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Inhibitory effect of lithium on nucleotide hydrolysis and acetylcholinesterase activity in zebrafish (*Danio rerio*) brain

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ABSTRACT

Lithium has been used as an effective antimanic drug in humans and it is well known for its effects on neuropsychiatric disorders and neuronal communication. ATP and adenosine are important signaling molecules, and most nerves release ATP as a fast co-transmitter together with classical neurotransmitters such as acetylcholine. In this study, we evaluated the *in vitro* and *in vivo* effects of lithium on acetylcholinesterase and ectonucleotidase activities in zebrafish brain. There was a significant inhibition of ADP hydrolysis after *in vivo* exposure to lithium at 5 and 10 mg/l (27.6% and 29% inhibition, respectively), whereas an inhibitory effect was observed for AMP hydrolysis only at 10 mg/l (30%). Lithium treatment *in vivo* also significantly decreased the acetylcholinesterase activity at 10 mg/l (21.9%). The mRNA transcript levels of the genes encoding for these enzymes were unchanged after exposure to 5 and 10 mg/l lithium chloride. In order to directly evaluate the action of lithium on enzyme activities, we tested the *in vitro* effect of lithium at concentrations ranging from 1 to 1000 μM. There were no significant changes in zebrafish brain ectonucleotidase and acetylcholinesterase activities at all concentrations tested *in vitro*. Our findings show that lithium treatment can alter ectonucleotidase and acetylcholinesterase activities, which may regulate extracellular nucleotide, nucleoside, and acetylcholine levels. These data suggest that cholinergic and purinergic signaling may be targets of the pharmacological effects induced by this compound.

1. Introduction

Lithium is widely used in the treatment of bipolar disorder. Recent evidence has demonstrated that lithium can act through several signaling pathways and presents neuroprotective effects against a variety of insults in cultured neurons, in animal models of neurodegenerative diseases, and in human studies (Chakraborty et al., 2008; Yucel et al., 2008). One of the most extensively studied signaling pathways associated with the neuroprotective action of lithium is the inactivation of glycogen synthase kinase-3b (GSK3b), a proapoptotic enzyme responsible for hyperphosphorylation in Alzheimer's disease (Chakraborty et al., 2008).

Bipolar disorder, a neurological condition that causes cyclic variation in mood, drastically affects quality of life and significantly increases the chance of suicide in patients (Altamura et al., 2011; Ludtmann et al., 2011). This disease is defined by episodes of mania and hypomania, and its estimated worldwide occurrence is approximately 4% (Calabrese et al., 2003; Ketter, 2010).

ATP and adenosine are important signaling molecules in the central nervous system (Ralevic and Burnstock, 1998). The adenine nucleotide ATP is released at the synaptic cleft after nerve terminal depolarization, acting as a neurotransmitter or as a co-transmitter (Burnstock, 2009). ATP signaling is mediated by the cell-surface P2 receptors P2X and P2Y, which are a ligand-gated ion channel and metabotropic G protein-coupled receptor, respectively (reviewed in Burnstock, 2006). Extracellular ATP signaling is inactivated by the degradation of this nucleotide to adenosine by the action of ectonucleotidases. This group of enzymes includes the nucleoside triphosphate diphosphohydrolase (NTPDase) family that hydrolyzes both tri- and di-phosphonucleosides, and an ecto-5′-nucleotidase,

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which cleaves monophosphonucleosides to the respective adenosine nucleoside and controls purinergic neurotransmission (Robson et al., 2006; Schetinger et al., 2007). Adenosine may exert its action via the P1 receptors A_1, A_{2A}, A_{2B}, and A_3, which can inhibit (A_1 and A_3) or facilitate (A_{2A} and A_{2B}) neuronal communication (Fredholm et al., 2001;Fredholm, 2010). *In vivo* studies have shown that lithium alters ectonucleotidase activity in hippocampal synaptosomes (Wilot et al., 2004). However, no changes in these enzymes activities were observed in *in vitro* studies (Barcellos et al., 1998).

Most nerves release ATP as a fast co-transmitter together with classical fast transmitters such as acetylcholine, noradrenaline, and glutamate (Burnstock, 2004). Acetylcholine is a neurotransmitter secreted from the presynaptic nerve terminal and binds to acetylcholine receptors, which are clustered in the postsynaptic membrane. After being released, acetylcholine is cleaved into choline and acetate by acetylcholinesterase (AChE, EC 3.1.1.7), a fast serine hydrolase enzyme that regulates the concentration of the transmitter at the synapse (Soreq and Seidman, 2001). Studies have shown that adenosine is able to modulate acetylcholine release through inhibitory A_1 or facilitatory A_{2A} receptors (Rebola et al., 2002). Lithium is known to synergize the action of cholinomimetics in the central nervous system (Chaudhary and Gupta, 2001) and previous studies have shown that lithium treatment may alter the concentration of acetylcholine in the rat brain (Ronai and Vizi, 1975).

There has been growing interest in the development of novel animal models that could mimic human disease features and uncover cellular mechanisms involved in these pathologies (Rubinstein, 2003; Best and Alderton, 2008). The zebrafish, together with forward genetics and pharmacological interventions, has become a promising model to study many human diseases. In addition, drug mechanisms and several neurotransmitter systems, such as the purinergic and cholinergic systems, have been identified in zebrafish (Bertrand et al., 2001; Kucenas et al., 2006; Yi et al., 2006).

Due to the use of lithium for the treatment of mood disorders and the involvement of cholinergic and purinergic systems in several neuropsychiatric diseases, such as depression (Furey and Drevets, 2006; Burnstock, 2008), it is important to investigate whether these neurotransmitter systems may be involved in the therapeutic actions promoted by lithium. Therefore, the aim of this study was to test the *in vivo* and *in vitro* effects of lithium chloride on ectonucleotidase and acetylcholinesterase activities in zebrafish brain followed by a gene expression pattern analysis.

2. Methods

2.1. Animals

Adult (5–7 month-old), outbred, wildtype short-fin zebrafish of both sexes were obtained from a specialized commercial supplier (Redfish Agroloja Ltda., RS, Brazil) from a genetically heterogeneous (randomly bred) stock. The fish were acclimated to the laboratory environment for at least 14 days and housed in a 50-l tank with controlled water quality at 28 ± 2 °C and a density of up to five animals per liter. Animals were kept at a day/night cycle of 14:10 h and fed three times a day with commercial flakes. Fish were manipulated when healthy and free of any signs of disease, 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). The Ethics Committee of the Pontifícia Universidade Católica do Rio Grande do Sul (PUCRS) approved the protocol under the number CEP 07/03854.

2.2. Chemicals

Lithium chloride (CAS No. 7447-41-8), Trizma Base, ethylenedioxy-diethylene-dinitrilo-tetraacetic acid (EDTA), ethylene glycol bis (beta-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA), sodium citrate, Coomassie blue G, bovine serum albumin, malachite green, ammonium molybdate, polyvinyl alcohol, nucleotides, calcium, magnesium chloride, acetylthiocholine, and 5,5′-dithiobis-2-nitrobenzoic acid (DTNB) were purchased from Sigma (St. Louis, MO, USA). All other reagents used were of analytical grade.

2.3. In vitro and in vivo treatments

For the *in vitro* assays, lithium chloride was added to reaction medium before the preincubation with the enzyme and was maintained throughout the enzyme assays. Lithium chloride was tested at final concentrations of 1, 10, 25, 50, 100 and 1000 μM. The range of doses tested was chosen according to previous studies performed in Wistar rats (Barcellos et al., 1998). For the control group, the enzyme assay was performed in the absence of lithium chloride (no drug added in the reaction medium).

For the *in vivo* assays, fish were kept in 10-l aquariums and exposed to water with 1, 5 and 10 mg/l lithium chloride (corresponding to 23, 118 and 236 μM, respectively) because lithium chloride is highly soluble in water (water solubility: 83.5 g/100 ml at 20 °C). The lithium chloride doses were chosen based on those used in previous studies with aquatic organisms (Kszos et al., 2003). For the control group, animals were exposed only to water. The lithium solution was replaced on the third treatment day, and the animals were maintained in the test aquarium for 7 days. After lithium exposure, the fish were euthanized and the brains were dissected.

2.4. Determination of ectonucleotidase activities

Preparation of brain membranes was performed as described previously by Barnes et al. (1993). For each membrane preparation, a pool of five whole zebrafish brains was used, which were homogenized briefly 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 samples were centrifuged at $1000 \times g$ for 10 min and the pellet was discarded. The supernatant was centrifuged for 25 min at $40{,}000 \times g$. The resultant pellet was frozen in liquid nitrogen, thawed, resuspended in Tris-citrate buffer, and centrifuged for 20 min at $40{,}000 \times g$. This freeze–thaw–wash procedure was used to ensure the lysis of the brain membranes. The final pellet, containing a mixture of intra- and extracellular brain membranes, was resuspended and used in the enzyme assays. All samples were maintained at 2–4 °C throughout preparation.

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 5′-nucleotidase) were added to the reaction mixture 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 5′-nucleotidase activity) in 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 composed of 2.3% polyvinyl alcohol, 5.7% ammonium molybdate, and 0.08% malachite green was added in order to determine the amount of inorganic phosphate released (Pi) (Chan et al., 1986). After 20 min, quantification of Pi released was done 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 inactivated with trichloroacetic acid were used to correct for any non-enzymatic hydrolysis of substrates. Specific activity was expressed as nanomoles of Pi released per minute per

milligram of protein. Four different experiments were performed and the assays were run in triplicate.

2.5. Determination of acetylcholinesterase activity

Three whole zebrafish brains were pooled and homogenized on ice in 60 volumes (v/w) of Tris-citrate buffer in a motor-driven Teflon-glass homogenizer. The rate of hydrolysis of 0.8 mM acetylthiocholine was determined in a final volume of 2 ml with 100 mM phosphate buffer, pH 7.5, and 1.0 mM DTNB using a previously described method (Ellman et al., 1961). Before the addition of substrate, 10 μg of the protein sample was preincubated with the reaction medium described above for 10 min at 25 °C.

Acetylthiocholine hydrolysis was monitored by the formation of the thiolate dianion of DTNB at 412 nm for 2–3 min at 30-s intervals. Controls without the homogenate preparation were performed in order to determine the non-enzymatic hydrolysis of acetylthiocholine. The linearity of absorbance related to time and protein concentration was previously determined. Acetylcholinesterase activity was expressed as micromoles of thiocholine (SCh) released per hour per milligram of protein. Four different experiments were performed and the assays were run in triplicate.

2.6. Determination of protein concentration

Protein was measured using Coomassie Blue as the color reagent and bovine serum albumin as the standard (Bradford, 1976).

2.7. Semi-quantitative reverse transcription polymerase chain reaction (RT-PCR)

Specific primers used were as follows: *entpd1* (DrNTPDase1F 5′CCCATGGC ACAGGCCGGTTG-3′ and DrNTPDase1R 5′GCAGTCT-CATGCCAGCCGTG-3′); *entpd2_mg* (DrNTPDase2_mgF 5′GG-AAGTGTTTGACTCGCCTTGCACG-3′ and DrNTPDase2_mgR 5′-CAGGACACAAGCCCTTCCGGATC-3′); *entpd2_mq* (DrNTPDase2_mqF 5′- CCAGCGGAT TTAGAGCACGCTG-3′ and DrNTPDase2_mqR 5′-GAAGAACGGCGGCACGCCAC-3′); *entpd2_mv* (DrNTPDase2_mvF 5′ GCTCATTTAGAGGACGCTGCTCGTG-3′ and DrNTPDase2_mvR 5′-GCAACGTTT TCGGCAGGCAGC-3′); *entpd3* (DrNTPDase3F 5′ TACT-TTCTTTGGACAGAGCAACCCTG-3′ and DrNTPDase3R 5′-AAGCATATA GCCCAGGGACCAGG-3′); *5′-nucleotidase* (DrCD73F 5′-ACCTCCGAG-GAGTGTCGC TTTCG-3′ and DrCD73R 5′-CCTTGTTGGGGACCAGCGGT-TC-3′); and *ache* (Forward 5′ CCAAAAGAATAGAGATGCCATGGACG-3′ and Reverse 5′TGTGATGTTAAGCAGACGAGGCAGG-3′). Optimal conditions for RT-PCR using these primers were determined as described previously (Rico et al., 2006; Appelbaum et al., 2007; Rosemberg et al., 2007). The *β-actin* primers (Forward 5′GTCCCT-GTACGCCTCTGGTCG-3′ and Reverse 5′-GCCGGACTCATCGTACTC-CTG-3′) were used according to Chen et al. (2004).

Immediately following *in vivo* treatments with 5 and 10 mg/l lithium chloride (described above), the animals were euthanized by decapitation and their brains were dissected from the cranial skull. For each sample, a pool of five zebrafish brains was used. Total RNA was isolated from zebrafish brain using the TRIzol reagent (Invitrogen) according to manufacturer's instructions. RNA was quantified by spectrophotometry and all samples were adjusted to 160 ng/μl. cDNA was synthesized using the SuperScript III First-Strand™ (Synthesis System for RT-PCR) Invitrogen Kit following supplier's instructions. One microliter of RT reaction mix was used as a template for each PCR reaction. PCR reactions for the *entpd2*, *entpd3*, *5′-nucleotidase*, and *β-actin* genes were performed in a total volume of 20 μl with a final concentration of 0.1 μM primers, 0.2 μM dNTPs, 2 mM $MgCl_2$ and 0.5 U Taq DNA polymerase (Invitrogen). The PCR conditions for NTPDase1 were as above, except that 1.5 mM $MgCl_2$ was used. PCR reactions for acetylcholinesterase were performed in a total volume of 25 μl, with a final concentration of 0.08 μM primers, 0.2 μM dNTPs, 2 mM $MgCl_2$ and 1 U Taq DNA polymerase (Invitrogen). The following conditions were used for the PCR reactions: 1 min at 94 °C, 1 min at the appropriate annealing temperature (*entpd1*, *β-actin* and *5′-nucleotidase*: 54 °C; *entpd2* and *entpd3*: 64 °C; *ache*: 60 °C) and 1 min at 72 °C for 35 cycles. Post-extension at 72 °C was performed for 10 min. For each set of PCR reactions, a negative control was included. PCR products were analyzed on a 1.5% agarose gel containing ethidium bromide and visualized with ultraviolet light. The Low DNA Mass Ladder (Invitrogen) was used as a molecular marker and normalization was performed against the *β-actin* gene for quantification.

2.8. Statistical analysis

Data were analyzed by one-way analysis of variance (ANOVA) and expressed as the mean ± SD of four different experiments ($n = 4$). Post hoc analysis using the Tukey multiple test range was performed, considering $P<0.05$ as significant.

3. Results

We tested the *in vivo* effects of three concentrations of lithium chloride (1, 5, and 10 mg/l, corresponding to 23, 118, and 236 μM, respectively) on ectonucleotidase and acetylcholinesterase activities in zebrafish brain. There were no significant changes in ATP hydrolysis at all lithium chloride concentrations tested (Fig. 1A). However, after 7 days, lithium chloride exposure inhibited ADP hydrolysis at 5 and 10 mg/l (27.6 and 29%, respectively, $P<0.05$) and AMP hydrolysis at 10 mg/l (30%, $P<0.05$) when compared to the control group (Fig. 1B and C). This same treatment decreased acetylcholinesterase activity from zebrafish brain homogenates at 10 mg/l (21.9%; $P<0.05$) (Fig. 1D).

The inhibition of ADP, AMP, and acetylthiocholine hydrolysis by lithium chloride exposure could be a consequence of transcriptional control and/or post-translational regulation. RT-PCR analyses were performed when kinetic alterations were observed. The results demonstrate that the *entpd, 5′-nucleotidase* (Fig. 2A and C), and *ache* *m*RNA transcript levels (Fig. 2B and C) were unchanged after exposure to 5 or 10 mg/l lithium chloride.

To evaluate a possible direct effect of lithium chloride on ectonucleotidase and acetylcholinesterase activities, we have performed *in vitro* assays with lithium chloride concentrations ranging from 1 to 1000 μM. There were no significant changes to NTPDase and 5′-nucleotidase activities in zebrafish brain membranes in the presence of lithium chloride at all concentrations tested (Fig. 3A–C). In addition, acetylcholinesterase activity from zebrafish brain was also unaltered after lithium chloride exposure when compared to the control group (Fig. 3D).

4. Discussion

In the present study, we have shown that lithium chloride can alter *in vivo* ectonucleotidase and acetylcholinesterase activities in zebrafish brain. Lithium treatment inhibited ADP hydrolysis at 5 and 10 mg/l and AMP hydrolysis at 10 mg/l. Changes were not observed in ATP hydrolysis after *in vivo* exposure to lithium chloride. Interestingly, the exposure of zebrafish to 10 mg/l lithium chloride also inhibited acetylcholinesterase activity when compared to control group. Conversely, when directly added to the *in vitro* enzyme assays, it did not induce significant changes on ectonucleotidase and acetylcholinesterase activities. These results could be related to the fact that the *in vitro* experiments evaluate the direct effect of the drug on the enzyme without the influence of outside mechanisms, such as other cell signaling pathways. Indeed, the mechanism of lithium action may be related to the inhibition of inositol monophosphatase, which would affect the function of the phosphatidylinositol cycle (PI cycle)

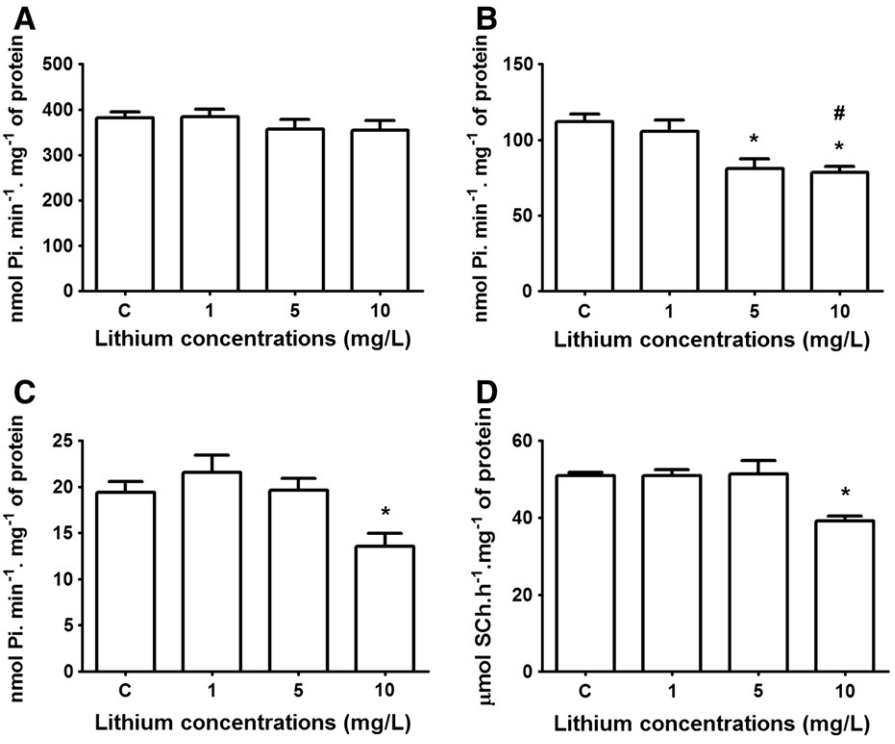

Fig. 1. *In vivo* effect of treatment with 1, 5, and 10 mg/l lithium chloride on NTPDase using ATP (A) or ADP (B) as substrates, 5′-nucleotidase (C) and acetylcholinesterase (D) activities in zebrafish brain. Data represent mean ± SEM of four different experiments ($n = 4$) performed in triplicate. *, difference when compared to the control group; #, difference when compared to the 1 mg/l lithium chloride-treated group. Data were analyzed statistically by one-way ANOVA followed by the post-hoc Tukey test; $P \leq 0.05$ was considered significant.

to cause accumulation of inositol phosphates and depletion of inositol (Shaldubina et al., 2001). Previous studies have shown that lithium decreased free inositol concentrations and increased inositol monophosphate (IP) concentrations in brain (Allison and Stewart, 1971). Therefore, our results indicate that the effect of lithium on ectonucleotidase and acetylcholinesterase activities is not related to a

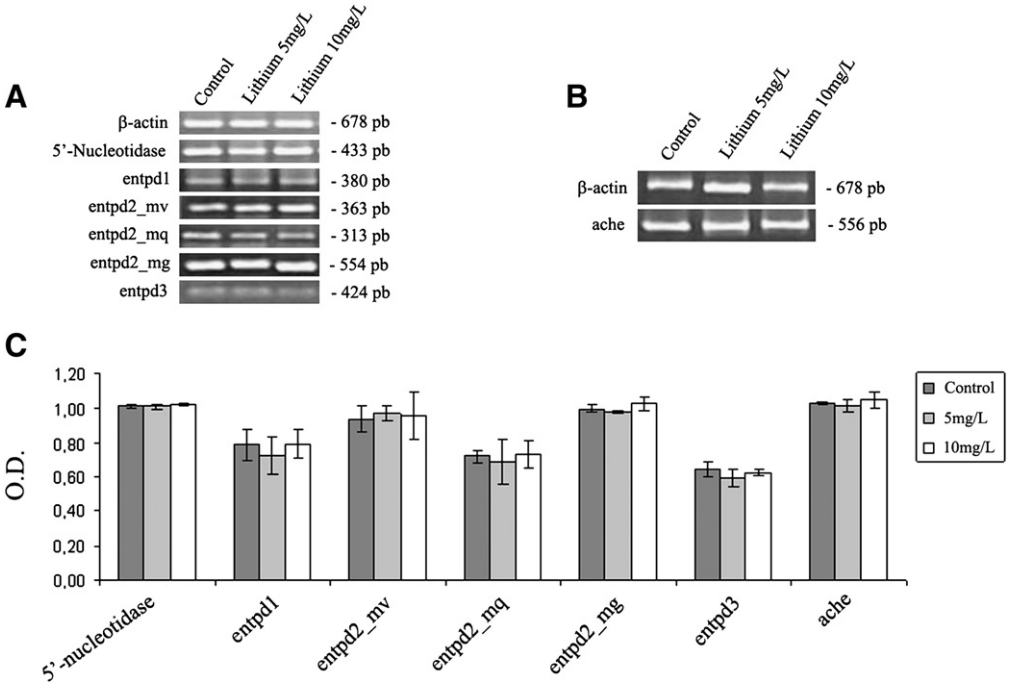

Fig. 2. Effect of treatment with 5 and 10 mg/l lithium chloride on ectonucleotidase and acetylcholinesterase mRNA transcripts. (A) A representative gel of *β-actin*, *entpd1*, *entpd2_mg*, *entpd2_mq*, *entpd2_mv*, *entpd3* and *5′-nucleotidase* mRNA expression in adult zebrafish. (B) A representative gel of acetylcholinesterase (*ache*) and *β-actin* mRNA expression in adult zebrafish brain. (C) Quantification using optical densitometry (O.D.) of the *entpd1*, *entpd2_mg*, *entpd2_mq*, *entpd2_mv*, *entpd3*, *5′-nucleotidase*, and *ache* genes versus *β-actin* (mean ± SD) of three independent experiments. The data were analyzed statistically by one-way ANOVA followed by the post-hoc Tukey test; $P < 0.05$ was considered significant.

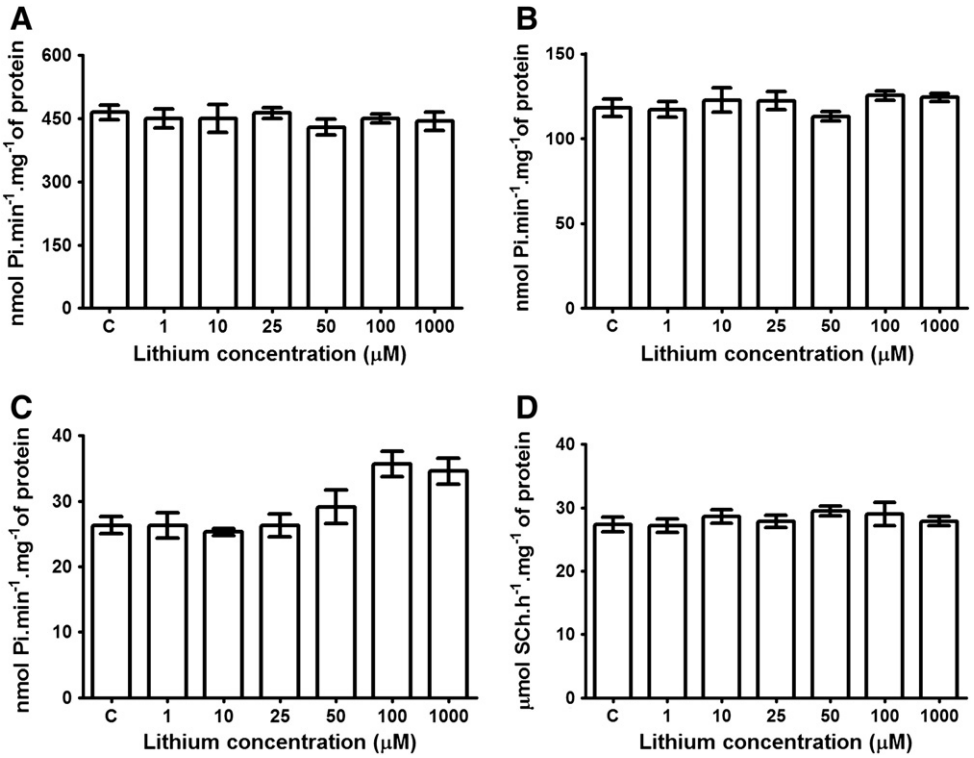

Fig. 3. *In vitro* effect of lithium chloride (1 to 1000 μM) on NTPDase using ATP (A) or ADP (B) as substrates, 5′-nucleotidase (C) and acetylcholinesterase (D) activities in zebrafish brain. Data represent mean ± SEM of four different experiments, each performed in triplicate.

direct action of this metal on the protein, but probably involves a post-transcriptional or post-translational modulation of these enzymatic activities.

The cholinergic system is one of the neurotransmitter systems implicated in the pathophysiologic mechanism of mood disorders (Shytle et al., 2002; Bertrand, 2005; Furey and Drevets, 2006). Acetylcholine is a neurotransmitter involved in essential brain functions, including memory and learning (Shaked et al., 2008). Lithium has been shown to modulate the levels of different neurotransmitters and could therefore improve learning, memory, cognition, and motor functions (Bhalla et al., 2010). Studies show that lithium may selectively interact with the cholinergic system (Williams and Jope, 1995; Bhalla et al., 2007). Lithium has been shown to potentiate seizures induced by pilocarpine, physostigmine, neostigmine and other cholinomimetics in the central nervous system, which may be prevented by either cholinergic antagonists or anticonvulsive drugs (Marinho et al., 1998; Chaudhary and Gupta, 2001). These findings indicate that lithium treatment stimulates cholinergic activity in certain brain regions, which may play a significant role on the therapeutic effect of lithium in neuropsychiatric disorders. Our results are in agreement with previous studies, as we observed a significant decrease in acetylcholine hydrolysis after lithium exposure. This finding reinforces the hypothesis that acetylcholine levels can be increased after lithium treatment, thereby modulating its effects on muscarinic receptors.

The roles of ATP as a neurotransmitter and adenosine as a neuromodulator have been studied extensively in the central and peripheral nervous systems. After ATP is released in the synaptic cleft, it can be hydrolyzed to ADP, AMP, and adenosine by ectonucleotidases, which is an important pathway for adenosine production (Zimmermann, 2006). Previous studies have shown hydrolysis of ATP and AMP was significantly increased in hippocampal synaptosomes of rats chronically treated with lithium, whereas no significant differences were observed in cortical synaptosomes (Wilot et al., 2004). In contrast, lithium did not affect the activity of these enzymes in *in vitro* studies (Barcellos et al., 1998). Acute and chronic lithium chloride exposure altered ATPase activities in several brain regions (McNulty et al., 1978). Studies have shown that chronic dietary lithium treatment appeared to reduce Na^+,K^+-ATPase activity in rat brain (Swann et al., 1980). In other studies, the activity of Na^+,K^+-ATPase is increased in membranes of intact synaptosomes in mouse brain after lithium treatment (Wood et al., 1989). Yildiz et al. (2005) have shown that lithium-induced alterations in nucleoside triphosphate levels in human brain caused a 25% reduction in Pi levels. Although lithium treatment induces controversial effects on ATP-metabolizing enzymes in brain, our findings have shown a significant inhibition of ADP and AMP hydrolysis, suggesting that lithium can exert a modulatory effect on ectonucleotidase activities and, consequently, on adenosine levels. Adenosine affects numerous physiological processes, including platelet aggregation, coronary vasodilation, lipolysis, and neuronal function in brain (Sebastião and Ribeiro, 2009). Studies have demonstrated neuroprotective actions of lithium against various insults in cultured cerebellar granule cells of rats and show that lithium protects against neuronal death caused by phenytoin and carbamazepine (Nonaka et al., 1998; Zhong et al., 2006). Therefore, the effect of lithium on this highly sophisticated pathway of ectonucleotidases may represent a tight control on adenosine levels, which can contribute to the neuroprotective effects of lithium.

Despite the neuroprotective actions described, lithium exposure can also induce toxic effects. Lithium has a profound effect on the development of diverse organisms (Klein and Melton, 1996). Most of the information on lithium toxicity related to aquatic organisms comes from studies on embryonic development (Selderslaghs et al., 2009). The irreversible neurologic lesions caused by lithium, particularly ataxia and dysarthria, are generally in the cerebellum (Kores and Lader, 1997). There is growing evidence that lithium can induce

chronic neurological sequelae. It has been suggested that lithium, cytokines, and neuroleptics synergize to disrupt calcium homeostasis in Purkinje cells and elicit calcium-mediated neurotoxicity (Grignon and Bruguerolle, 1996). Lithium toxicity may be life threatening or result in persistent cognitive and neurological impairment (Waring, 2006). Therefore, further studies evaluating chronic exposure to lithium in different doses will allow the investigation of the susceptibility of cholinergic and purinergic signaling as a target of neurotoxicological effects induced by this compound.

There are several mechanisms by which lithium could regulate acetylcholinesterase and NTPDase activity during *in vivo* experiments, including modifications at the transcriptional level and direct effects on the protein. In order to verify whether *ache*, *entpd* and *5′ nucleotidase* gene expression patterns were modulated when zebrafish were exposed to lithium chloride, we performed semi-quantitative RT-PCR experiments. Our results showed that *ache*, *entpd* and *5′ nucleotidase m*RNA transcript levels were unchanged in the lithium-treated group, suggesting that the change in the enzyme activities observed with lithium exposure was not directly related to changes in expression level.

In summary, our results demonstrate that purinergic and cholinergic systems are affected by lithium chloride exposure due to the inhibitory effect observed on ectonucleotidase and acetylcholinesterase activities in zebrafish brain. These findings may be related to an indirect effect promoted by lithium on NTPDase, 5′-nucleotidase, and acetylcholinesterase, since lithium did not significantly affect enzyme activity *in vitro*. These observations may represent a new mechanism underlying the neuroprotective and therapeutic effects of lithium. Furthermore, these findings contribute to a better understanding of lithium pharmacology and its interaction with purinergic and cholinergic neurotransmission.

Conflict of interest statement

Nothing declared.

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