Comparative Biochemistry and Physiology, Part B 139 (2004) 203–207

CBP

www.elsevier.com/locate/cbpb

Ecto-5′-nucleotidase activity in brain membranes of zebrafish (*Danio rerio*)

Mario Roberto Senger[a,b,1], Eduardo Pacheco Rico[a,1], Renato Dutra Dias[a], Maurício Reis Bogo[a], Carla Denise Bonan[a,*]

[a]*Departamento de Ciências Fisiológicas, Laboratório de Pesquisa Bioquímica, Faculdade de Biociências, Pontifícia Universidade Católica do Rio Grande do Sul, Avenida Ipiranga 6681, 90619-900, Porto Alegre, RS, Brazil*
[b]*Departamento de Bioquímica, Instituto de Ciências Básicas da Saúde, Universidade Federal do Rio Grande do Sul, Rua Ramiro Barcelos 2600-Anexo, 90035-003, Porto Alegre, RS, Brazil*

Received 19 March 2004; received in revised form 10 July 2004; accepted 14 July 2004

Abstract

Adenosine, a well-known neuromodulator, may be formed intracellularly in the CNS from degradation of AMP and then exit via bi-directional nucleoside transporters, or extracellularly by the metabolism of released nucleotides. This study reports the enzymatic properties of an ecto-5′-nucleotidase activity in brain membranes of zebrafish (*Danio rerio*). This enzyme was cation-dependent, with a maximal rate for AMP hydrolysis in a pH range of 7.0–7.5 in the presence of Mg^{2+}. The enzyme presented a maximal activity for AMP hydrolysis at 37 °C. The apparent K_m and V_{max} values for Mg^{2+}-AMP were 135.3±16 μM and 29±4.2 nmol Pi · min^{-1} · mg^{-1} protein, respectively. The enzyme was able to hydrolyze both purine and pyrimidine monophosphate nucleotides, such as UMP, GMP and CMP. Levamisole and tetramisole (1 mM), specific inhibitors of alkaline phosphatases, did not alter the enzymatic activity. However, a significant inhibition of AMP hydrolysis (42%) was observed in the presence of 100 μM α,β-methylene-ADP, a known inhibitor of ecto-5′-nucleotidase. Since 5′-nucleotidase represents the major enzyme responsible for the formation of extracellular adenosine, the enzymatic characterization is important to understand its role in purinergic systems and the involvement of adenosine in the regulation of neurotransmitter release.

Keywords: Zebrafish; Ecto-5′-nucleotidase; Adenosine; Ectonucleotidases; Extracellular nucleotides; ATP; Fish; *Danio rerio*

1. Introduction

Several cell types, including neurons and glial cells, can release nucleotides and nucleosides into the extracellular space that can play important roles in physiological and/or pathological conditions (Lucchi et al., 1992; Magalhães-Cardoso et al., 2003). In neurons, ATP can be stored in axon terminals and co-released during depolarization in the synaptic cleft with several neurotransmitters (Rathbone et al., 1999; Bodin and Burnstock, 2001). ATP is the principal agonist of P2 receptors, which are subdivided in two major classes: ionotropic P2X receptors and metabotropic P2Y receptors (Ralevic and Burnstock, 1998).

After its release, the neurotransmitter ATP can be converted into adenosine by a family of surface-located enzymes called ectonucleotidases (Bonan et al., 2001; Cunha, 2001). The ectonucleotidase pathway comprises several enzymes able to degrade nucleoside triphosphates and diphosphates, which belong to the E-NTPDase family (ectonucleoside triphosphate diphosphohydrolase) and the ectophosphodiesterase/nucleotide pyrophosphatase (E-PNPP) family (Zimmermann and Braun, 1999; Zimmermann, 2001). These enzymes, together with an ecto-5′-nucleotidase, are involved in the removal of released ATP, which acts as a signaling molecule, but also in the production of another important messenger, adenosine.

* Corresponding author. Tel.: +55 51 3320 3500x4158; fax: +55 51 3320 3612.
E-mail address: bonan@portoweb.com.br (C.D. Bonan).
[1] Both are first authors.

1096-4959/$ - see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.cbpc.2004.07.011

5′-Nucleotidase activity is widely distributed in vertebrate tissues (Grondal and Zimmermann, 1987; Zimmermann, 1992) and catalyzes the hydrolysis of a variety of nucleoside 5′-monophosphates, such as AMP, CMP, UMP, IMP and GMP (Bianchi and Spychala, 2003). At least seven different 5′-nucleotidase activities have been described with different biochemical and molecular properties (Bianchi and Spychala, 2003). Beyond its enzymatic properties, 5′-nucleotidase is a lymphocyte surface protein CD73 and may be involved in cell adhesion (Zimmermann, 1992, 1996; Bianchi and Spychala, 2003). Adenosine, a product of AMP catabolism by the action of 5′-nucleotidase, is a well-known neuromodulator that can also reach the extracellular space by its release through bi-directional non-concentrative adenosine transporters (Deckert et al., 1988). Adenosine modifies cell functioning by operating G-protein-coupled receptors (A_1, A_{2A}, A_{2B}, A_3), which can inhibit (A_1) or enhance (A_2) neuronal communication (Dunwiddie and Masino, 2001; Fredholm et al., 2001).

Zebrafish is a consolidated model system in many research areas, including neuroscience (Lele and Krone, 1996; Vascotto et al., 1997; Ivetac et al., 2000) and it has been shown that its genome shares similarities with the human genome (Barbazuk et al., 2000). There is evidence indicating the role of ATP as a neurotransmitter in this specie, since P2X purinoceptors have been cloned and characterized in zebrafish (Boué-Grabot et al., 2000; Egan et al., 2000; Norton et al., 2000; Diaz-Hernandez et al., 2002). Furthermore, studies in teleost brain have shown that adenosine A_1 receptors appeared similar to that found in mammals (Lucchi et al., 1992, 1994; Beraudi et al., 2003; Kucenas et al., 2003). Recently, studies have demonstrated the presence of NTPDase activity in brain membranes of zebrafish (Rico et al., 2003), which could play an important role in the modulation of the nucleotide and nucleoside levels. Since there is evidence about purinoceptors in the brain of lower vertebrates and that 5′-nucleotidase represents the major enzyme responsible for the formation of extracellular adenosine, this study aims to investigate an ecto-5′-nucleotidase activity in brain membranes of zebrafish.

2. Materials and methods

2.1. Materials

Trizma Base, ammonium molybdate, polyvinyl alcohol, Malachite Green, AMP, EDTA, EGTA, sodium citrate, Coomassie Blue G, bovine serum albumin and magnesium chloride were purchased from Sigma (St. Louis, MO, USA). All other reagents used were of analytical grade.

2.2. Experimental animals

Zebrafish (*Danio rerio*) were obtained from commercial suppliers. The animals weighting 0.250–0.450 g were kept in 50-l aquarium at 25±5 °C for at least 7 days before the experiments. Fish were fed once daily with a commercial fish pellet and kept under a natural light–dark photoperiod. The procedure for maintenance and use of the animals were according to Colégio Brasileiro de Experimentação Animal (COBEA).

2.3. Brain membranes

Brain membranes were prepared as described by Barnes et al. (1993). Briefly, whole zebrafish brains were homogenized in 60 volumes (v/w) of chilled Tris–citrate buffer (50 mM Tris–citrate, 2 mM EDTA, 2 mM EGTA, pH 7.4, adjusted with citric acid) in a motor driven Teflon-glass homogenizer. The homogenate was centrifuged at 1000×*g* for 10 min and the pellet was discarded. After removing the nuclear and cell debris, the supernatant was centrifuged for 25 min at 40,000×*g*. The resultant pellet was frozen in liquid nitrogen, thawed, resuspended in Tris–citrate buffer and recentrifuged for 20 min at 40,000×*g*. This fresh–thaw–wash procedure was used to ensure lysis of the membranes. The final pellet, containing a mixture of intracellular and extracellular brain membranes, was resuspended and used in the enzyme assays. The material was maintained at 2–4 °C throughout preparation.

2.4. Enzyme activity

After membrane preparation, the optimum conditions for AMP hydrolysis were determined. Brain membranes of zebrafish (3–5 μg protein) were added to the reaction mixture containing 50 mM Tris–HCl (pH 7.2) and 5 mM $MgCl_2$ (or other cation as indicated) in a final volume of 200 μl. The samples were preincubated for 10 min at 37 °C. The reaction was initiated by the addition of substrate (AMP or other, as indicated) to a final concentration of 1 mM and stopped by the addition of 200 μl 10% trichloroacetic acid. The samples were chilled on ice for 10 min. Samples (0.4 ml) were removed and added to 1 ml of reagent used for determination of inorganic phosphate (Pi), composed of 5.7% ammonium molybdate, 2.3% polyvinyl alcohol and 0.08% Malachite Green and prepared as described previously (Chan et al., 1986). Incubation times and protein concentrations were chosen in order to ensure the linearity of the reactions. Controls with the addition of the enzyme preparation after mixing with trichloroacetic acid were used to correct non-enzymatic hydrolysis of substrates. Specific activity is expressed as nmol of Pi released $\cdot \min^{-1} \cdot$ mg protein^{-1}. All enzyme assays were run in duplicate.

2.5. Protein determination

Protein was measured by the Coomassie Blue method, using bovine serum albumin as a standard (Bradford, 1976).

2.6. Statistical analysis

Data were expressed as means±S.D. and analyzed by Student's *t*-test or one-way analysis of variance (ANOVA), considering $P<0.05$ as significant.

3. Results and discussion

An enzyme with characteristics of an ecto-5′-nucleotidase was detected in brain membranes of zebrafish. Previous experiments demonstrated that the time course of AMP hydrolysis is linear up to 30 min in the presence of Mg^{2+} (Fig. 1A). Mg^{2+}-AMP hydrolysis increased as a function of protein concentration and the product formation was linear in the range of 3–10 μg protein in the incubation medium (Fig. 1B).

The 5′-nucleotidase activity was divalent cation-dependent and its sensitivity to Mg^{2+}, Ca^{2+} and Zn^{2+} is demonstrated in Fig. 2. Mg^{2+} and Ca^{2+} were activators of AMP hydrolysis in brain membranes of zebrafish (Fig. 2A). AMP hydrolysis in the presence of Mg^{2+} was dose-dependent, increasing at 1 and 5 mM, but presented a decrease at 10 mM $MgCl_2$. AMP hydrolysis was activated by Ca^{2+}, but this effect was not concentration-dependent. Cation dependency was confirmed by a decrease in AMP hydrolysis in the absence of added cation. However, interestingly, when 5 mM EDTA was added to the incubation medium in the presence of 5 mM Ca^{2+} or Mg^{2+}, we did not observe a significant decrease in the enzyme activity. For this reason, we tested different concentrations of EDTA (1, 5, 10 and 20 mM) on AMP hydrolysis in the presence of 5 mM $MgCl_2$. EDTA did not promote changes in AMP hydrolysis and a substantial activity was measurable in the presence of different EDTA concentrations tested (data not shown). Therefore, it is possible to suggest that, after the chelation induced by EDTA, there still remained an amount of cation able to activate the enzyme activity. Considering that the ecto-5′-nucleotidase contains catalytically important Zn^{2+} in the active site (Knöfel and Sträter, 1999; McMillen et al., 2003; Bianchi and Spychala, 2003), we evaluated the effect of different Zn^{2+} concentrations (0.05–10 mM) on the AMP hydrolysis in brain membranes of zebrafish (Fig. 2B). Although EDTA produced no recognizable inhibition, which could be consistent with a possible lack of requirement by the enzyme for divalent cation, addition of $MgCl_2$ (1–10 mM), $CaCl_2$ (1–10 mM) or $ZnCl_2$ (0.5–1 mM) proved to be stimulatory (Fig. 2A,B). However, it is important to observe that a significant augmentation of activity was brought about by the addition of low concentrations of Zn^{2+} and, at higher concentrations, activity decreased strongly (Fig. 2B). The lack of inhibitory effect of EDTA suggests that, if the enzyme requires Mg^{2+} or Zn^{2+}, these divalent cations probably are tightly bound to it. Considering that AMPase activity exhibited a stable

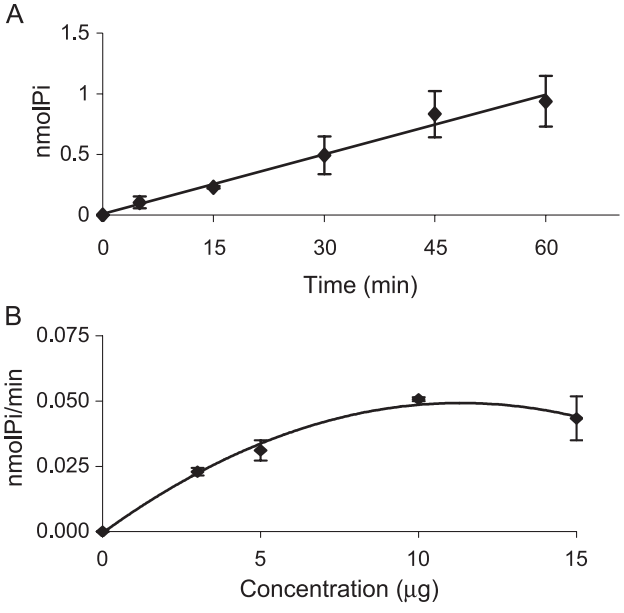

Fig. 1. (A) Time course for Mg^{2+}-AMP hydrolysis in 3–5 μg fractions of zebrafish (*D. rerio*) brain membranes. (B) Effect of different protein concentrations on Mg^{2+}-AMP hydrolysis in zebrafish brain membranes. Data represents means±S.D. of three different experiments, each in duplicate.

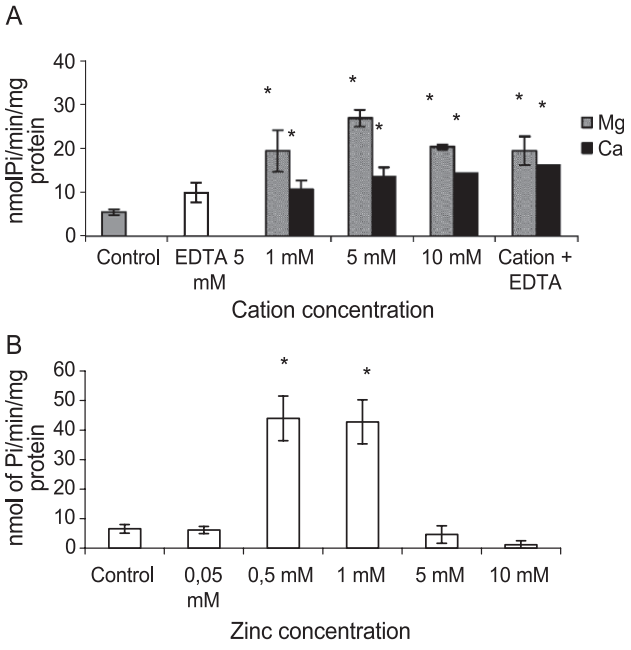

Fig. 2. (A) Effect of different concentrations of $MgCl_2$ and $CaCl_2$ on AMP hydrolysis in brain membranes of zebrafish (*D. rerio*). Control group was incubated without addition of cation. Cation+EDTA was incubated in the presence of 5 mM $MgCl_2$ or 5 mM $CaCl_2$ plus 5 mM EDTA. (B) Effect of different concentrations of $ZnCl_2$ on AMP hydrolysis in brain membranes of zebrafish. Control group was incubated without addition of $ZnCl_2$. Enzyme assays were carried out as described in Section 2. Data represent means±S.D. of four different experiments, each in duplicate. *Indicates difference when compared to the control group. Data were analyzed statistically by one-way ANOVA followed by Duncan test as a post-hoc test, considering a $P<0.05$ significant.

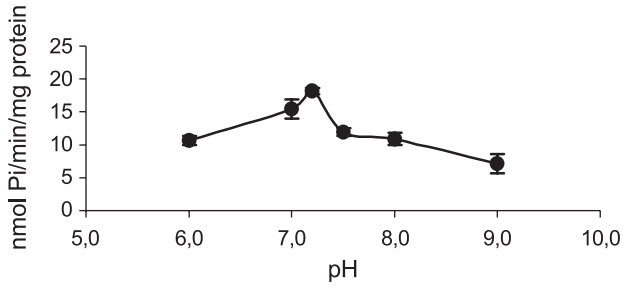

Fig. 3. Effect of pH on Mg^{2+}-AMP hydrolysis in brain membranes of zebrafish (*D. rerio*). Enzyme activity was described in Section 2, using the following buffers: 50 mM Tris–histidine, pH 6.0, 7.0, 7.2, 8.0, 9.0. Data represent means±S.D. of three different experiments, each in duplicate.

activation in the presence of $MgCl_2$, which is a classical activator of 5′-nucleotidase (Zimmermann, 1992), we used 5 mM $MgCl_2$ for subsequent enzyme assays.

Mg^{2+}-AMP hydrolysis was evaluated by measuring pH dependence on enzyme activity. In a medium containing 50 mM Tris–histidine (pH 6.0, 7.0, 7.2, 8.0 and 9.0), the maximal rate for AMP hydrolysis was observed at pH 7.2, similar to other 5′-nucleotidases described in the literature (Zimmermann, 1992, 1996) (Fig. 3). Mg^{2+}-hydrolysis was also assayed at different temperatures (10, 20, 37 and 45 °C). In the temperatures tested, the results showed that the enzyme displayed the highest activity at 37 °C (data not shown).

Mg^{2+}-AMP hydrolysis was determined at substrate concentrations in the range of 100–2000 μM. Enzyme activity increased with increasing concentrations of the nucleotide (Mg^{2+} fixed at 5 mM with variable concentrations of AMP) (Fig. 4). K_m and V_{max} for AMP hydrolysis in brain membranes of zebrafish were estimated from the Lineweaver-Burk plot with three different enzyme preparations. The apparent K_m and V_{max} values for Mg^{2+}-AMP were 135.3±16 μM (mean±S.D.) and 29±4.2 nmol $Pi \cdot min^{-1} \cdot mg^{-1}$ of protein (mean±S.D.), respectively.

Ecto-5′-nucleotidase has been described as an enzyme with a broad substrate specificity (Zimmermann, 1996). Our results showed that brain membranes of zebrafish were able

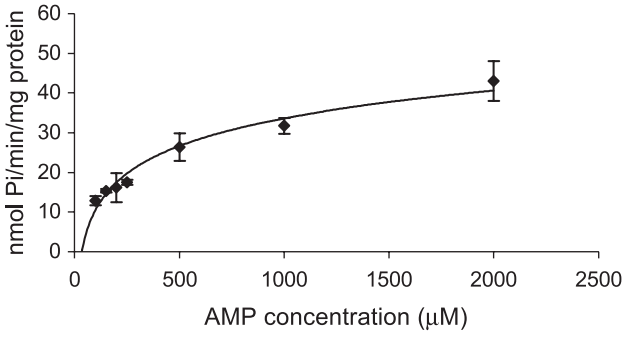

Fig. 4. Effect of different concentrations of substrate (100–2000 μM) on AMP hydrolysis in brain membranes of zebrafish (*D. rerio*). All experiments used fixed 5 mM Mg^{2+} with variable concentrations of nucleotide. Data represent means±S.D. of three different experiments, each in triplicate.

Table 1
Substrate specificity of 5′-nucleotidase in brain membranes of zebrafish (*D. rerio*)

Substrate	% Control activity
AMP	100±6
UMP	97±11
GMP	107±5
CMP	49±11

Results represent mean±S.D. of at least three experiments. Data are expressed as percentage of control activity (AMP hydrolysis—100%). Control Mg^{2+}-AMPase activity was 19.94±1.1 nmol $Pi \cdot min^{-1} \cdot mg^{-1}$. All substrates were used at 1 mM.

to hydrolyze all nucleoside monophosphates tested, presenting a relevant rate of AMP, GMP, UMP and CMP hydrolysis (Table 1).

To avoid the influence of other enzymes, such as alkaline phosphatase, in the AMP hydrolysis, inhibitors of this enzyme activity were tested. Levamisole and tetramisole, specific alkaline phosphatase inhibitors, had no effect upon AMP hydrolysis in brain membranes of zebrafish (Table 2). Conversely, the known 5′-nucleotidase inhibitor, adenosine 5′-[α,β-methylene]diphosphate (AMPCP), was also tested and inhibited enzyme activity significantly (42%, Table 2).

In this family of enzymes, the ecto-5′-nucleotidase (e-N), responsible for extracellular degradation of AMP, is the best-characterized enzymatic source of adenosine. It exhibits a K_m for AMP in the micromolar range and it is inhibited by AMPCP. Soluble 5′-nucleotidase with high K_m for AMP (millimolar range) is found in the cytosolic fraction (cN-I) and another form of 5′-nucleotidase activity (e-Ns), with a low K_m for AMP (K_m=15 μM in rat brain), has been observed in soluble fractions from rat liver, kidney and brain (Orford and Saggerson, 1996). Therefore, our results showed an enzyme in brain membranes of zebrafish that shares kinetic properties with an ecto-5′-nucleotidase.

Neurotransmitter signalling pathways require effective mechanisms for removing or metabolizing extracellular signalling molecules and, in the case of extracellular nucleotide signalling, a broad range of nucleotide-degrading and interconverting ecto- or extracellular enzymes have been identified (Zimmermann, 2001). The activity of the ectonucleotidase pathway appears critical to define the pattern of formation of extracellular ATP-derived adenosine to allow the activation of either inhibitory A_1 or facilitatory

Table 2
Effects of inhibitors on AMP hydrolysis in brain membranes of zebrafish (*D. rerio*)

Inhibitor	Concentration (mM)	% Control enzyme activity
AMPCP	0.1	57.90±0.86*
Tetramisole	1	95.61±9.54
Levamisole	1	81.58±12.4

Results are expressed as percentage of control activity (100%). Control AMPase activity was 22.64±2.64 nmol $Pi \cdot min^{-1} \cdot mg^{-1}$. AMPCP, adenosine 5′-[α,β-methylene]diphosphate. Data represent mean±S.D. of at least three experiments. *Significant difference from control activity (100%) by Student's *t*-test ($P<0.05$).

A_{2A} adenosine receptors (Magalhães-Cardoso et al., 2003). In summary, considering the previous identification of purinoreceptors and NTPDase in central nervous system of zebrafish, the presence of an ecto-5′-nucleotidase activity may be a key component in the control of this signalling pathway in this species.

Acknowledgements

This work was supported by Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul (FAPERGS), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Third World Academy of Sciences (TWAS). M.R.S. is recipient of a CNPq fellowship. E.P.R. is a recipient from FAPERGS. The authors would like to thank the Instituto de Pesquisas Biomédicas (PUCRS) for technical support.

References

Barbazuk, W.B., Korf, I., Kadavi, C., Heyen, J., Tate, S., Wun, E., Bedell, J.A., McPherson, J.D., Johnson, S.L., 2000. The syntenic relationship of the zebrafish and human genomes. Genome Res. 10, 1351–1358.

Barnes, J.M., Murphy, P.A., Kirkham, D., Henley, J.M., 1993. Interaction of guanine nucleotides with [^{3}H]kainate and 6-[^{3}H]cyano-7-nitroquinoxaline-2,3-dione binding in goldfish brain. J. Neurochem. 61, 1685–1691.

Beraudi, A., Traversa, U., Villani, L., Sekino, Y., Nagy, J.I., Poli, A., 2003. Distribution and expression of A_1 adenosine receptors, adenosine deaminase and adenosine deaminase-binding protein (CD26) in goldfish brain. Neurochem. Int. 42, 455–464.

Bianchi, V., Spychala, J., 2003. Mammalian 5′-nucleotidases. J. Biol. Chem. 278, 46195–46198.

Bodin, P., Burnstock, G., 2001. Purinergic signalling: ATP release. Neurochem. Res. 26, 959–969.

Bonan, C.D., Schetinger, M.R.C., Battastini, A.M.O., Sarkis, J.J.F., 2001. Ectonucleotidases and synpatic plasticity: implications in physiological and pathological conditions. Drug Dev. Res. 52, 57–65.

Boué-Grabot, E., Akimenko, M.A., Seguela, P., 2000. Unique functional properties of a sensory neuronal P2X ATP-gated channel from zebrafish. J. Neurochem. 75, 1600–1607.

Bradford, M.M., 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 218–254.

Chan, K., Delfert, D., Junguer, K.D., 1986. A direct colorimetric assay for Ca^{+2}-ATPase activity. Anal. Biochem. 157, 375–380.

Cunha, R.A., 2001. Adenosine as a neuromodulator and as a homeostatic regulator in the nervous system: different roles, different sources and different receptors. Neurochem. Int. 38, 107–125.

Deckert, J., Morgan, P.F., Marangos, P.J., 1988. Adenosine uptake site heterogeneity in the mammalian CNS? Uptake inhibitors as probes and potential neuropharmaceuticals. Life Sci. 42, 1331–1345.

Diaz-Hernandez, M., Cox, J.A., Migita, K., Haines, W., Egan, T.M., Voigt, M.M., 2002. Cloning and characterization of two novel zebrafish P2X receptor subunits. Biochem. Biophys. Res. Commun. 295, 849–853.

Dunwiddie, T.V., Masino, S.A., 2001. The role and regulation of adenosine in the central nervous system. Annu. Rev. Neurosci. 24, 31–55.

Egan, T.M., Cox, J.A., Voigt, M.M., 2000. Molecular cloning and functional characterization of the zebrafish ATP-gated ionotropic receptor $P2X_3$ subunit. FEBS Lett. 475, 287–290.

Fredholm, B.B., Ijzerman, A.P., Jacobson, K.A., Klotz, K.N., Linden, J., 2001. International Union of Pharmacology. XXV: nomenclature and classification of adenosine receptors. Pharmacol. Rev. 53, 527–552.

Grondal, E.J.M., Zimmermann, H., 1987. Purification, characterization and cellular localization of 5′-nucleotidase from Torpedo electric organ. Biochem. J. 245, 805–810.

Ivetac, I., Becanovic, J., Krishnapillai, V., 2000. Zebrafish: genetic tools and genomics. Asia–Pacific J. Mol. Biol. Biotechnol. 8, 1–11.

Knöfel, T., Sträter, N., 1999. X-ray structure of the *Escherichia coli* periplasmic 5′-nucleotidase containing a dimetal catalytic site. Nat. Struct. Biol. 6, 448–453.

Kucenas, S., Li, Z., Cox, J.A., Egan, T.M., Voig, M.M., 2003. Molecular characterization of the zebrafish P2X receptor subunit gene family. Neuroscience 121, 935–945.

Lele, Z., Krone, P.H., 1996. The zebrafish as a model system in developmental, toxicological and transgenic research. Biotechnol. Adv. 14, 57–72.

Lucchi, R., Poli, A., Traversa, U., Barnabei, O., 1992. Characterization of A_1 adenosine receptors in membranes from whole goldfish brain. Comp. Biochem. Physiol., B 102, 331–336.

Lucchi, R., Poli, A., Traversa, U., Barnabei, O., 1994. Functional adenosine A_1 receptors in goldfish brain: regional distribution and inhibition of K^+-evoked glutamate release from cerebellar slices. Neuroscience 58, 237–243.

Magalhães-Cardoso, M.T., Pereira, M.F., Oliveira, L., Ribeiro, J.A., Cunha, L., Ribeiro, J.A., Cunha, R.A., Correia-de-Sá, P., 2003. Ecto-AMP deaminase blunts the ATP-derived adenosine A_{2A} receptor facilitation of acetylcholine release at rat motor nerve endings. J. Physiol. 549, 399–408.

McMillen, L., Beacham, I.R., Burns, D.M., 2003. Cobalt activation of *Escherichia coli* 5′-nucleotidase is due to zinc ion displacement at only one of two metal-ion-binding sites. Biochem. J. 372, 625–630.

Norton, W.H., Rohr, K.B., Burnstock, G., 2000. Embryonic expression of a $P2X_3$ receptor-encoding gene in zebrafish. Mech. Dev. 9, 149–152.

Orford, M.R., Saggerson, E.D., 1996. A low-Km 5′-nucleotidase from rat brain cytosolic fraction: purification, kinetic properties, and description of regulation by a novel factor that increases sensitivity to inhibition by ATP and ADP. J. Neurochem. 67, 795–804.

Ralevic, V., Burnstock, G., 1998. Receptors of purines and pyrimidines. Pharmacol. Rev. 50, 413–492.

Rathbone, M.P., Middlemiss, P.L., Gysbers, J.W., Andrew, C., Herman, M.A., Reed, J.K., Ciccarelli, R., Di Iorio, P., Caciagli, F., 1999. Trophic effects of purines in neurons and glial cells. Prog. Neurobiol. 59, 663–690.

Rico, E.P., Senger, M.R., Fauth, M.G., Dias, R.D., Bogo, M.R., Bonan, C.D., 2003. ATP and ADP hydrolysis in brain membranes of zebrafish (*Danio rerio*). Life Sci. 73, 2071–2082.

Vascotto, S.G., Beckham, Y., Kelly, G.M., 1997. The zebrafish's swim to fame as an experimental model in biology. Biochem. Cell. Biol. 75, 479–485.

Zimmermann, H., 1992. 5′-Nucleotidase: molecular structure and functional aspects. Biochem. J. 15, 345–365.

Zimmermann, H., 1996. Biochemistry, localization and functional roles of ecto-nucleotidases in the nervous system. Prog. Neurobiol. 49, 589–618.

Zimmermann, H., 2001. Ectonucleotidases: some recent developments and a note on nomenclature. Drug Dev. Res. 52, 44–56.

Zimmermann, H., Braun, N., 1999. Ecto-nucleotidases: molecular structures, catalytic properties, and functional roles in the nervous system. Prog. Brain Res. 120, 371–385.