Comparative Biochemistry and Physiology, Part C 155 (2012) 566–572

Contents lists available at SciVerse ScienceDirect

Comparative Biochemistry and Physiology, Part C

journal homepage: www.elsevier.com/locate/cbpc

Arsenic alters behavioral parameters and brain ectonucleotidases activities in zebrafish (*Danio rerio*)

Luis Antonio Baldissarelli [a], Katiucia Marques Capiotti [a,c], Mauricio Reis Bogo [b,c], Gabriele Ghisleni [a], Carla Denise Bonan [a,c,*]

[a] *Laboratório de Neuroquímica e Psicofarmacologia, Departamento de Biologia Celular e Molecular, Faculdade de Biociências, Pontifícia Universidade Católica do Rio Grande do Sul, Porto Alegre, RS, Brazil*

[b] *Laboratório de Biologia Genômica e Molecular, Departamento de Biologia Celular e Molecular, Faculdade de Biociências, Pontifícia Universidade Católica do Rio Grande do Sul, Porto Alegre, RS, Brazil*

[c] *Instituto Nacional de Ciência e Tecnologia Translacional em Medicina (INCT-TM), 90035–003, Porto Alegre, RS, Brazil*

ARTICLE INFO

Article history:
Received 7 September 2011
Received in revised form 8 January 2012
Accepted 9 January 2012
Available online 13 January 2012

Keywords:
Arsenic
Ectonucleotidases
Locomotion
Zebrafish
Nucleoside triphosphate diphosphohydrolase
Ecto-5′-nucleotidase

ABSTRACT

Arsenic (As) exposure has been associated with serious chronic health risk to humans including cancer and neurological disturbances. However, there are limited studies about the mechanisms behind its toxicity. In this study, adult zebrafish were exposed to several concentrations of As (0.05, 5, and 15 mg As/L; Na_2HAsO_4 as As^V) during 96 h to evaluate the zebrafish locomotor activity, anxiety, and brain extracellular nucleotide hydrolysis. We showed that 5 mg/L As is able to promote significant decrease in the locomotor activity as evaluated by the number of line crossings. In addition, animals treated with 5 mg/L As presented an increase in time spent in the lower zone of the tank test, suggesting an anxiogenic effect. Considering that behavioral parameters, such as anxiety and locomotion, might be modulated by the purinergic system, we also evaluated the ectonucleotidase activities in zebrafish brain after a 96-h As exposure. A significant decrease in ATP, ADP, and AMP hydrolysis was observed at 0.05, 5, and 15 mg/L when compared to control group. These findings demonstrated that As might affect behavioral parameters and the ectonucleotidase activities in zebrafish, suggesting this enzyme pathway is a target for neurotoxic effects induced by As.

1. Introduction

Arsenic (As) is a heavy metal widely distributed in the environment due to its natural and anthropogenic sources. It exists in inorganic and organic forms and in different oxidation states. Inorganic As is metabolized by a sequential process involving a reduction of pentavalent As (arsenate; As^V) to trivalent As (arsenite; As^{III}) in the presence of a thiol, followed by oxidative methylation to pentavalent organic As (Hughes, 2002). High levels of inorganic As are found in ground water in many regions of the world as a result of geochemical processes promoting serious chronic health risks to humans (Bagli et al., 1996; Nickson et al., 1998; Hughes, 2006). In environmental exposure, toxicologists are primarily concerned with As in the trivalent and pentavalent oxidation state.

As is derived from natural and anthropogenic sources in the environment. The biogeochemical cycling of As and the natural flow of this element in the biosphere have been altered due to the increasing mining activity and use of pesticides and wood preservatives among others, resulting in contamination of soil and other ecosystem components. The high levels of As toxicity are well known, since compounds are easily absorbed, either orally or by inhalation, and the extent of absorption is related to the solubility of the compound (Nicolis et al., 2009; Ventura-Lima et al., 2009). Exposure to As leads to diseases, such as conjunctivitis, hyperkeratosis, hyperpigmentation, cardiovascular diseases, disorders of the peripheral and central nervous systems, skin cancer, and gangrene in the limbs (Anderson et al., 1986). The effects of As are observed weeks after first exposure as both central and peripheral neuropathy. Impairment of important neurological functions, such learning, short-term memory, and attention, can be caused by the central neuropathy due to As poisoning. Neuropsychological tests showed mildly impaired psychomotor and attentive processes whereas verbal learning and memory were severely damaged (Vahidnia et al., 2007). Although there is evidence that As exposure has a toxic effect on the nervous system, there are few studies addressing the neurochemical changes induced by this metal. Studies have shown that cholinergic, gabaergic, and monoaminergic systems may be a target of neurotoxic effects induced by As (Kannan et al., 2001; Rodríguez et al., 2003); however, there are no studies investigating the effects of As on purinergic signaling and in the control of extracellular nucleotide and nucleoside levels.

* Corresponding author at: Laboratório de Neuroquímica e Psicofarmacologia, Departamento de Biologia Celular e Molecular, Faculdade de Biociências, PUCRS. Av. Ipiranga, 6681 Prédio 12D, Sala 340, 90619–900 Porto Alegre, RS, Brazil. Tel.: +55 51 3353 4158; fax: +55 51 3320 3568.
E-mail address: cbonan@pucrs.br (C.D. Bonan).

1532-0456
doi:10.1016/j.cbpc.2012.01.006

ATP is an early signaling molecule, considered as a neurotransmitter in the CNS and performs its functions when it is released into the synaptic cleft in a calcium-dependent manner (Burnstock, 1997; Cunha and Ribeiro, 2000). It is stored in presynaptic vesicles and is released after depolarization acting on specific receptors (Ralevic and Burnstock, 1998) ATP can be co-released along with several other neurotransmitters, such as acetylcholine, glutamate, norepinephrine, serotonin, and acid γ-aminobutyric acid (GABA) (Burnstock, 1999, 2004). ATP activates P2 purinoceptors, which are subdivided into two distinct families: ionotropic P2X and metabotropic P2Y receptors (Ralevic and Burnstock, 1998).

ATP is degraded by a cascade of enzyme families located on the cell surface called ectonucleotidases. These enzymes constitute a highly refined system for the regulation of nucleotide-mediated signaling, controlling the rate, amount, and timing of nucleotide degradation and production. The hydrolysis of ATP to AMP is catalyzed mainly by a family of ectonucleotidases, named nucleoside triphosphate diphosphohydrolases (NTPDases). The nucleotide AMP is hydrolyzed to adenosine, an important neuromodulator, by the action of an ecto-5′-nucleotidase (CD73, EC 3.1.3.5) (Robson et al., 2006; Schetinger et al., 2007).

Zebrafish (*Danio rerio*) is a biological model consolidated in neuroscience and toxicology studies (Linney et al., 2004). The genome project of this animal is nearly completed, and studies have shown an alignment of the zebrafish genome with the human genome (Barbazuk et al., 2000; Stern and Zon, 2003). P2 purinoceptors have been identified in zebrafish (Kucenas et al., 2003) and we have also characterized the presence of NTPDases and ecto-5′-nucleotidase in brain membranes of zebrafish (Rico et al., 2003; Senger et al., 2004). Furthermore, previous studies from our laboratory have shown that metals, such as mercury, lead, and copper are able to induce significant changes in the enzymes involved in extracellular nucleotide hydrolysis in zebrafish brain (Senger et al., 2006; Rosemberg et al., 2007). Studies demonstrated that As(V) is able to induce impairment in long-term memory in zebrafish (De Castro et al., 2009); however, the neurochemical mechanisms involved in the cognitive deficit induced by As are still unclear.

Considering that As is an important environmental pollutant and induces impairment in several neurological functions, we investigated the effect of As exposure on locomotor activity and anxiety in zebrafish. Since these behavioral parameters can be modulated by the purinergic system, we also evaluated the extracellular nucleotide hydrolysis by assessing the NTPDases and ecto-5′-nucleotidase activities in zebrafish brain after exposure to As.

2. Material and methods

2.1. Animals

Adult wild type zebrafish (*Danio rerio*) strains (3–5 cm, 6–9 months old) of both sexes were obtained from specialized commercial supplier (Redfish, Porto Alegre, Brazil) and were of genetically heterogeneous (randomly bred) stock. The fish was acclimated for at least 14 days to the laboratory environment and housed in a 50-L thermostated aquarium filled with continuously unchlorinated water at a targeted temperature of 28 ± 2 °C with constant filtration and aeration (7.20 mg O_2/L) and up to a density of five animals per liter. Animals were kept at a day/night cycle of 14:10 h and fed twice a day with flake fish food and supplemented with live brine shrimp.

Fish were manipulated according to the "Guide for the Care and Use of Laboratory Animals" published by the US National Institutes of Health (NIH publication No. 85-23, revised 1996), being healthy and free of any signs of disease. The Ethics Committee of Pontifical Catholic University of Rio Grande do Sul (PUCRS) approved the protocol under the number 08/00058-CEUA.

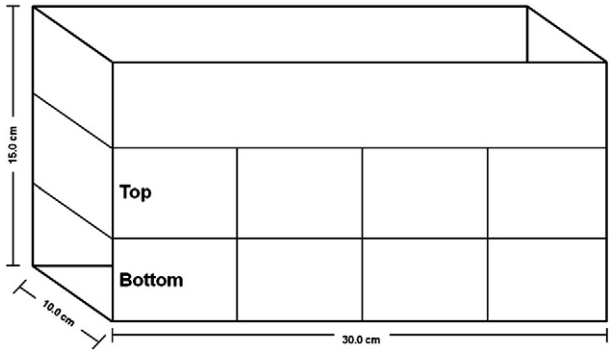

Fig. 1. Behavioral parameters were analysed in an apparatus, which consisted in a rectangular glass tank with the specific dimensions described above. Virtual divisions were used to evaluation of zebrafish swimming activity in the novel tank test, with two vertical areas (bottom and top) and eight horizontal sections with 4 sections per area.

2.2. In vivo treatment

A group of five animals were maintained during 96-h treatment in 5-L aquariums containing a range concentrations of As (0.05, 5, and 15 mg As/L; As as Na_2HAsO_4; As^V) (Chattopadhyay et al., 2002; Hermann and Kim, 2005; Ramirez and Gárcia, 2005). The pH and O_2 levels of water was measured daily and controlled to values of pH 7.0 and 7.20 mg O_2/L. After 96 h of exposure to arsenate, the animals were sacrificed by decapitation and the brain were shredded to perform molecular and enzymatic analysis. Control animals were maintained in group of five fish in 5-L aquariums employing only tap water with the same characteristics mentioned above.

2.3. Behavioral assessments

After the *in vivo* treatments described in Section 2.2, behavioral testing of As effects took place during the light phase between 10:00 h and 17:00 h. Animals were individually placed into the experimental tank (30 × 15 × 10 cm, length × height × width) after the 96-h As treatment and were first habituated to the test tank for 30 s, as previously described (Gerlai et al., 2000). There was no drug exposure during behavioral experiments. The locomotor activity was video recorded for 5 min after the habituation period and simultaneously analyzed using the ANY-

Table 1
Primer sequences and PCR amplification conditions.

Gene	Primer sequences (5′-3′)	Tm (°C)	PCR fragment (bp)
entpd1	CCCATGGCACAGGCCGGTTG (forward) GCAGTCTCATGCCAGCCGTG (reverse)	54	380
entpd2_mg[a]	GGAAGTGTTTGACTCGCCTTGCACG (forward) CAGGACACAAGCCCTTCCGGATC (reverse)	62	554
entpd2_mq[a]	CCAGCGGATTTAGAGCACGCTG (forward) GAAGAACGGCGGCACGCCAC (reverse)	62	313
entpd2_mv[a]	GCTCATTTAGAGGACGCTGCTCGTG (forward) GCAACGTTTTCGGCAGGCAGC (reverse)	62	263
entpd3	TACTTTCTTTGGACAGAGCAACCCTG (forward) AAGCATATAGCCCAGGGACCAGG (reverse)	62	424
5′-*nucleotidase*	5′-ACC TCC GAG GAG TGT CGC TTT CG-3′ (forward) 5′-CCT TGT TGG GGA CCA GCG GTT C-3′ (reverse)	54	433
β-actin	GTCCCTGTACGCCTCTGGTCG (forward) GCCGGACTCATCGTACTCCTG (reverse)	54	678

[a] Corresponds to the first two amino acid residues of the protein sequence.

Maze recording software (Stoelting Co., Wood Dale, IL, USA). The tank was divided into equal sections with four vertical lines and one horizontal line (Fig. 1), and the following behavior patterns were measured: number of line crossings (vertical and horizontal lines), distance travelled and mean speed. The time spent in tank position (bottom vs. upper levels) was considered the index of anxiety. This task exploits the natural tendency for zebrafish to spend the majority of time at the bottom when introduced into a novel environment and then gradually over a period of minutes expand their position of swimming to include the higher portions of the test tank. The longer time spent in the bottom and reduced time spent in the top of the tank indicate heightened anxiety (Seibt et al., 2010). The absolute turn angle represents the sum of all vectors angle of movements created from one position to animal's center point to the next. The anti-clockwise movement was considered negative and clockwise movement positive (−180° to 180°).

2.4. Preparation of brain membranes of zebrafish

The brain membranes were prepared as described previously by Barnes et al. (1993). Zebrafish were euthanized by decapitation, their brains were removed from the cranial skull by the dissection technique. For each sample (membrane preparation), a pool of five zebrafish brains was used which were briefly homogenized in 60 volumes (v/w) of chilled Tris–citrate buffer (50 mM Tris, 2 mM EDTA, 2 mM EGTA, pH 7.4, with citric acid) in a motor driven Teflon–glass homogenizer. The homogenate was centrifuged at 800 g for 10 min at 4 °C. 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 centrifuged for 20 min at 40,000 g. This fresh-thaw-wash procedure was used to ensure the lyses of the brain membranes. The final pellet was resuspended and used in the enzyme assays. All samples were maintained at 2–4 °C throughout preparation.

2.5. Enzyme assays

NTPDase and 5′-nucleotidase assays were performed as described previously (Rico et al., 2003; Senger et al., 2004). Zebrafish brain membranes (3 μg protein for NTPDase and 5 μg protein for ecto-5′-nucleotidase) were added to the reaction mixture

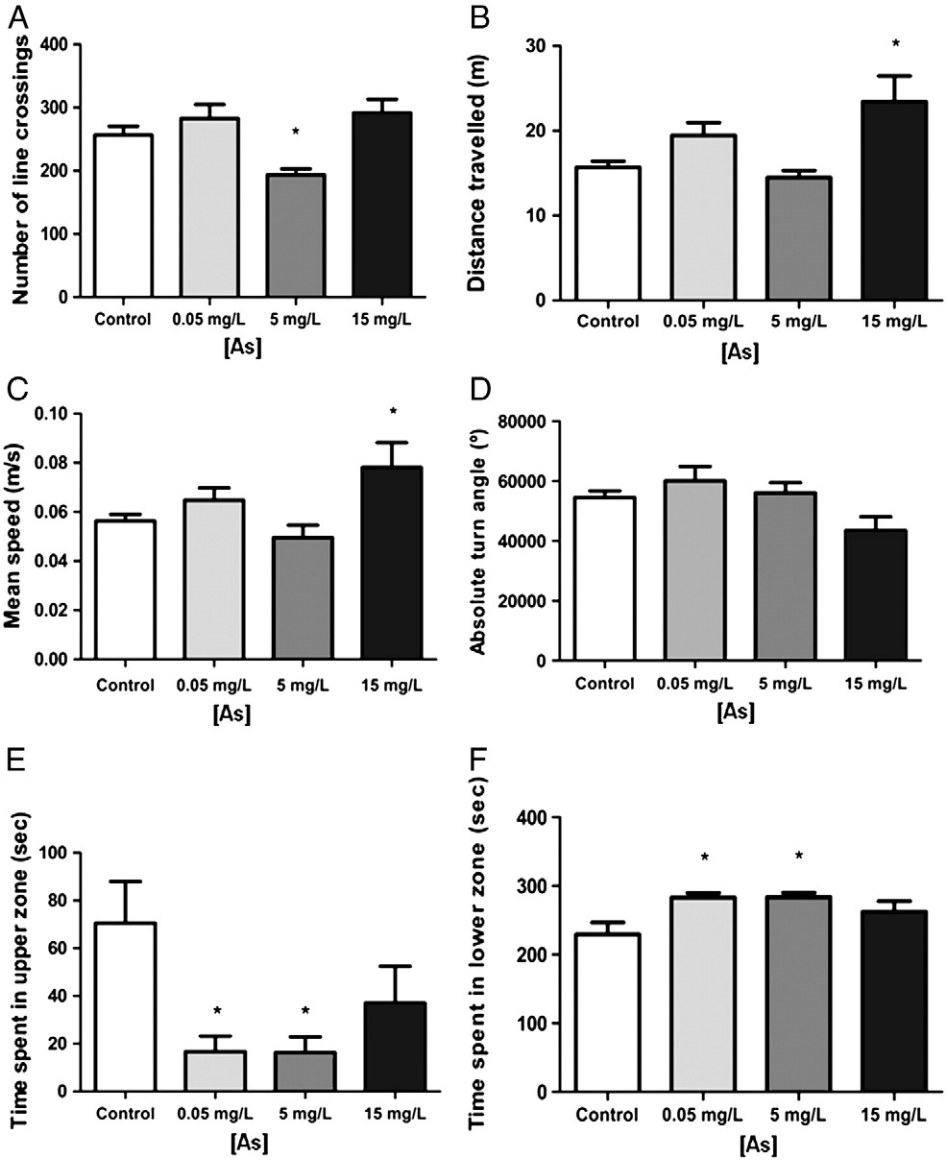

Fig. 2. Effect of different concentrations of As after a 96 h-exposure on number of line crossings (A), distance traveled (B), mean speed (C), absolute turn angle (D), time in upper zone (E), time in the lower zone (F) during 5 min of videorecording. Data were expressed as mean ± S.E.M from 10 animals for each group and analyzed by one-way ANOVA followed by Tukey post-hoc test. $^{*}p<0.05$ denotes a significant difference from control group.

containing 50 mM Tris–HCl (pH 8.0) and 5 mM $CaCl_2$ (for the NTPDase activity) or 50 mM Tris–HCl (pH 7.2) and 5 mM $MgCl_2$ (for the ecto-5′-nucleotidase activity) at a final volume of 200 μL. The samples were preincubated for 10 min at 37 °C and the reaction was initiated by the addition of substrate (ATP, ADP or AMP) to a final concentration of 1 mM. The reaction was stopped after 30 min by the addition of 200 μL trichloroacetic acid at a final concentration of 5%. The samples were chilled on ice for 10 min and 1 mL of a colorimetric reagent was added in order to determine the inorganic phosphate released (Pi) (Chan et al., 1986). After 20 min, the quantification of inorganic phosphate (Pi) released was determined spectrophotometrically at 630 nm. Incubation times and protein concentrations were chosen 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 was expressed as nanomoles of Pi released per min per mg of protein. All enzyme assays were run at least in triplicate.

2.6. Protein determination

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

2.7. Molecular analysis

After the treatments, animals were euthanized and the brain removed by dissection under sterile conditions and immediately subjected to a total RNA extraction by TRIzol reagent (Invitrogen, USA) in accordance with the manufacturer's instructions. The purity of the RNA was spectrophotometrically quantified by calculating the ratio between absorbance values at 260 and 280 nm. All samples were adjusted to 160 ng/μL and cDNA species were synthesized with SuperScript™ III First-Strand Synthesis SuperMix (Invitrogen, USA) following supplier's instructions. β-actin-PCR was performed as a cDNA synthesis control. PCR reactions were performed (total volume of 25 μL) using a concentration of 0.4 μM of each primer indicated below and 1 U platinum Taq DNA polymerase (Invitrogen) in the supplied reaction buffer. The zebrafish sequences encoding to ectonucleotidases retrieved from the GenBank database were *entpd1* (**AAH78240**), *entpd2_mg* (**XP_697600**), *entpd2_mq* (**XP_687722**), *entpd2_mv* (**AAH78419**), *entpdase3* (**ABR15509**), *ecto-5′-nucleotidase* (**NP_957226**), *β-actin* (**AAC13314**) and were used for searching specific primers, which were designed using Oligos 9.6 program (Table 1). In order to confirm primers specificity, each primer was compared with zebrafish genome and was able to recognize only its specific target sequence.

The following conditions were used for PCR reactions: 1 min at 94 °C, 1 min at the annealing temperature (see Table 1), 1 min at 72 °C for 30 cycles for *entpd3* and for 35 cycles (for *β-actin*, *entpd1*, *entpd2_mg*, *entpd2_mq*, *entpd2_mv*, *entpd1 and ecto-5′-nucleotidase*) with a further post-extension cycle at 72 °C for 10 min. A negative control was included for each set of PCR reactions. PCR products were separated on a 1.0% agarose gel with GelRed 10× and visualized with ultraviolet light. The fragment lengths expected for the PCR reactions were confirmed using Low DNA Mass Ladder and *β-actin* was determined as an internal standard. Band intensities were analyzed by optical densitometry using the software ImageJ 1.37 for Windows after running all PCR products in a single gel.

2.8. Statistical analysis

Data from behavioral parameters and ectonucleotidase activities for each substrate (ATP, ADP, and AMP) and gene expression were evaluated by one-way analysis of variance (ANOVA). Post-hoc comparisons were made using Tukey's HSD test. Significant difference in relation to control group was attributed to p values less than 0.05.

3. Results

3.1. Effect of As on behavioral patterns

Distinct parameters of zebrafish swimming activity were evaluated in an open field task after a 96-h exposure to different As concentrations (0.05 mg As/L, 5 mg/L, and 15 mg/L). As indexed by the number of line crossings in the apparatus there was a decrease in locomotor activity of animals submitted to 5 mg As/L (30.5%) when compared with control group (278.63 ± 64.34 line crossings) (Fig. 2A). We also showed that there is an increase in the distance traveled (49%) and the mean speed (39%) at 15 mg/L in relation of control animals (15.70 ± 0.703 meters and 0.056 ± 0.0027 m/s, respectively) (Fig. 2B and C). Considering the importance of routine turning and its pervasiveness throughout zebrafish life history, we analyzed the absolute turn angle as a measure of routine turns. This

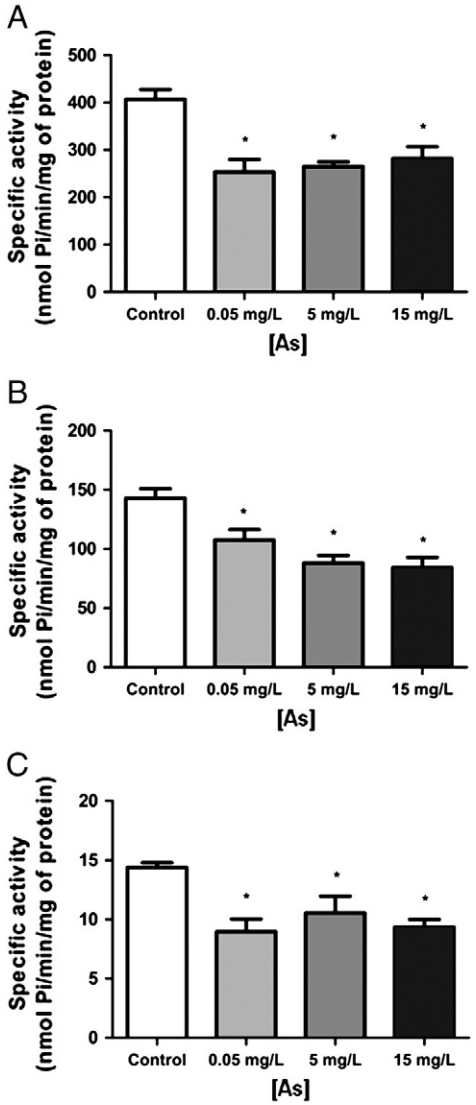

Fig. 3. Effect of different concentrations of As after 96 h-exposure on ATP (A), ADP (B), and AMP (C) hydrolysis in zebrafish brain membranes. Data were expressed as mean ± S.D. from 8 animals for each group and were statistically analyzed by one-way ANOVA followed by Tukey post hoc test. *$p<0.05$ denotes a significant difference from control group.

parameter has been analyzed through the measure of absolute turn angle that displays the degrees of fish turn per s, indicating the direction of the movement. Zebrafish exposed to all As concentrations tested did not show significant changes in the absolute turn angle during the 5-min evaluation of the task (Fig. 2D). In Fig. 2E, animals exposed to As at 0.05 mg/L (24%), 5 mg/L (23%) showed a significantly decrease in the time spent in the upper portion of the test tank when compared with the control group (70 ± 17 s). In addition, we observed as significant increase in the time spent in the lower zone of the test tank at 0.05 mg As/L (23%) and 5 mg As/L (23%) when compared with the control group (229.4 ± 17.4 s) (Fig. 2F). These parameters may be interpreted as an index of anxiogenic behavior.

3.2. Effects of As on extracellular nucleotide hydrolysis

The effects of As on NTPDase and ecto-5′-nucleotidase activities were observed in brain membranes of zebrafish after the 96-h exposure to As at three different concentrations (0.05 mg/L, 5 mg/L, and 15 mg/L). As promoted a significant decrease in ATP and ADP hydrolysis at 0.05 mg/L (37.6% and 25%, respectively), 5 mg/L (34.8% and 38%, respectively), and 15 mg/L (30.6% and 41%, respectively) when compared to control (Fig. 3A and B). In relation to ecto-5′-nucleotidase activity, the results showed that As also promoted a decrease of AMP hydrolysis at 0.05 mg/L (37.7%), 5 mg/L (26.7%), and 15 mg/L (35%) when compared to control group (Fig. 3C).

3.3. Effect of As on molecular analysis

Semiquantitative RT-PCR experiments of zebrafish brain ectonucleotidases and the enzyme/β-actin mRNA ratios were performed for each treatment. The results have shown that *entpd2_mg*, *entpd2_mq* and *ecto-5′-nucleotidase* transcript levels decreased after subchronic As exposure when compared to control group. A significant ($p<0.0001$) decrease on *entpd2_mg* expression was observed after subchronic treatment at 0.05 and 15 mg As/L (22% and 20%, respectively) whereas *entpd2_mq* transcript levels were decreased ($p<0.001$) at 0.05, 5, and 15 mg As/L (44%, 43% and 29%, respectively). *Ecto-5′-nucleotidase* gene expression was significant ($P<0.05$) decreased only in animals treated with 5 mg As/L (15%) (Fig. 4).

4. Discussion

This study shows that As promoted significant alterations in locomotion, anxiety, and extracellular nucleotide hydrolysis. There was a decrease in the locomotor activity at 5 mg As/L whereas there was an increase in the distance traveled and the mean speed at 15 mg As/L after a 96-h exposure. In addition, animals treated with As decreased

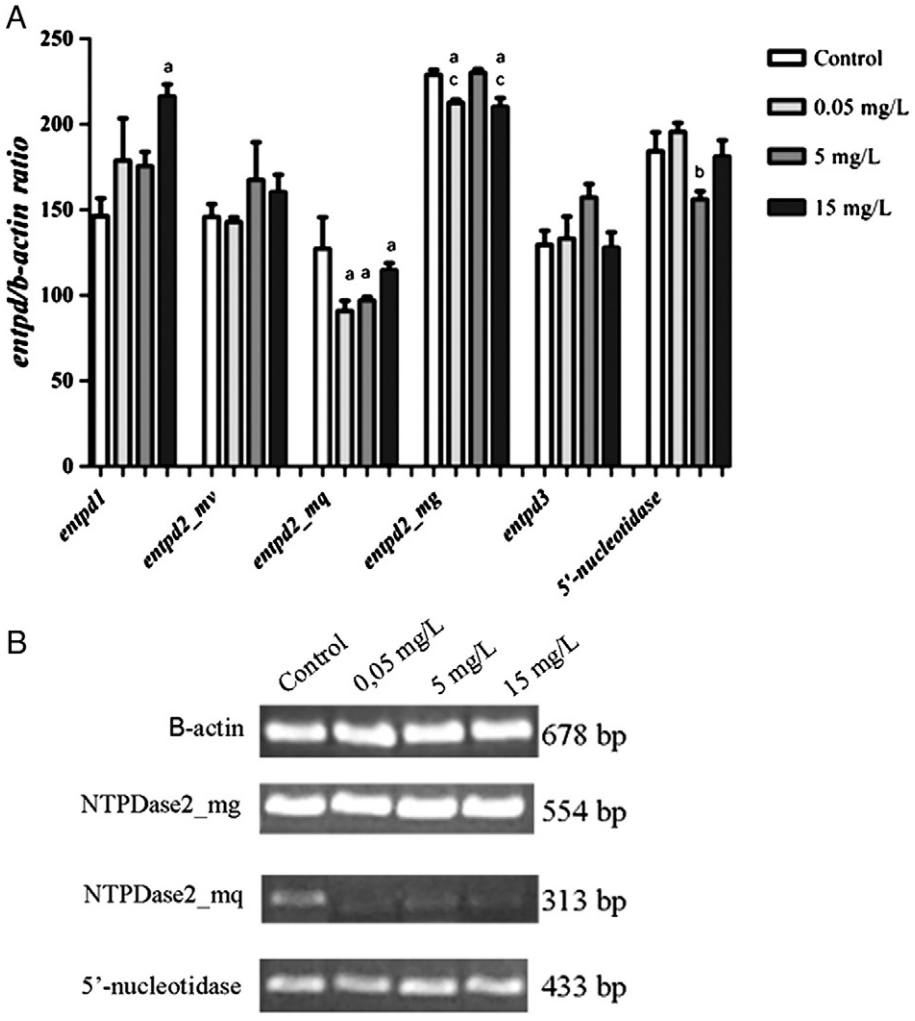

Fig. 4. Effect of subchronic (96 h) As exposure on ectonucleotidase transcripts. The figure shows β-*actin*, *entpd1*, *entpd2_mv*, *entpd2_mg*, *entpd2_mq*, *entpd3* and *5′-nucleotidase* mRNA expression in adult zebrafish (A) and the enzyme/β-actin mRNA ratios obtained by optical densitometry analysis (B). Three independent experiments were performed, with entirely consistent results. (a) significant difference from control group. (b) significant difference from 0.05 mg/L group. (c) significant difference from 5 mg/L group. ($p \leq 0.05$, ANOVA followed by Tukey's post hoc).

the time spent in the top and increase the time in the bottom zone of the tank, suggesting an anxiogenic effect of As. Previous studies have observed that As has a tendency to alter locomotor activity. Mice exposed to arsenic trioxide for 14 days showed a biphasic response in locomotor activity. Low doses of arsenic trioxide (3 mg/kg) increased locomotor activity whereas high doses (10 mg/kg) decreased this parameter (Itoh et al., 1990). Pryor et al. (1983) reported a decrease in locomotor activity of rats exposed to 1.5, 3.0, 6.0, and 12 mg/kg arsenic trioxide. This effect disappeared 3 weeks after the interruption of the administration. Similarly, rats exposed to sodium arsenite at 10 and 20 mg/kg for 15 or 30 days showed a reduction in locomotor activity, which was reversed after the interruption of arsenite exposure (Rodríguez et al., 2001). Therefore, the most consistent change in behavior of rats after administration of arsenic trioxide or sodium arsenite has been a decrease in locomotor activity (Rodríguez et al., 2001).

In addition, studies have also shown that As can alter other behavioral parameters, such as memory. De Castro et al. (2009) observed that the exposure to 0.01 mg As^V/L induced impairment in the long-term memory in zebrafish in an inhibitory avoidance task. Rodríguez et al. (2002) demonstrated that animals exposed to sodium arsenite from 15-day gestational period to approximately 4 months of age showed increased spontaneous locomotor activity and a delay in spatial learning task. Furthermore, As-exposed offspring showed longer latency to approach a novel object than controls in an object recognition task (Martinez-Finley et al., 2009). In the 8-way radial arm maze, As-exposed offspring had a significant increase in the number of entry errors compared to controls (Martinez-Finley et al., 2009), indicating adverse effects on learning and memory behavior (Martinez-Finley et al., 2009). However, there is no evidence on effects of As on anxiety parameters.

Zebrafish have a natural tendency to initially remain at the bottom of a novel environment (e.g., a test tank) and then gradually explore the higher portions of the test tank (Levin et al., 2007; Egan et al., 2009). Levin et al. (2007) have proposed that height in the tank may be a useful measure of anxiety that resembles thymogtaxis observed in rodents. Indeed, acute nicotine (Levin et al., 2007), buspirone (Bencan et al., 2009), and chronic fluoxetine (Egan et al., 2009) treatments reduce bottom-dwelling; however, results of the benzodiazepines, diazepam, and chlordiazepoxide were inconsistent in this task (Bencan et al., 2009). Our results showed that As can induce an anxiogenic effect on zebrafish, because these animals remained a longer time in the bottom of the tank.

In order to investigate the neurochemical mechanisms involved in the effects promoted by As on locomotion and anxiety, we evaluated the ectonucleotidase activities after a 96-h exposure to As, which regulate the concentration of ATP/ adenosine and P2/P1 receptor-mediated responses, and consequently, may modulate these behavioral parameters. Nucleotides are important extracellular messengers in both physiological and pathological conditions. After its release in the synaptic cleft, ATP can be catabolized to ADP, AMP, and adenosine. Our findings showed that As reduces significantly ATP, ADP, and AMP hydrolysis promoted by NTPDases and ecto-5′-nucleotidase in zebrafish brain membranes. The decrease in ectonucleotidase activities induced by As suggests an increase of ATP levels and, consequently, a decrease of adenosine levels in the synaptic cleft. Extracellular ATP can also induce apoptosis through $P2X_7$ receptors (Lepine et al., 2006) and the activation of these receptors involves PKC/MAPK signaling pathway in rat brain astrocytes (Wang et al., 2003). Therefore, an increase of ATP levels due to a decrease in ectonucleotidase activities could be one of the mechanisms involved in the neurotoxicity induced by As. Furthermore, a possible decrease of adenosine levels by As subchronic exposure could influence the interaction with P1 purinoreceptors and alter the neuromodulatory effect of this nucleoside. Recently, it was demonstrated that adenosine A_1 receptor blockade increases anxiety and autonomic arousal while a locomotor activating effect is probably mediated by A_2 receptors in zebrafish (Maximino et al., 2011). In this way, we could suggest that the decreased nucleotide hydrolysis demonstrated here by acute arsenic exposure could represent a reduction in adenosine levels and a subsequent activation of adenosine A_1 receptors (which present the lowest Kd for adenosine around 70 nM), triggering the anxiogenic effects and a decrease in locomotor activity in response to arsenic toxicity.

The alterations promoted by As in ectonucleotidase activity could be a consequence of transcriptional control. Interestingly, the results demonstrated that the relative gene expression levels of some NTPDase members (*entpd2_mg*, *entpd2_mq)* and ecto-5′-nucleotidase were significantly decreased after the treatment with As. It is possible to suggest that the changes in the transcriptional control induced by As exposure promote different alterations in ectonucleotidase mRNA transcription levels, which is the mechanism responsible for the decrease of ATP/ADP and AMP hydrolysis observed in this treatment.

Currently, the use of zebrafish is spreading to other areas of knowledge, such as Chemistry (Taylor and Raes, 2004), Neuroscience (Gerlai, 2003; Guo, 2004), Toxicology (Hill et al., 2005), and Pharmacology (Goldsmith, 2004). This teleost is able to quickly absorb the compounds that are added directly to the water and accumulate them in different tissues, especially in the central nervous system. Therefore, this species enables to accelerate the process of drug discovery and the understanding of toxicological effects induced by several aquatic contaminants. Exposure to various environmental contaminants such as TCDD (Dong et al., 2002), pesticides, carbamates and organophosphates (Senger et al., 2005), methanol (Rico et al., 2006), and heavy metals (Senger et al., 2006; Rosemberg et al., 2007) has been studied in zebrafish CNS. It makes this species an important biological model to be used as an indicator of water pollution. Thus, the evaluation of neurochemical and behavioral parameters in zebrafish is a valuable tool to increase the knowledge about the potential neurotoxic targets of As.

In summary, the alterations in ectonucleotidase activities and gene expression induced by As exposure might control adenosine levels, which modulate locomotion and anxiety responses in zebrafish. These findings demonstrated that As might affect behavioral parameters and the ectonucleotidase activities in zebrafish, suggesting this enzyme pathway is a target for neurotoxic effects induced by As.

Acknowledgments

This work was supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and by DECIT/SCTIE-MS through CNPq and Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul FAPERGS, (Proc. 10/0036-5 – PRONEX/ Conv. 700545/2008).

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