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# Chronic treatment with lithium increases the ecto-nucleotidase activities in rat hippocampal synatosomes

Leandre Carmem Wilot, Rosane Silva Da Silva, Otemar Júnior Ferreira, Carla Denise Bonan, João José Freitas Sarkis, Elizabete Rocha, Ana Maria Oliveira Battastini*

*Departamento de Bioquímica, Instituto de Ciências Básicas da Saúde, UFRGS, Rua Ramiro Barcellos 2600 – anexo, 90035-003, Porto Alegre, RS, Brasil*

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**Abstract**

Lithium is a mood-stabilizing treatment used in bipolar and other psychiatric disorders. The molecular mechanisms underlying lithium action remain poorly understood. Adenosine is a neuromodulator that possesses anticonvulsant and neuroprotective properties and the ecto-nucleotidase pathway is a metabolic source of the extracellular adenosine. Here we investigated the effect of lithium on the ecto-nucleotidase pathway in synaptosomes from hippocampus and cerebral cortex of adult rats. Male Wistar rats received standard rat chow with lithium chloride (2.5 mg/g of chow) and NaCl (17 mg/g of chow) during 4 weeks. The serum lithium levels were 1.18 ± 0.05 mEq./L. ATP and AMP hydrolysis was significantly increased (20 and 35%, respectively) in hippocampal synaptosomes of rats chronically treated with lithium chloride. No significant differences were observed in the hydrolysis of the three nucleotides by cortical synaptosomes. In conclusion, the modulation of the ecto-nucleotidase pathway may be a new explanation for the potential neuroprotective lithium action in hippocampal lesions.


*Classification codes:* Neurochemistry

*Keywords:* Lithium; Ectonucleotidases; Adenosine; Neuroprotection; Hippocampus; Cerebral cortex

Lithium is one of the most commonly-used drugs employed for the treatment of bipolar disorder and an increasing interest in the neuronal protective effects of this cation has been demonstrated in last few years [2,16,19]. Lithium has been demonstrated to protect the hippocampus against lesions in different ischemia models, suggesting its neuroprotective properties [9,21].

Adenosine is an endogenous neuromodulator that mediates neuroprotection, by decreasing membrane excitability and/or neurotransmitter release, limiting calcium influx, and exerting modulatory effects on glial cells [12,23]. Thus a possible role of adenosine has been suggested in several brain disorders, including epilepsy [24], Parkinson's disease [14], depression [30] and mania [17].

Extracellular adenosine can be derived from the bi-directional transporter system [13] or by the action of an enzymatic chain consisting of the ecto-ATPase and/or ecto-ATP-diphosphohydrolase and the ecto-5′-nucleotidase which hydrolyse ATP to AMP and AMP to adenosine, respectively [31].

Previous studies have shown that alterations in ecto-nucleotidase activities are associated with ischemia [6,26], learning, memory [4] and different models of epilepsy [3,22]. These findings clearly indicate the importance of this enzymatic cascade in controlling extracellular levels of ATP and adenosine in different physiological and pathological conditions. Adenosine produced from catabolism of released adenine nucleotides preferentially activates excitatory $A_{2A}$ receptors [11], which are particularly involved in brain disorders.

The objective of the present study is to investigate the effect of chronic lithium treatment on the ecto-nucleotidase

* Corresponding author. Tel.: +55 51 3316 5554; fax: +55 51 3316 5535.
*E-mail address:* batas@terra.com.br (A.M.O. Battastini).

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pathway in synaptosomes from hippocampus and cerebral cortex of adult rats.

Five adult male Wistar rats (aged 60–90 days; weighing 220–260 g) were housed per cage and kept on a 12-h light:12-h dark cycle (lights on at 7:00 a.m.) at a temperature of 23 ± 1 °C. The animals were divided into two groups: one group received standard rat chow and the other group had lithium chloride (2.5 mg/g of chow) and NaCl (17 mg/g of chow) added to the food. This previously described treatment lasted for 4 weeks [25], and the animals remained healthy and the serum lithium levels at the end of the period were determined by atomic absorption spectrometry. The serum lithium concentration were 1.18 ± 0.05 mEq./L (mean ± S.E.M., $n$ = 18), which is in the range observed in lithium treated patients. Rats were killed by decapitation and brain was isolated. Procedures for the care and use of animals were adopted according to the regulations published by the Brazilian Society for Neurosciences and Behavior (SBNeC).

Synaptosomes from hippocampus and cerebral cortex were isolated and ATP, ADP and AMP hydrolysis assayed as described previously [1]. Released inorganic phosphate was determined according to Chan et al. [7] and protein was measured by the Coomassie Blue method [5] using bovine serum albumin as standard. Differences in nucleotide hydrolysis from hippocampal and cortical synaptosomes between lithium treated and control rats were compared by Student's $t$-test.

As shown in Fig. 1, there was an increase by nearly 20% ($n$ = 12, $P < 0.05$) in ATP and 35% ($n$ = 12, $P < 0.01$) in AMP hydrolysis in synaptosomes from hippocampus of rats treated with lithium chloride when compared to control rats. There was no significant difference in ADP hydrolysis in the same structure between lithium-treated and control groups. Conversely, in synaptosomes from cerebral cortex of rats that received the same treatment, the hydrolysis of the three nucleotides was not significantly different in comparison to the control rats (Fig. 2).

The effects of lithium on neuronal transduction systems have been extensively studied in experimental animals models and many hypotheses have been proposed to explain its therapeutic action [18]. At the moment, to our knowledge, there is no evidence of the involvement of the extracellular catabolism of released ATP to adenosine, through the ecto-nucleotidase pathway, in the therapeutic and neuroprotective functions of lithium.

The extracellular hydrolysis of ATP is an important route for the production of adenosine. The ecto-5′-nucleotidase, which catalyzes the last step of this pathway is inhibited by ATP and ADP and activated by AMP. Thus, the first step catalyzed by ecto-ATPase/ATPdiphosphohydrolase may regulate the [ATP + ADP/AMP] ratio and consequently can regulate adenosine formation [10]. Although the regulation of ecto-ATPase/ATPdiphosphohydrolase activity has not yet been established, there is a number of reports showing that the ecto-5′-nucleotidase gene is transcriptionally regulated through a tissue-specific regulatory mechanism, which

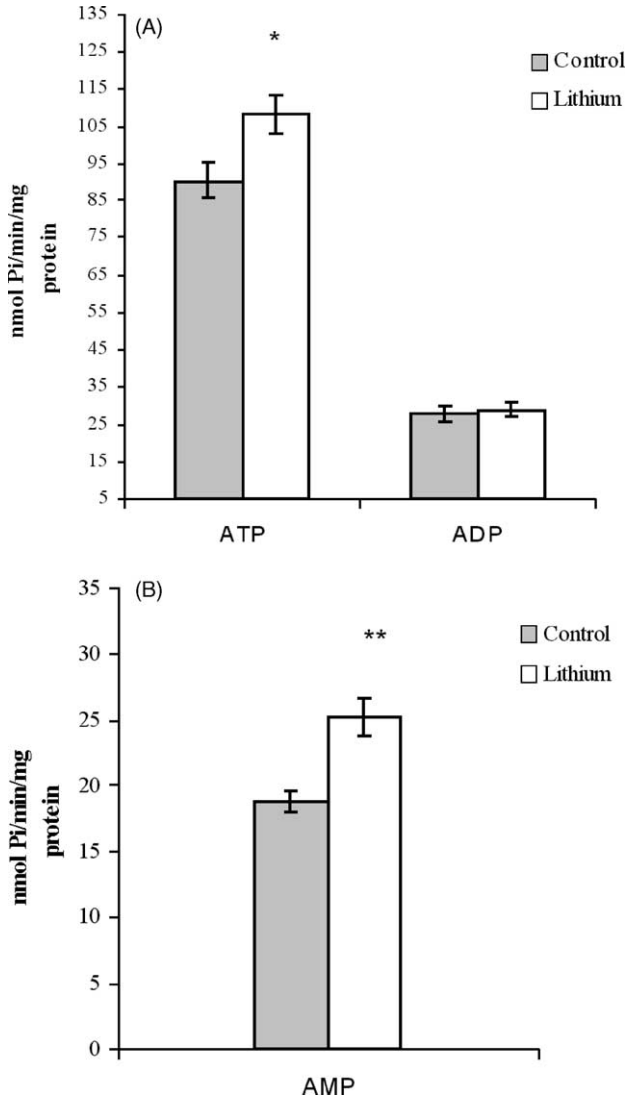


Fig. 1. Effect of chronic treatment with lithium on ATP, ADP (A) and AMP (B) hydrolysis in hippocampal synaptosomes of rats. Bars represent mean ± S.E.M. ($n$ = 12). Significantly different from the respective control group for $^{*}P < 0.05$ and $^{**}P < 0.01$ (Student's $t$-test).

involves cAMP response element binding protein (CREB) [20,28,29]. Considering that chronic treatment with lithium can regulate phosphorylated CREB levels [8,15] it is possible to suggest that the enhancement on ecto-5′-nucleotidase by lithium treatment may be through the control of gene expression.

Lithium is a multifocal drug, which acts on uptake and release of neurotransmitter involving synaptic and intracellular mechanisms [27]. Considering that the enzymes involved in adenosine formation are located in synaptic plasma membranes, it is important to investigate the effect of chronic treatment with lithium on the hydrolysis of nucleotides by synaptosomal preparations.

Another important finding from this study is the specific enhancement of ectonucleotidases in hippocampus comparing with cerebral cortex. Although further studies will be

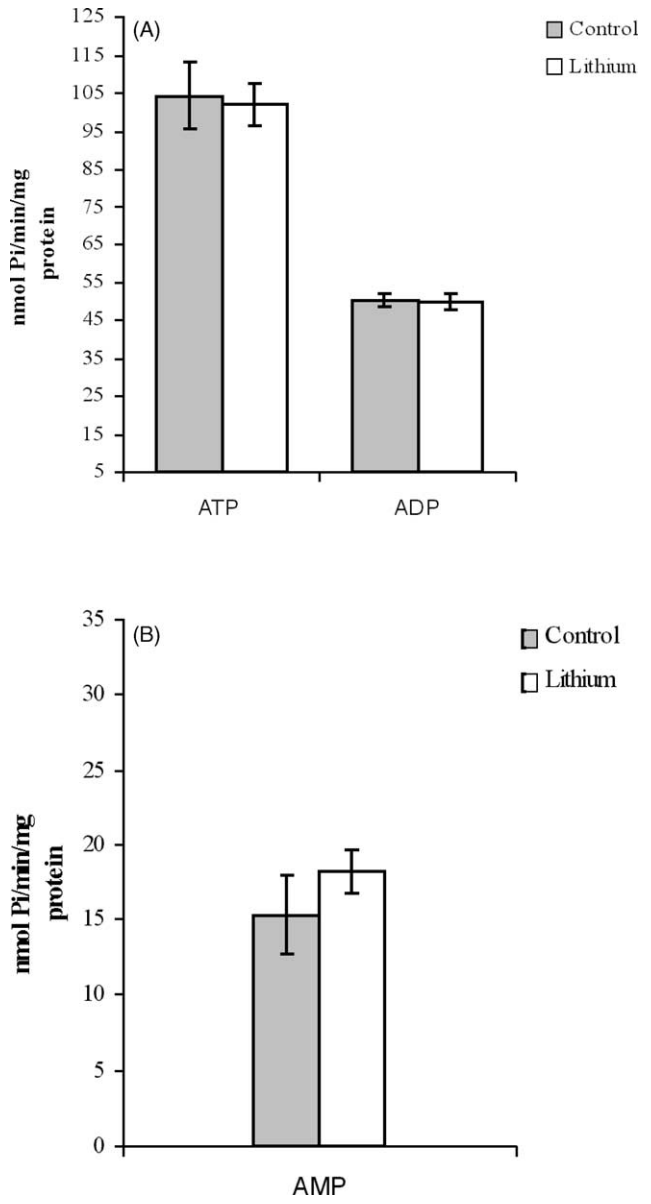


Fig. 2. Effect of chronic treatment with lithium on ATP, ADP (A) and AMP (B) hydrolysis in cortical synaptosomes of rats. Bars represent mean ± S.E.M. ($n$ = 6).

necessary to investigate the effect of lithium in other brain regions, this specificity may reflect a different modulation of the ecto-nucleotidases in hippocampus, which could be a relevant target to the long-term effect of lithium.

In summary, we have shown for the first time, that chronic treatment with lithium promoted an enhancement of ATP and AMP hydrolysis in synaptosomes from the hippocampus of rats. Thus, it seems that chronic lithium treatment can modulate the ecto-nucleotidase pathway in this brain structure, with a consequent decrease in ATP and increase in adenosine levels. These observations may represent a new mechanism underlying the neuroprotective as well as the therapeutic effects of lithium.

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