. The objective of this work was to determine the concordance of disc diffusion in comparison to the agar dilution method for: clarithromycin, metronidazole, levofloxacin, amoxicillin and tetracycline, using 44 strains of H. pylori from patients in the BioBío region. Univariate analysis was performed, and the Kappa test was applied for concordance using the Stata V.14 program. The resistance rates were for clarithromycin 29.5% and 25.0%; metronidazole 45.4% and 56.8%; Levofloxacin 31.8% and 25.0; Amoxicillin 2.2% and 0% respectively by disk diffusion and agar dilution. Tetracycline showed no resistance with any of the 2 methods used. Clarithromycin presented a considerable degree of concordance with a k = 0.6571 (p <0.0001). Metronidazole did not show concordance for the techniques under study (p=0.1586). Levofloxacin presented an almost perfect concordance with a k=0.8333 (p<0.0001). On the other hand, the Kappa test was not calculated for amoxicillin and tetracycline, since 97.3% and 100% concordance were obtained respectively. The disc diffusion method presented a high degree of agreement with the Gold Standard for clarithromycin, levofloxacin, amoxicillin and tetracycline. This is an easy method to assess susceptibility to H. pylori especially if it is performed routinely. For metronidazole there was a high degree of disagreement with agar dilution, which has already been reported. Finally, other studies with a greater number of isolations are necessary to assess whether the method of disk diffusion, which is simpler and cheaper, can be continued routinely in our region.

Pharmacology area: Farmacología gastrointestinal (Gastrointestinal Pharmacology)
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Acknowledgments: Beca Conicyt Magister Nacional 2019-22190334. PROYECTO VRID SEMILLA 219.036.049-5. Universidad de Concepción

80. STUDY OF THE INTERNALIZATION OF H. PYLORI J99 IN C. ALBICANS ATCC 10231 AGAINST CLAROTHROMYCIN AND AMOXICILLIN AS STRESS FACTORS.

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Helicobacter pylori is a pathogenic microorganism responsible for a large number of gastrointestinal pathologies. First-line therapy against H. pylori include clarithromycin, amoxicillin and a proton pump inhibitor. This treatment has lost effectiveness over time due to antibiotic resistance. On the other hand, in vivo evidence suggests an endosymbiotic interaction between H. pylori and Candida albicans that could avoid the direct action of antibiotics. These processes have not been reported in vitro. The objective of this work was to determine the incorporation of H. pylori J99 into C. albicans ATCC 10231 under in vitro conditions of stress to clarithromycin and amoxicillin. For this, a growth curve was performed for H. pylori and C. albicans quantified by 600nm optical density. The minimum inhibitory concentration of the study antibiotics was determined according to the CLSI. Co-cultures were performed at subinhibitory concentrations of antibiotics taking samples at 0, 1, 3, 6, 12, 24 and 48 hours, bacterial internalization was evaluated by optical microscopy, at the same time these samples were seeded on Sabouraud agar and incubated at 37 ° C under aerobic conditions for 48 h. With these cultures, DNA extraction was performed, by PCR, the 16S rDNA gene of H. pylori was amplified. In the samples analyzed by optical microscopy, bacterial internalization was observed inside C. albicans vacuoles when co-culture was performed with amoxicillin. The PCR analyzes were positive for the co-cultures of both antibiotics used. These results suggest that H. pylori can

enter inside C. albicans to protect against the presence of antibiotics as stressors.

Pharmacology area: Farmacología gastrointestinal (Gastrointestinal Pharmacology)
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Acknowledgments: Beca Conicyt Magister Nacional 2019-22190334. Proyecto VRID-Enlace, 218.036.047-1.0. Universidad de Concepción

81. POLYMERIC BIOCOMPATIBLE NANOCARRIERS FOR DRUG DELIVERY APPLICATIONS SHOWS PRESERVED BIOLOGICAL ACTIVITY OF LOADED PROTEINS, IN VITRO AND IN VIVO.
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In the search for new and more effective therapies, polymeric nanoparticulate systems which protect the drugs and increase bioavailability have been developed. The use of biocompatible and biodegradable polymers could guarantee the harmless character of the formulation while allows the controlled release of the active principle. Using ionotropic gelation method, we synthesized chitosan-TPP nanoparticles loading recombinant and model proteins, in a reproducible way. This nanoparticulate system showed a peak of protein released around the fourth day, in vitro, and promoted the internalization of loaded BSA-FITC conjugates by Hep-2 cells after 24 hours of incubation. Cytotoxicity assay evidenced the benign character of the formulation, while experiments of biological activity in vitro and in vivo, showed a specific biological response due to the system loading. Visualization of the nanoparticles was possible thanks to transmission electronic microscopy. This procedure proved to be an effective method to formulate proteins, and, potentially, other molecules, in a safe way, while the release of the active principle can be delayed over time. This type of systems can be used in drug delivery applications in which pharmacological interaction with the cell is required.

Pharmacology area: Otros (Others)
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Acknowledgments: The authors would like to thank Conicyt and Universidad de Concepción.

82. LEUKEMIA INHIBITORY FACTOR, A NEW MODULATOR OF THE OVARIAN CHOLINERGIC SYSTEM IN SUBFERTILE RAT.
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Leukemia Inhibitory Factor (LIF) is a proinflammatory cytokine that participates the regulation of ovarian functions. LIF participation in the subfertility period has not been described and the mechanism of action is unknown. In vitro studies have shown that LIF increase the acetylcholine (ACh) synthesis, choline acetyltransferase (ChAT) expression and its activity in the upper cervical ganglion. In our laboratory, an intrinsic ovarian cholinergic system has been determined recently, which participates in the regulation of ovarian function. The aim was to evaluate the LIF/LIF Receptor (LIFR) levels and its effect on the ovarian cholinergic system in subfertile rats. We measured LIF and LIFR mRNA and protein levels by qRT-PCR and western-blot at 3 (fertile) and 9 months old (subfertile) Sprague-Dawley rats. To evaluate the LIF effect on the ovarian cholinergic system, rat ovaries were incubated in vitro for 3 and 8 h with LIF (100ng/ml) and buffer Krebs (vehicle). ACh production and the mRNA content of the genes encoding the ChAT and AChE enzymes it was determined by fluorometry and qRT-PCR respectively. The results show increase in LIF protein levels and increase of LIFR mRNA in ovaries in fertile period in subfertil period. Incubation with LIF increases ACh in incubation medium, without observing changes in ovarian ACh levels. The ChAT and AChE mRNA content enzymes significantly decrease at 3h of incubation (40% and 50%, respectively). In contrast, in ovaries incubated for 8 h, LIF does not affect ovarian ACh levels nor in the incubation medium. These results suggest that LIF regulates ovarian cholinergic function during reproductive aging, pending as LIF and the cholinergic system regulate follicular development at this period.

Pharmacology area: Farmacología endocrina-reproductiva (Endocrine/Reproductive Pharmacology)
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83. IDENTIFICATION OF RESIDUES INVOLVED IN THE DOPAMINE TRANSPORTER-GBETAGAMMA PHYSICAL/FUNCTIONAL INTERACTION.
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The dopamine transporter (DAT) plays a crucial role in the regulation of brain dopamine (DA) homeostasis. Through re-uptake of DA, DAT serves two important functions: the termination of synaptic transmission at dopaminergic terminals, and the replenishment of vesicular DA pools. In addition to uptake, DAT can also function to release DA. This process, which is referred to as DAT-mediated efflux, is the mechanism used by potent and highly addictive psychostimulants, such as amphetamine and its analogues, to increase extracellular DA levels in motivational and reward areas of the brain. It has long being recognized that DA neurons release DA through exocytotic and non-exocytotic processes. However, the exact mechanism by which physiological signals or psychostimulants, such as amphetamine, induce DA efflux through DAT still remains a complex and not completely understood area of research. Recently, we discovered that the

G protein betagamma subunits bind to the intracellular carboxy-terminus of DAT and regulate transporter activity. More importantly, we have observed that activation of Gbetagamma promotes DAT-mediated DA efflux. However, the amino acid residues involved in Gbetagamma interaction site(s) in DAT and their role in transporter regulation remain largely unknown. Here, we used a combination of bioinformatics, mutagenesis, immunoprecipitations, and functional assays to identify the Gbetagamma binding site on DAT and its role in transporter regulation. Preliminary functional studies are consistent with previous biochemical evidence indicating that the sequence FREKL located in the carboxy-terminus of DAT plays a role in Gbetagamma interaction with DAT and promotion of DA efflux. Thus, this study provides a starting point for a further detailed characterization of the DAT-Gbetagamma interaction and a better understanding of its contribution to DAT-mediated efflux.

Pharmacology area: Farmacología molecular (Molecular Pharmacology)
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84. EVALUATION OF SINAPTIC COMPONENTS DURING NEURULATION OF XENOPUS LAEVIS EMBRYOS.

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In chordates, neurulation and neural tube formation is the first step in the central nervous system (CNS) development. Failures, by genetic or environmental alterations, in this process may induce neural tube defects (NTDs). It has been described in the literature, that the use of anti-epileptic drugs (AEDs) during pregnancy, increase the probability of NTDs by mechanisms not completely identified. Recently, glutamate signaling through NMDAR has been proposed to participate in neurulation process. Here we hypothesized that glutamate would be released by a vesicular-related mechanisms which contribute to cellular responses necessary for normal neural tube formation. For evaluate this, we use Xenopus laevis embryos, collected in different neurulation stages (9 - 20 hours post-fertilization (hpf)) for obtain RNA transcripts. Then we performed PCR and qPCR for assess relative expression studies, focalized in evaluate the presence of vesicular release-related and synaptic receptor proteins. We observe the presence of vesicle related proteins, such as SNAP25, VAMP2, Syntaxin and VGLUT1, as well as glutamate receptor MGLuR2 and AMPAR during neurulation. Then, to evaluate the functionality of AMPAR in neurulation, we perform pharmacological studies, using the antagonist CNQX. We observe that CNQX don't provoke any evident alteration in neural tube formation. Finally, we performed an induction of epileptogenic behavior using Pentylenetetrazol to evaluate CNS health after neurula-treatments. We observe a decrease of almost ~50% in the seizure latency onset necessary for epileptogenic behavior vs not treated controls. Our results suggest that, glutamate could be released using vesicles proteins and the expression of AMPAR don't participate in the normal neural tube development but could regulate additional later process important for the CNS establishment on Xenopus laevis.

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Acknowledgments: FONDECYT project N°11160562.

85. TRIPANOCIDAL ACTIVITY OF CASTANEDIA SANTAMARTENSIS (ASTERACEAE) AGAINST TRYPANOSOMA CRUZI.

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Chagas disease (CD) an endemic disease from Latin America, is caused by Trypanosoma cruzi infection. More than 7 million people are infected. Currently, there are two drugs derived from nitro compounds for the treatment of CD with important side effects, that cause the treatment to be abandoned, furthermore, the effectiveness in the chronic phase is still controversial. Therefore, is necessary the search for new drugs that are more effective and better tolerated. Castanedia santamartensis R. M. King & H. Rob, is known for their properties to treat skin sores. The objective of this study was to evaluate the trypanocidal activity of an ethanolic extract (ETE) of C. santamartensis and its fractions. Air-dried and powdered leaves, were extracted at room temperature with ethanol and concentrated and dried by evaporation at reduced pressure. The fractionation was obtained by chromatographic separation methods, using solvents of different polarity. The in vitro trypanocidal activity of the ETE and the fractions was determined against T. cruzi trypomastigotes (Dm28 strain) using MTT and flow cytometry techniques. Nifurtimox was used as a reference drug. The IC50 (concentration that produce a 50% parasitic death) was calculated using the least squares method. The ETE presented trypanocidal activity (IC50 of 197.3 micrograms/mL). The CS200; CS300 and CS400 fractions, presented trypanocidal activity with IC50 values of 91.2; 63.9 and 15,4 microgramos/mL respectively. The IC50 of the reference drug was 5.7 micrograms/mL. The results indicate that C. santamartensis contains secondary metabolites with activity against T. cruzi.

Pharmacology area: Farmacología de Productos Naturales (Natural Products Pharmacology)
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86. EFFECTS OF P2X2R OVEREXPRESSION ON AMPK AND PGC-1α DEPENDENT SIGNALING PATHWAYS

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One of the main toxic agents in Alzheimer's Disease (AD) are the soluble oligomers of the Aß peptide (OS-Aß). Chronic treatments with OS-Aß have been shown to increase the expression of the P2X2 receptor (P2X2R) in PC12 cells and rat hippocampal cells, participating in increasing intracellular calcium and allowing a leak of ATP to the extracellular environment. AMPK protein kinase has several roles on protein, energy and mitochondrial metabolism and is regulated by changes in the levels of intracellular Ca2+ and AMP/ATP ratio. AMPK is capable of phosphorylate PGC-1a, which is a transcription co-activator that, when is phosphorylated, is activated and translocates to the nucleus, promoting mitochondrial biogenesis. Using PC12 cells to overexpress P2X2R, the effect of its activation was assessed by ATP treatments, on AMPK activity and the subcellular distribution of PGC-1a. From functional experiments (calcium microfluorimetry and electrophysiology), immunocytochemistry and Western blot, it was concluded that overexpression and activation of P2X2R by ATP, prevents an increase in AMPK activity and generates changes in the subcellular distribution of PGC-1a, which suggests that P2X2R would be related to the toxicity generated by the Aß peptide and the intracellular calcium overload.

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87. NEW LIPOPHILIC CATIONS DERIVED FROM CAFFEIC ACID INDUCE CYTOTOXIC EFFECT IN HUMAN COLORECTAL CANCER CELLS.

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One of the deadliest pathologies worldwide is cancer. Colorectal cancer is the third most common type of cancer. A few drugs are provided for treatment this disease, like 5-fluorouracil, oxaliplatin and irinotecan, as standard therapy. However, this therapy several times failed due to high drug resistance and side effects, leading cancer progression. There are several risk factors both exogenous and endogenous that increase the incidence of this disease in the organism, hence the importance of characterize the cancer cells from cytological, metabolic and molecular aspects. These features give them the differences between normal epithelium cells, becoming with high proliferative rates in an uncontrolled manner. In this sense, the mitochondria appear as a new target for new molecules against cancer, since they have high mitochondrial-transmembrane potential than normal cells, capable to accumulate cationic compounds. This work is focused in the evaluation of a new set of molecules derivatives from caffeic acid attached to a different size length of triphenylphosphonium-aliphatic chain and their effect on human colorectal cancer cells. We evaluated cytotoxic effect by MTT assay, the decrease of mitochondrial potential by flow cytometry and the decrease of cellular of ATP levels by luminescence. The results showed that the compounds were cytotoxic in colorectal cell lines (HCT-15 and COLO 205), decreasing mitochondrial-transmembrane potential and cellular ATP levels. In conclusion, these new compounds may induce cytotoxic effect by a mitochondrial mechanism, inducing bioenergetics stress, suggesting the importance of studying new pharmacological agents taking advantage of the cellular singularities like mitochondrial metabolism.

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88. P2Y2R AND P2X4R EXPRESSION PROFILE AND ITS ROLE IN PROLIFERATION AND METASTATIC POTENTIAL IN GASTRIC CANCER CELL LINES.

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Gastric cancer is considered a major health concern due to its unspecific symptomatology on early stages and complex pathophysiology that hinders any attempts for targeted therapeutic approaches. This disease has high incidence and mortality rates worldwide, being the third cancer-related cause of death in Chile, which focuses scientific work in understanding the mechanisms that trigger abnormal proliferation rates and subsequent tumor migration in gastric epithelia. Between these possibilities, purinergic signaling emerges as promising pathway that regulate cell growth, proliferation and migration according to the expression rates of its many receptor classes and subclasses. Among these, P2Y2 receptor (P2Y2R) is widely known by its contribution to cell invasion and metastasis in prostate, colorectal and colon cancer; which is in contrast to the antiproliferative effects reported for P2X4 receptor (P2X4R) on cancer models in vitro. Despite all this background, purinergic signaling involvement in gastric cancer remains unknown. For this reason, our investigation was focused to characterize the expression profile of P2Y2R and P2X4R, in terms of protein levels by western blot and gene expression by qPCR, in cell lines derived from primary gastric adenocarcinoma (AGS), moderately and mildly differentiated metastatic gastric adenocarcinoma (MKN-74 and MKN-45, respectively) and healthy gastric epithelia (GES-1). Moreover, to assess P2Y2R and P2X4R contribution to gastric cancer growth and invasion, we evaluated the effect of different agonists and antagonists on cell proliferation by Resazurin assay, and stablished metastatic potential by transepithelial electrical resistance (TEER) measurements in

the gastric cell lines described above after overexpressing or silencing P2Y2R and P2X4R. Our results provide preliminary insights on gastric cancer pathophysiology that can be used as future pharmacological approaches for treatment.

Pharmacology area: Farmacología gastrointestinal (Gastrointestinal Pharmacology)
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89. INTRACELLULAR AMYLOID-BETA OLIGOMERS DECREASE EXCITABILITY AND AMPA MEDIATED CURRENT IN NUCLEUS ACCUMBENS NEURONS.
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Alzheimer disease (AD) is a progressive neurological disorder that causes dementia in an increasingly aging worldwide population. Despite the current dogma of AD, where extracellular aggregates of amyloid beta peptide (Aß) initiates neurotoxicity, growing evidence shows synaptic dysfunction and loss of limbic functions in early stages before amyloid plaque deposition, where the presence of intracellular Aß has been reported. The nucleus accumbens (NAc), a central integrative brain area of the limbic system, is particularly affected in AD in humans and transgenic mice models. However, the effect that intracellular Aß may have on neuronal function has still not been examined. Therefore, in this study we analyzed the effects of intracellular Aß oligomers (iAßo) on acutely dissociated NAc neurons. To evaluate if iAßo could modulate components of the neurotransmission, we used a modified whole-cell patch clamp technique to dialyze Aßo intracellularly through the recording electrode. The effects of iAßo were study on the maximum evoked current (Imax) where under control conditions, the AMPA current was 149 ± 18 pA and decreased to 73 ± 15 pA after the application of iAßo 1 µM. Interestingly, GABA and GLY currents were not affected. Furthermore, iAßo was able to decrease accumbal neurons excitability, diminishing the number of action potential spikes and its amplitude. Overall, these findings showed that iAßo inhibited the amplitude of AMPA receptors in accumbal neurons and also decreased neuronal excitability. These effects support the notion that iAβo is able to impair neurotransmission in limbic areas.

Pharmacology area: Fisiología (Physiology)
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90. FUNCTIONAL MODULATION AND MOLECULAR INTERACTION OF THE ALKALOID KOUMINE WITH GLYCINE RECEPTORS.
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Koumine is one of the main alkaloids of the Gelsemium genus plants. Behavioral studies have reported that the administration of koumine exerted analgesic and anxiolytic effects. The mechanisms underlying these beneficial effects are not well defined. However, behavioral studies have shown that the analgesic and anxiolytic effects of koumine are inhibited by strychnine, a selective antagonist of inhibitory glycine receptors (GlyRs), which are chloride-permeable pentameric ligand-gated ion channels expressed in the central nervous system. To date, whether koumine is able to modulate the function of GlyRs is unknown. Here, by using biochemical, electrophysiological and bioinformatics approaches, we studied the potential modulation of GlyRs by koumine. Our electrophysiological studies showed that koumine negatively modulates the GlyR function. For example, the acute application of 25 micromolar of koumine inhibited the glycine-activated current through recombinant alpha1-GlyRs and alpha3-GlyRs by 35%. Molecular docking studies based on the alpha3-GlyR crystal structure suggest that koumine interacts with the orthosteric pocket of the receptor, favoring a closed state of the ion channel. Ongoing biochemical studies will determine whether koumine directly interacts whit the extracellular domain of alpha3-GlyRs. Overall, these results demonstrate the actions of koumine on the GlyR function. These results, together with ongoing studies, may contribute to understand the mechanisms underlying the koumine-induced analgesia and anxiolysis.

Pharmacology area: Farmacología molecular (Molecular Pharmacology)
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91. PHARMACOLOGICAL INHIBITION "IN VIVO" OF OVARIAN ACETILCOLESTERASE REVERTS POLIQUISTIC OVARY PHENOTYPE IN RAT.
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Ovarian function is subject to endocrine and nerve regulation. An increase in sympathetic tone by cold stress (CS) induces a phenotype like polycystic ovarian condition (PCO). On the other hand, a local cholinergic system has been described in rat ovary, in which we find acetylcholine (ACh), the muscarinic receptor M1, and the enzyme acetylcholinesterase (AChE). Chronic treatment with the AChE inhibitor Huperzine A (Hup-A) has been reported to increase the fertility on the rat. In this context, the purpose of this study is to determine whether the long-term changes induced by CS on ovarian function can be reversed by increasing ACh chronically by administering Hup-A. In this study, Sprague-Dawley rats were subjected to CS subsequently hemiovarioectomized and implanted with a miniosmotic pump with Huperzine A (10 µM) or subjected to the procedure but without the implantation of miniosmotic pump (Sham). 28 days after the procedure the ovary and the serum were collected to measure steroid hormones

Testosterone (T), Progesterone (P4) and Estradiol (E2) by enzyme immunoassay and the follicular development by morphometry. A second group of rats were used to measure the fertility after mate with males of proven fertility. The results show that CS generates a polycystic phenotype with cysts, hyperandrogenism and low fertility. The administration of Hup-A reverses the alterations in follicular development and hyperandrogenism produced by CS but not increase the fertility. The pharmacological potential of these findings gives to the cholinergic local system relevance in the treatment of PCO.

Pharmacology area: Farmacología endocrina-reproductiva (Endocrine/Reproductive Pharmacology)
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Acknowledgments: This work was funded by FONDECYT Grant N° 1170291 (HEL) and Fellowship CONICYT N° 21170073 (RR).

92. DEVELOPMENT OF ACPV-56 LOADED MICROPARTICLES, FOR THE TREATMENT OF RHEUMATOID ARTHRITIS. PRELIMINARY STUDY.

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Rheumatoid arthritis (RA) is a chronic disease whose worldwide incidence is increasing. Currently, there are non-pharmacological and pharmacological approaches for the therapeutic management of RA. From the latter, the drug ACPV-56 has demonstrated anti-arthritical activity due to its ability to inhibit the proliferation of activated lymphocytes, which results in a marked anti-inflammatory effect. Clinical studies have proven that upon oral administration of therapeutic doses, ACPV-56 causes gastrointestinal adverse effects that negatively influence the compliance to chronic treatments. A strategy to avoid the adverse gastrointestinal effects of ACPV-56 and increase the dosing time intervals is by incorporating the drug into a controlled release system administered by intramuscular injection. As none of the available formulations containing ACPV-56 is intended for parenteral administration, the objective of this research is to develop a drug delivery system based on biodegradable microparticles (MPs) encapsulating ACPV-56. The MPs, elaborated by spray drying of a mixture of anionic polysaccharides, cationic and phospholipids, were characterized in terms of its in vitro release kinetics, employing conditions that emulate the physiological environment. The formulation parameters were optimized in order to obtain MPs suitable for injection. The obtained micro particles were spherical, with a medium diameter close 20 µm, relatively mono-disperse and with a minor tendency to aggregation. The incorporation of ACPV-56 did not affect the physicochemical properties of the developed MPs. Preliminary findings of the release kinetics showed that the encapsulation of ACPV-56 within MPs delays its release at least 10 times when compared to the free drug. As a conclusion, the developed microparticles represent a promising alternative for treatment of RA.

Pharmacology area: Tecnología farmacológica (Pharmaceutical Technology)
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93. DEVELOPMENT OF CHEMICALLY CROSS-LINKED HYDROGELS WITH POTENTIAL BIOMEDICAL APPLICATIONS.

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Hydrogels based on natural origin polymers have shown promising results as scaffolds for cell encapsulation and drug delivery in tissue engineering. In this work, biopolymeric hydrogels, from natural and biocompatible polymers, were produced by two methods, chemical cross-linking with glutaraldehyde and freeze-thawing. The hydrogels were characterized using scanning electron microscopy (SEM) and Fourier-transform infrared spectroscopy (FTIR). The SEM images showed that the structure and morphology of the hydrogels produced by chemical cross-linking differed from those produced by freeze-thawing, while FTIR analysis also revealed different chemical composition between them. Their potential biomedical application was also assessed. First, their biocompatibility with HEP-2 cell line was tested using an MTT assay. The results showed that the chemically cross-linked hydrogels did not affect the cell viability compared to the freeze-thawing-produced hydrogels. We further tested the potential of chemically cross-linked hydrogels to retain and release bioactive compounds in the cells by loading the hydrogels with BSA protein conjugated with FITC. Using a fluorescence microscope, we observed that the HEP-2 cells were stained green, indicating a successful release of the conjugate. These data suggest that our chemically cross-linked hydrogels have the potential to be used for drug delivery in tissue engineering applications.

Pharmacology area: Tecnología farmacológica (Pharmaceutical Technology)
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94. ANTIDEPRESSIVE LIKE EFFECT INDUCED BY THE ACUTE ADMINISTRATION OF IBOGAINE AND NORIBOGAINE IN RATS AND ITS POSSIBLE MECHANISM OF ACTION.

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Previous human subjective data and animal studies demonstrated that the psychedelic alkaloid ibogaine, and its metabolite noribogaine, have potent antiaddictive effects. The biological mechanism through both compounds elicit this beneficial effect remains still unclear. Among several molecular targets, ibogaine and noribogaine inhibit the serotonin transporter (SERT). This action and a longterm increase of brain-derived neurotrophic factor RNAm levels in the rat prefrontal cortex that we found in our previous study after the acute ibogaine i.p. administration, lead us to hypothesize that the anti-addictive property of ibogaine and noribogaine could be related to a potent putative antidepressant-like effect. Consistent with this possibility we characterized the behavioral effects (dose and time-dependence) induced by the acute ibogaine (20 and 40 mg/kg) and noribogaine (20 and 40 mg/kg) administration in rats using the forced swimming test (FST). Fluoxetine (40 mg/kg/i.p.) a standard antidepressant drug, was used as a control. We found that ibogaine and noribogaine induced a dose- and timedependent antidepressive-like effect. To know if the antidepressive-like effect induced by ibogaine was due to an effect per se or by the presence of its metabolite (noribogaine) we intravenously injected animals with ibogaine. Ibogaine 1 and 5 mg/kg after an i.v. injection on animal behavior immediately evaluated in the FST. Under these conditions, ibogaine did not generate an antidepressant effect. Ibogaine seems to depend on noribogaine content to induce the beneficial effect. All the behavioral responses were consistent with the pharmacokinetic data. Interestingly, noribogaine 40 mg/kg elicited an antidepressant-like effect per se with a higher potency than fluoxetine. Our data support the possibility that this potent antidepressive-like action could collaborate, at least in part, to explain the ibogaine´s previous anti-addictive effects.

95. MODULATION OF ANTIOXIDANT ACTIVITY IN DERIVATIVES OF AMINOETHYL PHENANTRENE AND HALO-APORPHINES WITH ANTINEOPLASTIC ACTIVITY.

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In the search for new molecules with antineoplastic and antioxidant properties derived from the natural product boldine (1), it was proposed to increase the molecular lipophilicity of the precursor molecule with the expectation of improving the above mentioned bioactivities. Using (1) as starting matherial was prepared: secoboldine (N, N- (methyl) (ethyl) phenanthrene; seco-boldine (2)) and a series of halo derivatives (halo = Cl or Br) (3-6), which were characterized by NMR-1H and -13C and the measurement of their melting point. Boldine (1) has the lowest dissociation energy in the phenolic group of C-9 with respect to C2-OH which is consistent with the highest acidity recorded for first phenolic group. The insertion of one halogen atom (3,4) maintains the energy value to break the O-H bond, but in compounds that bearing two halogen atom (5,6) the dissociation energy markedly increases while antioxidant capacity decrease in these compounds. This experimental evidence is correlated with the "local softness" (LS) exhibited by boldine and halogenated compounds. In conclusion, the antioxidant activity in these compounds can be modulated by the insertion of halogen atoms in the appropriate positions.

Pharmacology area: Farmacología de Productos Naturales (Natural Products Pharmacology)
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96. GALLIC AND GENTISIC ACID DERIVATES INDUCE AUTOPHAGY IN MURINE AND HUMAN COLORECTAL CANCER CELL LINES.

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Colorectal cancer is the third most common neoplasm in the world. The standard treatment consists mainly in surgery and the use of three first-line chemotherapies, 5-fluorouracil, oxaliplatin and irinotecan. The high drug resistance, several side effects and high costs of the treatments, give an opportunity to search for new molecules with new pharmacological targets. In the recent years, cancer cell mitochondria have become in an interesting pharmacological target due to their high mitochondrial-transmembrane potential that allows accumulated cationic probes conjugated with cytotoxic pharmacophore. In our laboratory, the gallic and gentisic acid derivatives as conjugated with lipophilic cationic triphenylphosphonium through an aliphatic chain of ten carbons have been tested in breast and colorectal cancer cells. These compounds triggered a series of events that leads cell apoptosis. The objective of this work is described how the decrease of ATP levels induces the activation of AMPK, which promotes death by autophagy in colorectal cancer lines. Through western blot and luminescence assay, we observed that in human and murine colorectal cell lines, the derivatives induce the reduction in cellular ATP levels followed by the activation of AMPK and LC3B, leading to cell death by autophagy. In conclusion, our compounds may be inducing apoptosis by triggering mitochondrial unbalance, energy stress and autophagy.

Pharmacology area: Farmacología molecular (Molecular Pharmacology)
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97. ANTIPROTOZOAL ACTIVITY OF CHICORY (CICHORIUM INTYBUS) AGAINST TRYPANOSOMA CRUZI.

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Chagas disease is an endemic parasitosis in Latin America. However, its drug treatment frequently induces adverse effects. Thus, it is urgent to develop new therapies. Chicory is a bioactive plant with antiparasitic activity related with its content of sesquiterpene lactones (SL). Most antiparasitic studies of chicory have been focused on nematodes, but poorly explored against parasitic protozoa. The aim of this study was to evaluate the antiprotozoal effects of SL-rich chicory extracts against Trypanosoma cruzi, the etiological agent of Chagas disease. SL of chicory leaves and roots were extracted from 5 chicory cultivars (Spadona, Goldine, Larigot, Measeto and Benulite) using methanol/water and purified by solid-phase extraction. The extracts were dissolved in DMSO. SL profiles of the extracts were characterised by UHPLC-MS metabolomics. The cytotoxicity of extracts was tested on T. cruzi trypomastigotes (Y strain) and mammalian Vero cells. Trypomastigotes were incubated for 24 h with serial dilutions of extracts (100-6.3 µg/mL), and benznidazole was used as positive control. Vero cells were exposed to extracts for 24 h at 100 and 50 µg/mL to evaluate cytotoxicity. Cell viability was evaluated by resazurin reduction test. Chemical profiling showed that chicory extracts have distinct content of SL among cultivars and between plant parts. All the extracts had dose-dependent effect against isolated trypomastigotes. However, Spadona leaf extract was the only with no toxicity against Vero cells at 100 µg/mL, suggesting a selective trypanocidal activity. Taken together, these results revealed that chicory Spadona leaf warrants deeper exploration regarding the relationship between its chemical profile and antiprotozoal activity. Consequently, these results encourage further investigation of chicory as a source of SL with antiparasitic therapeutic potential.

Pharmacology area: Farmacología de Productos Naturales (Natural Products Pharmacology)
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98. AUTISTIC AUTOANTIBODIES ABSORBED FROM BREAST MILK GENERATES COGNITIVE IMPAIRMENT IN BREEDING FEMALE BUT NOT MALE RATS.

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Autism spectrum disorders (ASD) involve a range of complex neurodevelopmental disorders, characterized by social impairments, communication difficulties, and restricted, repetitive and stereotyped patterns of behavior. ASD exerts a significant physiological, emotional and financial burden on the families of the individual and society as a whole. Recently, beside the knowledge about genetic factors involved in this pathology, there is new evidence related to immunological causes of ASD. Therefore, it is of outmost importance to elucidate the molecular and physiological mechanisms of ASD pathology. Data from us and others have shown that normal young rat hippocampal slices incubated with purified IgA autoantibodies from ASD patients and breeding rats from pregnant mothers injected with the same antibodies, impairs LTP as well as disrupts learning and memory. Taking this into account, we hypothesized that ASD autoantibodies are absorbed from breast milk and generates autoimmune-related cognitive impairment characteristic of ASD pathology. To achieve this aim, we used a rat model where mothers were injected with ASD autoantibodies during breast milk period and the breeding was tested after that period using learning and memory test together with electrophysiological and immunohistochemical studies. We found that both LTP and learning and memory were significantly impaired in female but not male breeding rats and this alteration are correlated with the presence of ASD autoantibodies in hippocampus and Cortex. These results demonstrate that ASD autoantibodies are incorporated from breeding milk, cross both intestinal and blood-brain barrier and impairs learning and memory in a sex-preference fashion. They also give us new knowledge about possible causes of autism and opening a new line in pharmacological therapies.

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99. MDMA (3,4-METHYLENEDIOXYMETHAMPHETAMINE) AND HELPING BEHAVIOR: PRELIMINARY CHARACTERIZATION IN SPRAGUE-DAWLEY RATS.

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MDMA (3,4-methylenedioxymethamphetamine, “ecstasy”) is a psychotropic drug that induces an “open mind” state in healthy humans characterized by heightened self-acceptance, openness to communication and a fear threshold decrease known as the entactogenic syndrome. Disregarding its therapeutic potential, direct evaluation of MDMA-like effects in animal models remained limited to the pro-social paradigm, a model that recreates different stereotyped rodent behaviors. In contrast to these classical pharmacological criteria, helping behavior is a complex type of pro-social paradigm that has been recently described in rodents. It stands out because of its pertinence to develop a more sophisticated pharmacological model to study human-like behaviors, as it may occur in rats as a result of the interaction between psychomotor capabilities and the amount of stress experienced by the animal, even in

the absence of reward. Despite of its relevance, the behavioral characterization of the effects of MDMA in this model remains unexplored. In the present work, a preliminary characterization of the effects of MDMA on helping behavior in male rat pairs (helper rat + victim rat; with/without previous individual housing) after 12 days-administration/training cycles at two different dose levels (5 mg/kg; 10 mg/kg i.p.) has been attempted using a slightly modified water-trap model developed ad hoc. The results obtained indicated that MDMA might not enhance helping behavior compared to controls when the acting roles of each pair member has not been interchanged. In contrast, current data seems to be in agreement with the notion that helping assistance may rather depend on if the rat pair met each other previously or not and/or the experience of being trapped in the water trap, at least at the dose ranges evaluated.

Pharmacology area: Neuropsicofarmacología (Neuropsycopharmacology)
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100. EFFECT OF A LACTOBACILLUS ADMINISTRATION ON ANXIETY-LIKE BEHAVIORS IN ADULT RATS.
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There is increasing evidence that gut microbes affect central nervous system (CNS) function. For instance, there are some Lactobacillus strains with proven anxiolytic and antidepressant like effects in rodents. However, there is also evidence that other Lactobacillus have anxiogenic effects in rats. In this regard, our previous findings show that 2 week administration of the potential probiotic bacteria Lactobacillus casei L-54-2-33 to healthy pre-pubescent Sprague-Dawley male rats, increased anxiety like behaviors, while lowering hippocampal expression of 5-HT1A receptor. Therefore, we asked if this anxiogenic-like effect was due to the rat's young age (post-natal day [PND] 35). To test this, we administered 104 CFU/ml of L. casei L-54-2-33 in the drinking water of male Sprague-Dawley rats from PND65 till PND76, and compared their anxiety-like behaviors with age and sex matched control rats fed with vehicle (sucralose and MRS broth) in the drinking water for the same amount of time. Anxiety-like behaviors were then evaluated using the open field (OF) test and elevated plus maze (EPM). Rats fed with the bacteria spent significantly less time in the central zone of the OF in comparison to controls, while there were no differences between bacteria fed and controls rats in the EPM. These results match with our previous findings in younger male rats, suggesting that the anxiogenic effects of L. casei L 54 2 33 are strain specific, and that these effect do not depend on the age of the animal. Together our findings suggest that bacteria known to promote changes in the CNS, might exert its strain-specific effects regardless of the age of the host, which is a novel feature in probiotic (or in this case psychobiotic) interventions

Pharmacology area: Neuropsicofarmacología (Neuropsycopharmacology)
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101. ANXIOLYTIC EFFECTS OF A CHILEAN EXTRACT OF HUMULUS LUPULUS.
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Introduction: Humulus lupulus is broadly cultivated in the world both for beer manufacture, but also for its medicinal properties, for the treatment of excitability and restlessness. There are important regional differences in metabolome composition of the plants that influence its response. The present study investigated whether a Chilean Humulus lupulus extract, previously selected by demonstrate antioxidant properties (enzymatic and in vitro assays), can elicit effects on the central nervous system, using various experimental models in rats.
Methods: a) Treatments: Two different doses (low or high) of Humulus lupulus extract from a regional variety, or Medi-Drop-sucralose used as vehicle (control group), were orally administered for 42 days to adults male Sprague-Dawley rats. Extracts were administrated one hour before the behavioral tests performed in this study. b) Open field was used for the evaluation of locomotor activity. Anxiety was evaluated by elevated plus-maze test (EPM). Results: No significant differences were observed on the locomotor behavior of rats, following the oral administration of two doses of extract or control, measured by total distance travelled and average speed, on the open field apparatus. But rats treated with low dose of extract significantly increased (16,75 +5,1 sec) the time spent on the central zone of the open field, compared to control (4,7+ 1,7 sec); this parameter is correlated with statistically difference observed with open arm spent time on the EPM between control and medium dose of extract. Conclusion: These results show that the low dose of this Chilean lupulus extract, could exert and anxiolytic effect.

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102. PRODUCTION OF RECOMBINANT HUMAN INTERLEUKIN-4 EXPRESSED IN ESCHERICHIA COLI AS INCLUSION BODIES.
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Interleukin-4 (IL-4) is a potent lymphoid cell growth factor that stimulates the growth and survivability of certain B cells and T cells. It exhibits anti-inflammatory responses and participates in immune processes by providing protection from intracellular pathogens. IL-4 also plays an important role in T helper cells differentiation. Additionally, it can suppress pro-inflammatory cytokines. In this work, a His-tagged recombinant human IL-4 was overexpressed in Escherichia coli under the control of a T7 promoter. The resulting inclusion bodies were separated from cellular debris by centrifugation and solubilized by 8M urea. The denatured IL-4 was refolded in a single chromatographic step by gradual removal of denaturant agent. This protocol yielded 4.5 mg of IL-4 from 40g of biomass. The refolded protein was highly pure and subsequent biological activity assay that was measured in the human erythroleukemia cell line TF-1 suggested that IL-4 had similar activity profile to the commercial produced protein. The results of this study suggest that on-column refolding represent a convenient and low-cost process for the refolding of IL-4 and may be a promising candidate for development as commercial reactive for cancer research.

Pharmacology area: Tecnología farmacológica (Pharmaceutical Technology)
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103. A-KINASE ANCHORING PROTEIN AKAP79 INTERACTS WITH THE INTRACELLULAR DOMAIN OF THE GLYCINE RECEPTORS ALPHA SUBUNITS.

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Glycinergic inhibition is critical for breathing control, muscle tone regulation and nociception. Studies showed that PKA phosphorylation in the residue S346 located in the intracellular domain (ICD) of the glycine receptor (GlyRs) containing the alpha 3 subunit. This posttranslational modification produces inhibition of glycinergic function in the spinal cord, which was related to the generation of inflammatory chronic pain. Noteworthy, the molecular mechanisms associated with the inhibition of a3GlyR by phosphorylation are not yet elucidated. In this context, the interaction of the A-kinase anchoring protein (AKAP79) to partners involved in nociceptive pathways has been recently reported. Specifically, it has been observed that disruption of the interaction between AKAP79 and TRPV1 decreases sensitization in nociceptive neurons. Furthermore, the direct interaction between AKAP79 and the beta3 and beta2 subunits of the GABAAR promote the PKA-mediated phosphorylation of serine residues located in the ICD of those subunits. Nonetheless, whether AKAP79 is able to bind GlyRs and modulates its function is still unknown. Here, by using immunocytochemical and GST pull-down assays, we reported a direct association between the ?1, ?2 and the ?3 subunits of the GlyRs with AKAP79. Confocal imaging showed that AKAP79 specifically co-localized with all the GlyRs subunits. In addition, in pull-down studies we observed that a GST-ICD?3GlyR fusion protein are able to bind AKAP79.Thus, our experimental data contributes to the characterization of a new intracellular partner of the GlyRs. This open new avenues in the searching of new therapeutic targets for the inflammatory chronic pain treatments.

Pharmacology area: Farmacología del dolor (Pharmacology of Pain)
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104. PERINATAL ASPHYXIA INDUCES LONG-TERM DEMYELINATION, OLIGODENDROCYTES DAMAGE AND NEUROINFLAMMATION IN RAT BRAIN: PREVENTION BY NEONATAL MESENCHYMAL STEM CELLS TREATMENT.

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The effect of perinatal asphyxia (PA) was evaluated on myelination, oligodendrocytes, neuroinflammation and cell death in rat telencephalon and hippocampus from postnatal (P)1 up to 14 days, a period characterized by a spur of neuronal networking, finding a sustained injury that may have profound adverse effects on neuronal development. The study evaluated whether that injury could be prevented by mesenchymal stem cells (MSCs) treatment. PA was induced by immersing foetus-containing uterine horns into a water bath at 37°C for 21 min. Asphyxia-exposed (AS) and sibling caesarean-delivered (CS) foetuses were resuscitated and nurtured by surrogate dams. Animals were euthanized at P1, 7 or 14, dissecting samples from telencephalon and hippocampus to be assayed for (i) myelin (MBP and transcriptional factors involved in repairing demyelination, Olig-1and 2; immunofluorescence, RT-PCR); (ii) oligodendrocyte density (immunofluorescence); (iii) neuroinflammation (RT-PCR, ELISA, immunofluorescence), and (iv) cell death (TUNEL). Two hours after delivery, AS and CS neonates were injected with either 5 µl of vehicle or 5x104 MSCs into the left lateral ventricle. It was found that PA produced: (i) a decrease of MBP density and oligodendrocyte/mm3 at P7 in telencephalon, but not in hippocampus; (ii) an increase of Olig-1, in telencephalon at P7; (iii) an increase of IL-6 mRNA levels in telencephalon at P7, and of IL-1β mRNA in hippocampus at P14; (v) an increase of cell death, including oligodendrocyte at P7 in telencephalon; (vi) MSCs treatment prevented the effect of PA on demyelination, oligodendrocyte density, neuroinflammation and cell death. It is proposed that PA induces regionally and developmental-dependent changes in brain regions, and MSCs treatment can prevent the changes induced by PA on myelination, oligodendrocyte density, neuroinflammation and cells death.

Pharmacology area: Neuropsicofarmacología (Neuropsycopharmacology)
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105. MHC-CLASS I POLYPEPTIDE-RELATED SEQUENCE A (MICA) AS AN IMMUNOTHERAPEUTIC TARGET IN CANCER.
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MICA is a ligand to NKG2D, an activation receptor that triggers natural killer (NK) cells effector functions for early tumor elimination; however, the normal function of MICA/NKG2D axis is compromised in cancer, including gastric adenocarcinoma (GA). Several mechanisms have been proposed to explain this response, including the presence of released MICA, either as soluble proteins or in exovesicles, which may favor down-modulation of NKG2D in cytolytic cells, resulting in desensitization of NK cells as the tumors progress. MICA is a highly polymorphic molecule that codifies allelic variants, which have been described to affect NKG2D binding avidity and cell cytotoxicity, while some MICA-STR variants located in the transmembrane domain promote NKG2D internalization. MICA-STR A5.1 variant acquires a GPI-anchor which is recruited in exosomes. The aim in this work was to evaluate the MICA expression in gastric adenocarcinoma and their relationship with the allelic variants and effect on the regulation of NKG2D receptor. We study the MICA expression and release in samples of tumor tissue by flow cytometry and ELISA assay. We isolated DNA genomic to determinate the MICA allele by sequence based-typing PCR using specific primers. Also, we evaluate the expression of NKG2D in tumor-infiltrating NK cells by flow cytometry. Our results indicated that the expression of NKG2D on NK cells was inversely proportional to the levels of MICA on tumor cells, and that not all patients showed detectable levels of soluble MICA (sMICA) in their serum and, while the diminished expression of NKG2D on cytolytic cells did not correlate with the concentration of sMICA in the serum of GA patients, this could be due to the presence of MICA in exovesicles as the MICA-STR A5.1 variant. In conclusion, we propose that MICA is an immunotherapeutic target in gastric adenocarcinoma and the MICA allelic variants should be considered in the therapeutic strategies.

Pharmacology area: Farmacología gastrointestinal (Gastrointestinal Pharmacology)
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106. INTERNALIZATION MECHANISM OF FOLATE-MODIFIED PAMAM DENDRIMERS IS MEDIATED BY MORE THAN ONE ENDOCYTOSIS PATHWAY.
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Nowadays, central nervous system (CNS) diseases affect 1.5 billion people worldwide and there is a continuous development of new therapies. However, in many cases efficiency of therapies is low because of biological barriers and deficient biodistribution of drugs. New advances in the nanomedicine have allowed the creation of nanotransporter systems. Among them, polyamidoamine (PAMAM) dendrimers have demonstrated a great potential in drug delivery to CNS. PAMAM dendrimers are polymeric structures composed by an ethylenediamine core that branches creating layers, called generations, which end in primary amines protonated at physiological pH and can be modified with other terminal groups, such as folate. Considering the current difficulty of delivering drugs to the CNS, we examined the internalization mechanism of folate-conjugated PAMAM dendrimers mediated by folate receptor α (FRα), a membrane protein overexpressed in choroid plexus that once it binds to folate is internalized by the caveolae endocytosis pathway, and is postulated as a target tissue for drug delivery to CNS. In this study, we selected the HeLa cell line for internalization experiments, based on confocal and western-blot results. One unmodified (G4) and two folate-modified (PFO25 and PFO50) fourth generation PAMAM dendrimers were used. Confocal images showed that PFO50 was not able to entry HeLa cells, unlike PFO25 and G4, which were visualized after one hour incubation. Quantification of Mander's coefficients indicated only a slight increase of colocalization of PFO25 with FRα than unmodified G4, which suggests that the internalization pathway of folate-modified dendrimers is possibly mediated by more than one endocytosis mechanism.

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107. EFFECT AQUEOU EXTRACT OBTAINED FROM LEAVES OF U. MOLINAE AND THEIR RESPECTIVE PRODUCTS OF GASTOINTESTINAL DIGESTION ON THE VIABILITY OF COLON CANCER CELLS.
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Colorectal cancer is the third most common diagnosis in men (10%) and second in women (9.4%). The use of chemotherapy to fight this disease leads side effects, this the main reason for investigations of possible antiproliferativity of different natural sources. In that regard, active compounds U. molinae and their products the gastrointestinal metabolized could act as prophylactic and complementary because effcts anticancer has been reported.The aims is To asses the effect aqueou extract obtained from leaves of U. molinae and their respective products of in vitro gastrointestinal digestion on the viability of colon cancer cells. Ugni molinae leaves were used to prepare

an aqueous extract, with this a gastrointestinal disgestion was performed, obtaining also a final residue. These samples were evaluated in different viability tests, such as trypan blue exclusion, metabolic activity (MTT) and cytotoxicity (LDH), on colorectal cancer cells (CaCo-2) and healthy cells (HEK) for a period of 24 hours. When treating the cells, it is observed that the count of viable CaCo-2 cells decreases as the concentration increases. In the case of HEK cells no changes in the count are observed. MTT assay only with the gastrointestinal digestion samples observated an effect of inhibition of the metabolic activity in the case of caco-2 cells, in hek cells there is no significant effect. Cytotoxicity assays using LDH do not show significant changes in the activity of the enzyme lactate dehydrogenase in any case. Finally, it is concluded that the samples have positive effect on viable cell count and MTT assay, because damage colorectal cancer cells (caco-2) but not healthy cells (HEK), while very mild cytotoxicity was observed at through the LDH assay.

Pharmacology area: Farmacología de Productos Naturales (Natural Products Pharmacology)
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108. ASSESSMENT OF DIFFERENT PHARMACOLOGICAL ACTIVITIES OF PEUMUS BOLDUS EXTRACTS USING CHEMICAL SUBTRACTION STRATEGY.
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Peumus boldus Mol., (Monimiaceae) is a Chilean medicinal three used for gastrointestinal and liver diseases. Phytochemical profiling of this plant is based on its aporphine alkaloids, phenolic compounds and essential oil. However, in herbal infusions some authors thought that flavonoids are responsible for its antioxidant and chemopreventive effects rather than alkaloids and essential oil. The objective of this study was to evaluate different knock-out extracts prepared by chemical subtraction oriented to selectively remove alkaloids and essential oils from crude extracts. These extracts were obtained by means of conventional centrifugal partition chromatography (CPC) and pH-zone-refining CPC. DPPH bleaching test, cytotoxicity in AGS cells, DNA damage in monocytes (Comet assay) and inhibition of Acetylcholinesterase were determined for all extracts. Solutions of the different lyophilized extracts were prepared at different concentrations (1-1000 ug/mL). The results of DPPH assay indicated an IC50 of 63.05, 109 and 43.73 ug/ml for total extracts, alkaloids and polyphenols respectively, the fraction containing the polyphenols having greater antioxidant capacity. In turn, cytotoxicity tests showed that polyphenols at concentrations lower than 1000 ug/mL protected AGS cells. On the contrary, alkaloid fraction reduced cell viability from 400 ug/mL whereas fraction containing essential oil displayed higher toxicity from 125 ug/mL. Only the fraction with alkaloids displayed an expected acetylcholinesterase inhibition.

Pharmacology area: Farmacología de Productos Naturales (Natural Products Pharmacology)
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109. PARTICIPATION AND ROLE OF CONNEXINS IN THE RELEASE OF GLUTAMATE AND ATP IN THE NEURULATION PROCESS IN XENOPUS LAEVIS.
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Neurulation is an important process in the formation and development of CNS. This event correspond to the first step of neural embryonic development (stg 12,5–20 in Xenopus laevis) and implicates different cellular process like, migration and proliferation. Alterations in the signaling of this period could result in neural tube defects (NTDs). Several studies has demonstrated the participation of connexins (Cxs) as hemichannels in cellular communication through the release of ATP and glutamate, regulating cellular migration and stabilizing synaptic transmission. In this investigation, we identified the presence of several Cxs during neurulation. To evaluate its relative expression, we identified their RNA transcripts in different stages of Xenopus laevis neural embryonic development such as: stg 10; stg 12,5; stg 14 and stg 20. Our results revealed the presence of transcripts of Cxs 43, 45, 46, 32 and 26 in different stages of Xenopus laevis development. The more important proteins correspond to Cx 46 (GJA3) which has 6 fold expression vs Cx 45 (GJA7) and Cx 43 (GJA1). In turn, Cx 32 (GJB1) have a significant presence of 3 fold vs Cx 26 (GJB2), the second more abundant, during neurulation. Later, we decided to evaluate the functionality of these Cxs as hemichannels through pharmacological blockage assays, using inhibitors such as carbenoxolone (cbx) and enoxolone (enx). We found values of IC50 of ~30 µM and ~20 µM for cbx and enx respectively in their capacity to induce neural tube defects. In addition, in silico studies using molecular docking techniques, we determine the possible site of cbx and enx association in the protein, located in the extracellular domain (E2). Taken together these results, we suggest that Cx 46 and Cx 32 will participates as hemichannel in neural tube closure and their blockade results in NTDs.

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110. INHIBITION OF ENDOPLASMIC RETICULUM EXIT RESCUES A NIEMANN PICK TYPE C DISEASE MODEL.
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Currently, more than 70 lysosomal diseases have been identified, accumulating substrates in lysosomes and late endosomes. Within this group we find the Niemann-Pick type C (NPC) disease, that generates aberrant accumulations of cholesterol and other lipids within cells, resulting in early neuronal death. NP-C1, the most common protein showing disease-causing mutations, codes for a transmembrane protein NPC1, present in lysosomes and late endosomes membranes. NP-C2, is caused by a mutation in the NPC2 gene that encodes the NPC2 protein, which is soluble and present in the same organelles. The disease produced by loss of function of NP proteins generates hepatomegaly and splenomegaly. Based on preliminary laboratory data, we hypothesize that proteins related to the organization of the endoplasmic reticulum (ER) are necessary to maintain the disease phenotype. In order to identify other proteins involved, we studied Tango1, a transmembrane ER protein that organizes vesicle cargo. Its loss of function produces disorganization and stress of the ER. It was analyzed in a model of Drosophila melanogaster where NP-C1 is replicated with a knock-down of dnpc1a gene, NPC1 ortholog, through RNAi. Using this system, we determined that tango1 loss of function reverts NP-C1 phenotype, improving Drosophila larval development progression. Also, the effect of the inhibition of ER secretion was analyzed using Fli-06, a compound that inhibits exportation. We tested a pharmacological model that phenocopies NP-C1, completely reverting NPC phenotype. The study corroborates that the organization of the secretory pathway is determinant to maintain the phenotype of this disease and that by itself an alteration in it results in a phenotype equivalent to the deficiency of NPC1.

Pharmacology area: Otros (Others)
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111. ADDITIVE EFFECT OF MODAFINIL AND CAFFEINE ON THE LOCOMOTIVE ACTIVITY OF ADULT RATS.

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Currently, many people are subject to a high work and academic load that leads them to consume psychotropic substances to be able to carry out these highly demanding activities. In Chile, it has been observed that some of these substances commonly used are modafinil and caffeine to promote wakefulness and concentration. Concomitant use of modafinil and caffeine could trigger anxious symptoms and psychomotor agitation in humans. Modafinil has different action mechanisms, being the blocking of dopamine transporter (DAT) the most known and relevant. On the other hand, caffeine is an adenosine receptor antagonist and inhibitor of phosphodiesterase. Therefore, the concomitant use of modafinil and caffeine could promote a higher effect on neural activity compare to the use of caffeine or modafinil alone. The objective of this work was to measure the additive effects of the administration of modafinil and caffeine on the horizontal and vertical locomotor activity. To assess the traveled distance and number of bipedestation, we used rats treated with caffeine (20 mg/kg, i.p.), modafinil (80 mg/kg, i.p.) and caffeine plus modafinil. Our preliminary results show that rats treated with caffeine plus modafinil produce an increase on locomotor activity (horizontal and vertical) compared to the administration of caffeine or modafinil alone. The effect induced by caffeine plus modafinil was additive. To correlate these behavioral effects with an increase in dopaminergic activity in the mesolimbic and nigrostriatal pathways, we will measure the dopamine release in striatum and nucleus accumbens using in vivo brain microdialysis and fast scan cyclic voltammetry.

Pharmacology area: Neuropsicofarmacología (Neuropsycopharmacology)
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112. CLINICAL CHARACTERISTICS OF NEUROLOGICAL PATIENTS INFECTED WITH HTLV-1 AND DETERMINATION OF THE LOCATION OF TAX VIRAL PROTEIN.

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Tropical Spastic Paraparesis neuropathogenesis (abbreviated HAM / TSP, "HTLV-1-associated myelopathy / tropical spastic paraparesis"), endemic in Chile, shows by anatomo-pathological studies spinal cord injuries due to axonal loss and demyelination of cortical spinal beams, visualized by NMR. 70% of patients start their disease with paretospastic gait. Since 2009 the screening of HTLV-1 in blood banks. The prevalence in donors studied in the ISP in PBMCs ("Peripheral Blood Mononuclear Cells") containing T-CD4+ lymphocytes, the main viral reservoir, showed real-time prevalence of 1.2 healthy / 1000 individuals. Neuropathogenesis is attributed to the viral protein Tax because the virus does not infect neurons and 40% of patients with paraparesis are seronegative for the virus, but express a tax gene. In cerebrospinal fluid (CSF) of patients we detect Tax (by ELISA) and in isolated cells (by immunofluorescence) and in plasma (by "Western Blot"). Tax secreted from patient PBMCs agrees with the extracellular role that we propose, because we know that it interacts with semaphorin-4D that triggers the disassembly of microtubules and actin fibers through Plexin-B1.

Pharmacology area: Farmacología molecular (Molecular Pharmacology)
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113. POLYMERIZATION ACTIVITY AND CYTOTOXICITY OF MOLECULES WITH AFFINITY FOR LAU/PLA BINDING SITE OF TUBULIN AS NOVEL STABILIZING AGENTS.

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The importance of microtubules in cellular division set these proteins as pharmacological targets for antimitotic agents, known as tubulin binding agents (TBA), which can promote stabilization or destabilization of tubulin polymerization. The occurrence of adverse drug reaction associated to several of these agents drives the need for the development of new TBAs with a safer pharmacological profile. In this regard, a combination of computational virtual screening, molecular dynamics and binding free energy estimations was performed by our group, based on the stabilizing LAU/PLA binding site of tubulin. A set of 7 candidates were proposed as potential stabilizing agents with affinity for the site. In this work, we confirm the polymerization capacity for these 7 candidates in vitro at concentrations of 50 and 100 µM. Also, we observed an additive effect of the compounds when co-treating with Taxol, confirming a non-competitive binding with taxane-site binders. Finally, viability assays in a cancer cell line were developed showing a cytotoxic effect of molecules at 100 µM. These results set a starting point of further studies for the characterization of the novel agents that will open possibilities for the rational screening of new tubulin stabilizing agents.

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114. RELEASE AND UPTAKE KINETICS OF DOPAMINE ARE PRESERVED IN STRIATUM OF ADULT FEMALE RATS EXPOSED DURING FIRST HOURS OF POSTNATAL LIFE TO ESTRADIOL VALERATE.

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Sex hormones play an important role in regulating reproductive and non-reproductive tissues, such as the brain. In the nervous system, sex hormones are important in its development and neural plasticity, however changes in the sex hormones milieu during fetal or neonatal stages affect brain function and generate persistent changes until adulthood. During last 7 years our lab has been interested in study how neonatal exposure to sex hormones such as estradiol valerate (EV) affect the functionality of midbrain dopaminergic neurons of adult male and female rats. The aim of this work was to evaluate the release and uptake kinetics of striatal dopamine (DA) induced by methylphenidate (MPH: 5.0 mg/kg i.p.) of adult female rats exposed during the first hours of postnatal life to estradiol valerate (EV: 0.1 mg/50 uL of sesame oil s.c.) or vehicle (50 uL of sesame oil s.c.). Our results did not show significant differences in the voltammetry parameters such as peak amplitude, area and tau (time constant of decay). Despite these results, we cannot rule out changes in the voltammetry parameters in nucleus accumbens, a key nucleus of the reward circuit, where we have previously observed a reduction in DAT expression of animals programmed with sex hormones.

Pharmacology area: Neuropsicofarmacología (Neuropsycopharmacology)
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115. CYTOTOXIC EFFECTS CAUSED BY DELOCALIZED LIPOPHILIC CATIONS DERIVED FROM POLYHYDROXY-BENZOIC ACIDS IN COMBINATION WITH DOXYCYCLINE ON LUNG CANCER CELLS.

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Lung cancer has the highest mortality between all neoplasms, being the second leading cause of death in Chile. These cancers are classified as small cell carcinoma or non-small cell carcinoma, being Smoking the main risk factor. Conventional treatments are radiotherapy, chemotherapy and surgery; however, 5-year survival rates remain extremely poor, due to the development of resistance and eventual relapse. The Cancer Stem Cells hypothesis suggests that they are responsible for tumor initiation and growth and are resistant to conventional treatments. Therefore, it is necessary to develop new therapies that allow us to effectively eliminate resistant tumor cells (TC). Mitochondria may be considered as a therapeutic target in the treatment of cancer, because it exhibits a greater transmembrane potential in TC, being susceptible to being the target of positively charged molecules. The delocalized lipophilic cations of triphenylphosphonium (TPP+) are molecules synthesized from gallic acid, mono and polybenzoates decyl esters. Therefore, we evaluated 4 decyl polyhydroxybenzoate compounds linked to TPP+ as potential cytotoxic agents in two lung cancer cell lines (NCI-H727 and NCI-H1299) and in pulmonary fibroblasts (PH) as control. Doxycycline is an antibiotic of the tetracycline group; recently its has been studied this antineoplastic use producing the inhibition of mitochondrial biogenesis in TC. Cell viability assay was performed with the compounds at 24, 48 and 72 hours in normoxia and hypoxia with 5% oxygen to determine IC50, to subsequently perform a combination test of compounds with doxycycline. The results showed that the TCT analyzed are sensitive to the cytotoxic action of all compounds and this effect is increased as time goes by. In addition, there are no significant differences in IC50 between hypoxia and normoxia cultures.

Pharmacology area: Farmacología molecular (Molecular Pharmacology)
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116. TARGETING MITOCHONDRIAL METABOLISM IN NEURODEGENERATIVE DISEASES THROUGH NCLX BLOCKADE.

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The loss of mitochondrial function is part of the almost all neurodegenerative diseases. Therefore, there's a reduction in ATP synthesis ending up in a dysfunction of neuronal dynamics. At least three proteins from the Krebs' circle are calcium sensitive. In our laboratory, we're focus in the design and synthesis of CGP37157 derivatives, reference blocker drug of the mitochondrial sodium/calcium exchanger (NCLX), which also has neuroprotective properties. Our aim is the discovering of new pharmacological tools able to handle calcium flux between mitochondria and cytosol, lowering the calcium overload descried in the cytosol. Putting together these two ideas, we wondered if the partial blockade of mitochondrial calcium efflux could improve not only mitochondrial metabolism but also calcium handling. The CGP57137 derivative selected was ITH12575, a benzothiazepine with a different aromatic substitution. First, the calcium movements were studied by the fluorescent dye Fluo4, in the human neuroblastoma SH-SY5Y cell line and in embryonic cortical neuros of rat primary culture. In order to confirm ITH12575 selectivity for NCLX, the exchanger was silenced by a siRNA and neuroprotective assays were evaluated. Finally, mitochondrial stress assays were performed using the seahorse method, in presence/absence of both a toxic stimulus (high potassium concentration) and the compound. Data from ATP synthesis, mitochondrial respiration and respiratory maximal capacity were obtained. The results show that, by the regulation of mitochondrial calcium, the mitochondrial metabolism is partially recovered thanks to ITH12575.

Pharmacology area: Neuropsicofarmacología (Neuropsycopharmacology)
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117. DEVELOPMENT OF OXYTETRACYCLINE-CYCLODEXTRIN INCLUSION COMPLEXES AND PHARMACOKINETIC STUDY IN RAINBOW TROUT

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Oxytetracycline (OTC) is a bacteriostatic broad-spectrum antibiotic, widely used in aquaculture. However, it has variable bioavailability and low solubility. Being a tetracycline, it forms chelates with divalent cations (Ca+2, Mg+2), which could interact with membrane proteins, preventing absorption. To avoid interaction, inclusion complexes were made with HP-B-CD (OTC-HP-B-CD) by spray drying. The microparticle systems obtained were characterized physicochemical (XRD, DSC and FT-IR) and morphologically (SEM). From the characterization it was concluded that there was complete encapsulation of OTC into HP-B-CD. From studies of maximum solubility, in the case of OTC-HP-B-CD, a significant increase in solubility and dissolution rate were obtained. A pharmacokinetic study in Oncorhynchus mykiss (by cannulation to the stomach of OTC and OTC-HP-B-CD suspensions, and sampling within 72 hours), evaluated the relative bioavailability between the administration of OTC and OTC-HP-β-CD by quantifying OTC in serum by high perfomance liquid chromatography. The analysis of variance (ANOVA) and the comparation of areas under a curve (AUC; 18595 vs 14024), tmax (24 hours on both) and t1/2 (17,06 vs 18,09 hours) indicated that there are no significant differences between OTC and OTC-HP-β-CD. OTC-HP-B-CD presented a higher value in Cmax compared to OTC (328 vs 223 ng/mL).

Pharmacology area: Tecnología farmacológica (Pharmaceutical Technology)
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118. ALPHA4 AND ALPHA7 NICOTINIC RECEPTOR SUBUNITS DETECTION ON ZEBRAFISH USING PCR TOOLS.

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The zebrafish has been a widely used model organism in several fields for the past 40 years because of its remarkable features. It is cheap to maintain, easy to manipulate, fast to breed, and its genome is almost fully sequenced. Also the zebrafish manifests a series of complex behaviors that makes it useful for assessing the neuropsychological effects of novel drugs. Therefore, in previous works we have used the zebrafish as a tool to observe the rewarding properties of nicotine identifying the factors involved in the nicotine addiction and to test a novel nicotinic antagonist capable of negating the rewarding effect of nicotine. In order to identify some of these factors we aim to detect the mRNA of two of the most abundant nAChR subunits, α4 and α7, in the brain of fish treated with both nicotine and an antagonist. To do this we use the RT-PCR method in which we have been able to detect the expression of these subunits. Also we attempt to measure the expression changes using qPCR with comparative ΔΔCT with a calibrator sample (non-treated fish) and an endogenous reference gene (β-actin) to elucidate the transcriptional effects of the drugs over the zebrafish brain and thus understanding the similarities of the mammalian and zebrafish responses to nicotine.

Pharmacology area: Farmacología molecular (Molecular Pharmacology)
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Acknowledgments: The financial support for this research work, National Foundation of Science and Technology grants 1150615 (P.I.-V.), 1170662 (M.R.-P.)

119. FOXO1 MEDIATES HIGH GLUCOSE-CARDIAC FIBROBLASTS DIFFERENTIATION
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Normally cardiac fibroblasts (CF) maintain the homeostasis of the extracellular matrix (ECM) in the heart, whereas in pathological conditions such as diabetes, become more active promoting cardiac fibrosis. High glucose (HG) induces CF differentiation, where TGF-beta1 has a crucial role. TGF-beta1 requires FoxO1 to induce CF differentiation, whereas FoxO1 is deregulated in diabetes, resulting in its hyperactivation, oxidative stress and cell differentiation. Therefore, in this work we wanted to determine the role of FoxO1 in CF differentiation promoted by high glucose. CF obtained from adult Sprague-Dawley rats were incubated in HG, as a in vitro model of hyperglycemia. CTGF and alpha-SMA expression was determined by RT-PCR, whereas CTGF and alpha-SMA protein were evaluated by westernblot (WB). The activation of FoxO1 was analyzed evaluating its phosphorylated forms, its nuclear localization and the expression of FoxO1 specific genes targets (p21cip and p15ink) by RT-PCR. The oxidative stress was evaluated analyzing the expression of the FoxO3a, catalase and SOD2 proteins by WB, and ROS production by colorimetry. The role of FoxO1 was demonstrated using AS1842856 (FoxO1 inhibitor) and FoxO1 silencing using siRNA. HG increased the protein and mRNA of CTGF and alpha-SMA (CF differentiation marker), whereas HG decreased of AKT activation, decreased phospho-s256-FoxO1 level, increased FoxO1 nuclear localization and increased FoxO1 genes target expression (FoxO1 activation marker). Likewise, HG decreased FoxO3a, catalase and SOD2 protein, and increased ROS production. In addition CF differentiation induced by HG was completely abolished by FoxO1 inhibition using AS1845628 and FoxO1 silencing. Collectively these data suggest that FoxO1 is necessary to CF differentiation induced by high glucose and suggest that FoxO1 would be a pharmacological target for new treatments against diabetic cardiomyopathy.

Pharmacology area: Farmacología cardiovascular (Cardiovascular Pharmacology)
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120. PHARMACOLOGICAL COMPARISON BETWEEN PREFRONTAL CORTEX AND NUCLEUS ACCUMBENS NEUROTRANSMITTER LEVELS AFTER BASOLATERAL AMYGDALA STIMULATION.
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Glutamatergic neurons of the basolateral amygdala (BLA) innervate both, the nucleus accumbens (Nac) and prefrontal cortex (PFC) (McDonald, 1991). The relation between BLA and Nac has been implicated on the control of motivated behavior (Stuber et. al., 2011). Furthemore, the triade between BLA, Nac and PFC has also been shown to be critical in the regulation of goal-directed behavior by an inhibitory control of PFC on Nac dopamine release during amygdala activation (Jackson et. al. 2018). BLA has been implicated in fear, anxiety and stress (Simon et. al., 2014; Janak and Tye, 2015). The stress response is centered in the corticotrophin releasing factor (CRF) system (Bale and Vale, 2004). There are two major receptors for CRF in the brain, type-1 and type-2 CRF receptors (CRF-R1 and CRF-R2). Several studies have shown a significant role of CRF-R1 in the stress response; however, the role of PFC and Nac CRF-R2 in the stress response is poorly understood. We studied the role of CRF-R2 in PFC and Nac neurotransmitter levels after BLA stimulation by double in vivo microdialysis in PFC or Nac of anesthetized adult rats. Local infusion of antisauvagine 30 (CRF-R2 antagonist) in the PFC or Nac significantly increased PFC and Nac dopamine and glutamate extracellular levels induced by BLA stimulation. Our results suggest that there is an inhibitory tone mediated by CRF-R2 controlling dopamine and glutamate extracellular levels in PFC and Nac that dependon BLA stimulation.

Pharmacology area: Farmacodinamia (Pharmacodynamics)
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121. ACTIVATION OF NMDA RECEPTORS DURING CHRONIC PAIN RECRUITS PROTEIN SRC KINASES TO OPEN PANNEXIN1 CHANNEL IN NEUROPATHIC RAT MODEL.
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In experimental pain models the upregulation of NMDA receptor (NMDAR) appears to be crucial in the enhanced responsiveness of nociceptive neurons of dorsal horn of the spinal cord for initiation and maintenance of pain. In the chronic pain model performed by our laboratory, it was shown that intrathecal injection i.t. of 10Panx (Inhibitor panx1 channel peptide) prevents the effect of hyperalgesia caused by i.t of NMDA in neuropathic rats suggesting an interaction between Panx1 channel and NMDAR. Our work is based on elucidating whether the regulation of the Panx1 channel by post-translational modifications is carried out by Src kinases in neuropathic rats model. In dye uptake experiments (used to assess Panx1 channel opening) of the spinal cord slices of neuropathic rats have demonstrated that pharmacological inhibition of PP2 (Src tyrosine kinase protein inhibitor), and 10Panx decreased dye uptake in neurons stimulated by NMDA. Likewise, rats treated with 10panx-NMDA or PP2-NMDA decreases the expression of phosphorylated Panx1 (pPanx1) and phosphorylated Src527 (pSrc527) by western Blotting.

Algesymmetric test (Randall Selitto) results have shown that intrathecal administration of 10Panx (300 µM, 10µl i.t) -NMDA (0.6 mM, 10µl i.t) and PP2 (3.3 mM, 10µl i.t)-NMDA (0.6 mM, 10µl i.t) inhibit pain in neuropathic rats compared to control. We conclude that Panx1 or Src inhibition prevents nociceptive signaling induced by upregulation of NMDAR in neuropathic rats, suggesting that the pronociceptive effects of pharmacological activation of NMDAR induce the opening of the Panx1 channel probably mediated by Src kinase.

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122. PARTICIPATION OF VGLUT1 AND GLUTAMATE SIGNALING DURING NEURULATION IN XENOPUS LAEVIS.
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Introduction: The development of the nervous system begins with the closure of the neural tube, one of the morphological changes present in the neurulation process. Several signaling pathways participate in the formation of the neural tube, such as FGF, Wnt, Chordin and glutamate, recently described. Failures in this process lead to the formation of neural tube defects (NTDs), the second more prevalent birth defect worldwide. Use of antiepileptic drugs during pregnancy had shown an increase in the incidence of NTDs by mechanism not fully understood yet. Here, we propose that glutamate, the principal excitatory neurotransmitter of the nervous system and specifically its vesicular transporter VGLUT1, participate in the normal closure of neural tube. Methods: X. laevis embryos were treated with Rose Bengal, a VGLUT1 antagonist, at early neurula stage (14 hpf, neural plate) until end of neurulation (21 hpf). Then, we perform a morphological evaluation of neural tube closure and immunofluorescence experiments. Additionally, after neurula treatments, behavior studies using Xenopus tadpoles (stg 45-49), were performed to evaluate epileptic sensibility by measure seizure latency onset using Pentylenetetrazol (PTZ). Results: We observe and incomplete neural tube closure in embryos treated with Rose Bengal in a dose-response manner, with an EC50 of 3.5±0.5µM. Furthermore, we observe a decrease of ~43% ($p<0.01$, ANOVA) in the seizure latency onset necessary for epileptogenic behavior induced by PTZ and a 5-fold increase (0.4±0.3 m vs 5±0.2m) in the distance traveled at 2 minutes after treatment ($p<0.05$, ANOVA). Conclusions: VGLUT1-mediated glutamate signaling participate in normal neural tube development. Partial blocking of this pathway at neurula modifies the epileptogenic-induce response in tadpoles, possibly by alter the normal establishment of the nervous system.

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