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Changes in cortical and hippocampal ectonucleotidase activities in mice lacking cellular prion protein

Grace Schenatto Pereira[a], Roger Walz[b,*], Carla Denise Bonan[c], Ana M.O. Battastini[a], Ivan Izquierdo[a], Vilma R. Martins[d], Ricardo R. Brentani[e], João J.F. Sarkis[a]

[a]Departamento de Bioquímica, ICBS, UFRGS, Avenida Ramiro Barcellos 2600, 90035–003, Porto Alegre, RS, Brazil
[b]CIREP, Centro de Cirurgia de Epilepsia, Hospital de Clínicas, Universidade de São Paulo, Campus Universitário, Monte Alegre, 14048–900 Ribeirão Preto, SP, Brazil
[c]Departamento de Ciências Fisiológicas, Faculdade de Biociências, Pontifícia Universidade Católica do Rio Grande do Sul, Avenida Ipiranga, 6681, 90619–900, Porto Alegre, RS, Brazil
[d]Centro de Tratamento e Pesquisa Hospital do Câncer, Rua Prof. Antônio Prudente 109/4 A, 01509–010, São Paulo, SP, Brazil
[e]Ludwig Institute for Câncer Research, São Paulo Branch, Rua Prof. Antônio Prudente 109/4 A, 01509–010, São Paulo, SP, Brazil

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Abstract

Animals lacking cellular prion protein (PrP^c) expression are more susceptible to seizures. Adenosine is an endogenous anticonvulsant agent and it levels in the synaptic cleft are regulated by ectonucleotidases. We evaluated ectonucleotidase activities in synaptosomes from hippocampus and cerebral cortex of adult PrP^c null mice and wild-type mice (genetic background 129/Sv X C57BL/6J). There was an increase (47%) in adenosine triphosphate (ATP) hydrolysis in hippocampal synaptosomes of PrP^c knockout mice as compared with the wild-type animals. In cortical synaptosomes, ATP hydrolysis was similar in both PrP^c mice and controls. However, there was a significant decrease in adenosine diphosphate (ADP) hydrolysis in both hippocampal (−39%) and cortical (−25%) synaptosomes in PrP^c null animals compared to wild-type mice. Changes in brain ectonucleotidases activities related to modifications in the PrP^c expression may contribute, at least in part, to the higher sensitivity to seizures of PrP^c null mice.

Keywords: Prion; Prion protein PrP^c; Ectonucleotidases; Adenosine triphosphate diphosphohidrolase; Apyrase

Pathologic prion protein has long been known as the causative agents of spongiform encephalopathies in animals and humans [15]. It has the same amino acid sequences as its physiologic counterpart, cellular prion protein (PrP^c), whose physiological function is not known [15]. Since PrP^c is located in the outer surface of cells anchored to a phosphatidylinositol glycolipid, it is a candidate for either a signalling function or, less likely, a transport function [1]. Mice devoid of PrP^c develop normally [8] and present normal learning [8,16] and anxiety levels at an adult age, showing only minor abnormalities in locomotor activity [16]. We demonstrated that animals lacking cellular prion protein expression are more susceptible to seizures induced by various convulsant agents, including pentylenetetrazol, pilocarpine and kainic acid [17].

Adenosine is an endogenous nucleoside with anticonvulsant and neuroprotective properties [11]. These involve probably an inhibition of seizure initiation and propagation, an effect which has been attributed to the activation of A1 receptors [4,7]. Extracellular nucleotides are subject to degradation by ectonucleotidases. Breakdown of the nucleotides by these ecto-enzymes, like adenosine triphosphate (ATP) diphosphohydrolase (Apyrase, ATPDase, E.C. 3.6.1.5) and 5′-nucleotidase (EC 3.1.3.5) is a pathway for the complete ATP dephosphorylation to adenosine. [2].

Results from our laboratory have demonstrated that ectonucleotidase activities are altered in physiological and pathological events involving neuroplasticity in animals, such as memory formation [3] and chronic epilepsy [4]. Here we investigate whether ATP, adenosine diphosphate (ADP) and adenosine monophosphate (AMP) hydrolysis in synaptosomes from cortex and hippocampus of mice lacking cellular prion protein.

Three to five month-old male PrP^c null mice ($n = 5$) and wild-type mice ($n = 5$) (genetic background 129/Sv X C57BL/6J) were housed five per cage with water and food

* Corresponding author.

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ad libitum. Animals were kept on a 12 h light/dark cycle (lights on at 07:00 h) at a temperature of 23 ± 1°C. Procedures for the care and use of animals were according to the regulations of the Brazilian Society for Neurosciences and Behaviour.

Synaptosomes from hippocampus and cerebral cortex were isolated [13], and ATP, ADP and AMP hydrolysis assayed as described previously [4]. Released inorganic phosphate was determined according to Chan et al. [9]. Protein was measured by the Coomassie Blue method [5], using bovine serum albumin as standard. Differences in ATP, ADP and AMP hydrolysis from cortical and hippocampal synaptosomes between wild-type and knockout animals were compared by Student's *t*-test. $P < 0.05$ was considered to represent a significant difference in the statistical analysis used.

As shown in Fig. 1, there was a 47% increase in the rate of ATP hydrolysis in hippocampal synaptosomes of PrP^c knockout mice when compared to the wild-type animals ($P < 0.05$). However, in the same structure there was a significant decrease of 39% for the rate of ADP hydrolysis in the PrP^c knockout mice compared with the wild-type animals ($P < 0.05$). In synaptosomes from cerebral cortex, ATP hydrolysis was similar in the cortical synaptosomes of both the PrP^c knockout and wild-type mice (Fig. 2); however, ADP hydrolysis was significantly decreased (25%) in the animals lacking PrP^c in comparison to the wild-type animals ($P < 0.05$) (Fig. 2). AMP hydrolysis showed no significant changes in hippocampal and cortical synaptosomes of PrP^c knockout and wild-type mice (data not shown).

Thus, ATP and ADP hydrolysis by ectonucleotidases in synaptosomes from animals lacking cellular prion protein are different from those of the wild-type group. In hippocampal synaptosomes there is an enhancement of ATP hydrolysis and an impairment of ADP hydrolysis; in cortical synaptosomes, only ADP hydrolysis was altered, showing a decrease in PrP^c null mice.

ATP is a chemical mediator of fast excitatory transmission and its actions occur through P2-purinoceptors. Once ATP reaches the extracellular space, it is converted to adenosine by the conjugated action of ectonucleotidases [3]. Taken to account that AMP hydrolisis was normal, our findings lead us to the hypothesis that a decrease in ADP hydrolysis by an ecto-ATP diphosphohydrolase in the cortex and hippocampus of knockout PrP^c animals, can result in reduced levels of AMP which, by a stoichiometric effect, would be slowly hydrolyzed by 5′-nucleotidase, thus producing a decrease in adenosine levels. Based on the present results, it is tentative to speculate that the decrease in adenosine levels in animals lacking cellular prion protein can contribute, at least in part, to their higher sensitivity to seizures. In fact, the behavioral pattern of seizures observed in those animals characterized by a generalized tonic component of the trunk and four limbs [17] suggests a fast neocortical spread [10], although brainstem influences can not be ruled out [14]

As ADP is a marker substrate of apyrase, one can conclude that this enzyme is affected in the same sense in hippocampus and cortex by the PrP^c gene knockout. The recently cloned ecto-apyrase (ATP diphosphohydrolase, apyrase, ATPDase, EC 3.6.1.5), expressed in primary neurons, presents a wide distribution in cerebral cortex, hippocampus, cerebellum, glial and endotelial cells [18]. The increase of ATP hydrolysis observed in hippocampus but not in the cortex could be due to differential compensa-

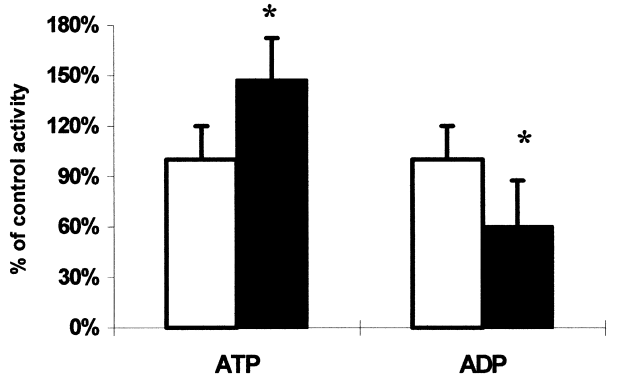

Fig. 1. ATPase and ADPase activities in synaptosomes from hippocampus of knockout (PrP^c 0/0) and wild-type mice. Open bars represent ATP or ADP hydrolysis in wild-type animals. Closed bars represent ATP or ADP hydrolysis in knockout (PrP^c 0/0) animals. The control activities in synaptosomes from hippocampus of wild-type mice were 155.32 ± 31.7 and 51.18 ± 12.6 nmol $Pi.min^{-1}.mg^{-1}$ protein for ATP and ADP hydrolysis, respectively. Bars represent means ± SD of at least five animals. Asterisks indicate significant differences between knockout and wild-type animals ($P < 0.05$, Duncan multiple range test).

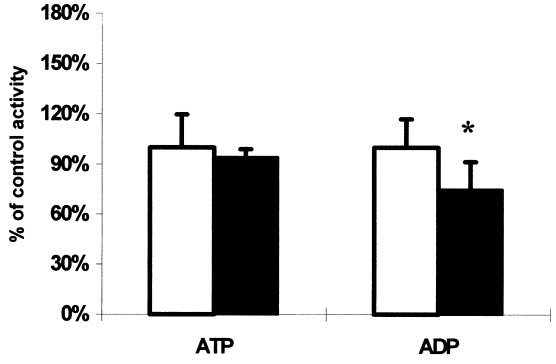

Fig. 2. ATPase and ADPase activities in synaptosomes from cerebral cortex of knockout (PrP^c 0/0) and wild-type mice. Open bars represent ATP or ADP hydrolysis in wild-type animals. Closed bars represent ATP or ADP hydrolysis in knockout (PrP^c 0/0) animals. The control activities in synaptosomes from cerebral cortex of wild-type mice were 220.18 ± 43.2 and 68.89 ± 3.42 nmol $Pi.min^{-1}.mg^{-1}$ protein for ATP and ADP hydrolysis, respectively. Bars represent means (SD of at least five animals. Asterisks show significant differences between knockout and wild-type animals ($P < 0.05$, Duncan multiple range test).

tory mechanisms, including an enhancement in ecto-ATPase activity. This is co-expressed with ecto-apyrase in the rat brain [12].

PrP^c has a direct role in cellular resistance to oxidative stress [6]. Prion protein-deficient neurons reveal lower glutathione reductase activity and increased susceptibility to oxidative stress [19]. Adenosine can suppress epileptic discharges both by blocking free radical toxicity and its modulatory effects in neocortex [20]. The slower rate of ADP hydrolysis and the consequent decrease of adenosine levels, could facilitate the discharges progression in the presence of higher levels of oxidative stress observed in PrP^c null animals.

In summary, the ATP and ADP hydrolysis by ectonucleotidases in synaptosomes of mice lacking PrP^c differ from that in wild-type group. The differences vary according to the brain structure studied. The mechanisms involved in these findings are unknown. Changes in brain ectonucleotidases activities related to modifications in the PrP^c expression could contribute, at least in part, to the higher sensitive to seizure of PrP^c null mice.

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