calcium ions stimulated ATP and ADP hydrolysis in a similar way. Significant decreases in hydrolysis of both substrates were observed in presence of 1 mM EDTA, confirming cation dependence. The pH optimum was found to be 8.0 for ATP and 8.5 for ADP. Kinetic parameters were determined using substrates in range 0.05–5 mM. The apparent Km for ATP and ADP were 460.7±8.0 and 574.5±10.5 microM, respectively. The Vmax values were 3726.2±64.5 and 2656.7±48.1 nmol/min/mg for ATP and ADP, correspondingly. Chevillard plot demonstrated that one enzyme site was responsible for ATP and ADP hydrolysis. A significant ATP and ADP hydrolysis inhibition ($P<0.05$) were observed in the presence of 1–10 mM sodium azide, 1 mM sodium fluoride, 0.3 mM suramin and 0.1 mM sodium orthovanadate, while no inhibition with ouabain, oligomycine or theophylline were detected. Minor alkaline phosphatases contamination was confirmed by levamisole. Western blot verified presence of NTPDase1, while NTPDase2 and NTPDase3 were not detected.
According to our results, ATP and ADP hydrolysis observed in plasma membranes from rat uterus corresponded to NTPDase1 activity. These findings are consistent with literature data about NTPDase1 presence on the surface of myometrial smooth muscle cells and blood vessel endothelium.

P6-5
The NTPDase family in zebrafish

(Selected for Oral Poster Presentation)

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The nucleoside triphosphate diphosphohydrolase (NTPDase) family cleaves tri- and diphosphonucleosides to monophosphonucleosides and is responsible for terminating purinergic transmission. Since the NTPDase family in zebrafish is poorly understood, in the present study we evaluated the nucleotide hydrolysis in three tissues of adult zebrafish (brain, liver, and heart), confirmed the presence of distinct NTPDase members by a phylogenetic analysis and verified their relative gene expression profiles in the respective tissues. A different profile of ATP and ADP hydrolysis in the brain, liver, and heart as a function of time (0–60 min) and protein amount (3–15 ug) was observed. Sodium azide (20 mM), ARL 67156 (300 uM) and Suramin (300 uM) differently altered the nucleotide hydrolysis in zebrafish tissues, suggesting the contribution of distinct NTPDase activities. Homology-based searches identified the presence of NTPDase1–6 and NTPDase8 orthologs. In addition, the phylogeny also grouped three NTPDase2 and two NTPDase5 paralogs. The deduced amino acid sequences share the apyrase conserved regions (ACRs), conserved cysteine residues, putative N-glycosylation, phosphorylation, N-acetylation sites, and different numbers of transmembrane domains. RT-PCR experiments revealed the existence of a distinct relative entpd1–6 and entpd8 expression profile in brain, liver, and heart. Taken together, our results indicate that several NTPDase members might contribute to a tight regulation of nucleotide hydrolysis in zebrafish tissues.

P6-6
Ecto-5′-nucleotidase (e-5NT) switches from neurons to astrocytes after cortical stab injury in rat

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Background: Ecto-5′-nucleotidase (e-5NT) is ectoenzyme that controls extracellular levels of ATP and adenosine and thus, neuronal, astrocytic, and microglial function. We demonstrated in the rat model of cortical stab injury, that activity and expression of e-5NT changed in a biphasic manner, with an immediate decrease, followed by prominent up-regulation two weeks after the injury. In the present study we describe cellular distribution of e-5NT after the