plasma membrane called hemichannels or connexons, harbouring a central pore that permit the passage of ions and small molecules between cytoplasm and extracellular surroundings. Recent findings indicate that non-junctional hemichannels can open under both physiological and pathological conditions, depending on cell context. In the present work we have studied the currents generated by hemichannels formed by these human connexins Cx26, Cx32 and Cx43, expressed in Xenopus laevis oocytes and the simultaneous recording of ATP release. The activation of Cxs was done by depolarization pulses. The release of ATP is generally associated with tail currents. Connexin 26 is expressed in many tissues, and one of them is cochlea. Mutations in Cx26 cause nonsyndromic hearing loss and other syndromes affecting ectoderm-derived tissues. X-linked Charcot Marie Tooth (CMTX) is an inherited neurodegenerative disease affecting both motor and sensory peripheral nerves. CMTX is due to mutations in Cx32 and more than 200 mutations have been so far described. Schwann cells express Cx32 in the Schmidt-Lanterman incisures and in the paranodes in which may form hemichannels. Connexin 43 is found in astrocytes and is the major protein of cardiac ventricular gap junctions which are crucial to cell-cell communication and cardiac function. The protein level of Cx43 is reduced in patients with heart failure or dilated cardiomyopathy, pathophysiological conditions often associated with arrhythmias. Acknowledgments: This work is supported by grant SAF 2008/00732 of the Ministerio de Ciencia e Innovacion (MICINN) of the Spanish Government and by La Fundació La Marató de TV3. EMM is recipient of a Felloship of the MICINN.

P6. Purine extracellular metabolism

P6-1
Adenosine deaminase activity in intact trophozoites of Trichomonas vaginalis

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Background: To characterize the adenosine deaminase (ADA) activity from intact trophozoites of Trichomonas vaginalis, the etiologic agent of trichomonosis. Methods. ADA activity was characterized by a colorimetric assay carried out at 635 nm for measuring the ammonia released. The ADA protein sequences obtained from BLASTP function via the GenBank database were aligned with ClustalX program and a phylogenetic tree was built with MEGA 4.0 program using statistical Neighbor-Joining method with proportional (p) distance. Results. Considering adenosine as substrate, the protein curve was linear between 50 to 150 ug protein/mL, and the time course was linear up to 40 minutes. The optimal pH for deamination was 7.5. Adenosine and 2-deoxyadenosine were substrates for ADA, while guanosine and 2-deoxyguanosine were not deaminated. The apparent values for KM and Vmax were, respectively, 1.13 ± 0.07 mM and 2.61 ± 0.054 nmol NH3/min/mg of protein. Adenosine deamination was strongly inhibited in the presence of erythro-9-(2-hydroxy-3-nonyl)adenine (EHNA). Calcium and magnesium also inhibited the activity; effect prevented by EDTA. Furthermore, when ecto-5′-nucleotidase was inhibited, there also was no ADA activity, strongly suggesting the cascade association between these enzymes. The phylogenetic tree revelead four well-resolved terminal clades supported by high bootstrap values, confirming the presence of two ADA orthologues for T. vaginalis, which composed the second clade. Conclusion. Our data suggest the presence of an ecto-ADA in the parasite surface. The occurrence of ADA activity in T. vaginalis may represent important implications for the purinergic system in the immune response during trichomonosis. Financial support: CNPq (Brazil).

P6-2
Chronic ethanol treatment alters purine nucleotide hydrolysis and nucleotidase gene expression pattern in zebrafish brain

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The zebrafish is a model for the study of human diseases and the use of this species in biochemical and behavioral studies on alcoholism has increased recently. However, there are no data concerning the effects of chronic ethanol exposure on the purinergic system. The aim of this study was to evaluate nucleotide hydrolysis by NTPDases and ecto-5′-nucleotidase after long-term ethanol exposure. Additionally, the gene expression patterns of NTPDases1-3 and 5′-nucleotidase were determined. Animals were exposed to 0.5% ethanol for 7, 14, and 28 days. Zebrafish were euthanized, their brains were removed by dissection and brain membranes were prepared. NTPDase and 5′-nucleotidase activity were determined by the measurement of inorganic phosphate released from nucleotide hydrolysis. There were no significant changes in ATP and GTP hydrolysis after all treatments. However, a decrease in ADP (46% and 34%) and GDP (48% and 36%) hydrolysis was verified after 7 and 14 days, respectively. After 7 and 14 days of ethanol exposure, a significant decrease in AMP hydrolysis (48% and 36%) was also observed, whereas GMP hydrolysis was inhibited only after 7 days (46%). NTPDase2_mv and NTPDase3 mRNA transcript levels decreased after 7 and 14 days, respectively. In contrast, ethanol increased NTPDase1, NTPDase2_mq and NTPDase3 transcript levels after 28 days of exposure. NTPDase2_mg and 5′-nucleotidase gene expression was not altered. The ecto-nucleotidase pathway may be a target of chronic ethanol toxicity. Therefore, regulation of the purinergic system may play a role in the mechanisms underlying the effects of ethanol on the central nervous system.

P6-3
Antipsychotic drugs alter adenine nucleotide hydrolysis in zebrafish (Danio rerio) brain

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Zebrafish is an ideal vertebrate model system to study human diseases and drug screening. We evaluated the in vitro and in vivo effects of typical (haloperidol) and atypical (olanzapine and sulpiride) antipsychotic drugs on NTPDase and 5′-nucleotidase activities from zebrafish brain followed by a gene expression pattern analysis. For in vitro experiments, antipsychotics (1–250 uM) were added to reaction medium and maintained throughout the enzyme assays. For in vivo exposure, zebrafish were submitted to haloperidol (9 uM), olanzapine (100 uM), or sulpiride (250 uM) treatment for 2 hours. Zebrafish were euthanized, their brains were removed by dissection, and brain membranes were obtained. Ectonucleotidase activities were determined by the measurement of inorganic phosphate released from nucleotide hydrolysis. The in vitro experiments demonstrated that ATP hydrolysis was inhibited at 250 uM haloperidol (28.9%) and olanzapine (60.7%) whereas ADP hydrolysis was decreased at 250 uM haloperidol (26.5%) and sulpiride (25.6%). After 2 h-exposure, only haloperidol was able to inhibit ATP hydrolysis (35%). Haloperidol was also able to decrease NTPDase3 and two isoforms NTPDase2 (NTPDase 2_mv and NTPDase2_mq) mRNA transcript levels. These findings demonstrated that antipsychotic drugs inhibited NTPDases whereas did not change 5′-nucleotidase activity. The evaluation of the effects of antipsychotics on purinergic system in zebrafish may contribute to a better understanding of mechanism of action of these drugs as well as discover potential pharmacological targets and accelerate the pace of psychiatric drug discovery.

P6-4
Characterization of ATP and ADP hydrolysis activity in plasma membranes isolated from rat uterus

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Extracellular nucleotides modulate female reproductive functions, fertilization and pregnancy. The aim of this study was to characterize ecto-nucleotidase activity in uterine plasma membranes isolated from Wistar albino rats. Enzyme activities were determined by measuring the amount of liberated inorganic phosphate using a colorimetric assay. Magnesium and