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

Carla Denise Bonan[1], Kelly Juliana Seibt[1], Renata Luz Oliveira[1], Eduardo Pacheco Rico[2], Tathyana Mar Amorim Franco[1], Renato Dutra Dias[1], Mauricio Reis Bogo[1]
[1]*Departamento de Biologia Celular e Molecular, Pontifícia Universidade Católica do Rio Grande do Sul, Brazil,* [2]*Departamento de Bioquímica, Universidade Federal do Rio Grande do Sul, Brazil*

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

Maja Milosevic, Ivana Stanojevic, Snjezana Petrovic, Dunja Drakulic, Natasa Velickovic, Anica Horvat
POBox 522 Vinca Institute of Nuclear Sciences Serbia

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