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ScienceDirect
Neuroscience Letters 413 (2007) 21–24
Neuroscience Letters
www.elsevier.com/locate/neulet

Habituation to an open field alters ecto-nucleotidase activities in rat hippocampal synaptosomes

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Received 25 September 2006; received in revised form 9 November 2006; accepted 12 November 2006

Abstract

ATP and adenosine may play a role in the mechanisms of synaptic plasticity and memory formation. Previous studies have shown that ecto-nucleotidase activities are altered during memory consolidation of an aversive task named step-down inhibitory avoidance. Here we investigate ecto-nucleotidase activities in hippocampal synaptosomes of rats submitted to training and test sessions of habituation to open field, which is one of the most elementary forms of learning. There were no significant alterations on ATP, ADP and AMP hydrolysis immediately after the training session. However, immediately after the test session (0 min), there was a significant increase of ATP hydrolysis (61%), but not of ADP and AMP hydrolysis. Sixty minutes after the test session, a significant increase of NTPDase (75% and 60.5% for ATP and ADP hydrolysis, respectively) and ecto-5′-nucleotidase (40%) activities was observed. This study reveals the involvement of ecto-nucleotidase activities in different learning paradigms during memory processing.
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Keywords: Memory; Habituation; Open field; Retrieval; Ecto-nucleotidases; Adenosine

ATP and adenosine are important signalling molecules in the central nervous system [30,12]. ATP is synthesized, stored and released by the central and peripheral nervous systems upon depolarization [6,29]. Extracellular ATP acts through two subclasses of P2 purinoreceptors, P2X and P2Y [30]. P2X receptors are coupled to ligand-gated Ca^{2+}-permeable channels, whereas the P2Y receptors are linked to a G-protein [11].

The signalling actions induced by extracellular ATP are directly correlated to the activity of ecto-nucleotidases once they trigger enzymatic conversion of ATP to adenosine [39,31]. Ecto-nucleotidases comprise a group of ecto-enzymes involved in the control of nucleotide and nucleoside levels in the synaptic cleft, which includes nucleoside triphosphate diphosphohydrolase (NTPDase) family and ecto-5′-nucleotidase [39]. Four members of the NTPDase family are tightly bound to the plasma membrane via two transmembrane domains, and have a large extracellular region with an active site facing the extracellular side. NTPDase1, 3 and 8 slightly prefer ATP over ADP by a ratio of 1, 3 and 2, respectively. Meanwhile, NTPDase2 prefers triphosphonucleosides [5,31]. Adenosine, a product of ATP catabolism, can exert its neuromodulatory effects through four subtypes of P_1-purinoreceptors named A_1, A_{2A}, A_{2B} and A_3 [10,15,16].

Previous studies from our laboratory have shown that one-trial inhibitory avoidance training is associated with a learning-specific, time-dependent decrease of ecto-nucleotidase activities in hippocampus and entorhinal cortex of rats [7,8]. In addition, studies have observed that ATP and ADP hydrolysis are increased in anterior and posterior cingulate cortex after

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0304-3940/$ – see front matter © 2006 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.neulet.2006.11.023

one-trial inhibitory avoidance training in rats [26]. These findings raise questions about the importance of the ecto-nucleotidase pathway in biochemical events related to the early phase of memory formation. Step-down inhibitory avoidance task involves the specific repression of the natural tendency of rats to explore beyond a safe platform without affecting their exploratory behavior while on the platform [20]. Recently, it has been shown that inhibitory avoidance training is restrained by treatments that inhibit long-term potentiation (LTP) in hippocampal area CA1 [18]. Moreover, inhibitory avoidance task is, indeed, accompanied by LTP in this brain area [37].

Another form of learning is habituation to an open-field, which has been very little studied in terms of pharmacological and biochemical mechanisms of memory consolidation and retrieval, clearly depending on the hippocampus [34,36]. Habituation to a novel environment is believed to be one of the most elementary forms of learning, in which the decreasing exploration, as a function of repeated exposure to the same environment, is taken as an index of memory [33,34]. This is normally studied in two or more brief sessions of exposure to an open field or similar environment [19,21].

Considering the involvement of the ecto-nucleotidase pathway in the formation of aversive memory induced by step-down inhibitory avoidance [7,8], we have evaluated ATP, ADP and AMP hydrolysis in rat hipocampal synaptosomes after habituation to an open-field. The influence of this enzyme pathway in a non-associative task [36], which presents biochemical differences in memory consolidation when compared to an aversive memory, was also discussed.

Male Wistar rats (age 60–90 days; weight 220–280 g) from our breeding colony were housed four to a cage, with water and food *ad libitum*. Animal were kept on a 12 h light/dark cycle (lights on at 07:00 h) at a constant temperature of 23 ± 1 °C. Procedures for the care and use of animals were adopted according to the regulations of Colégio Brasileiro de Experimentação Animal (COBEA), based on the Guide for the Care and Use of Laboratory Animals (National Research Council).

The animals were placed in a 40 cm × 60 cm open field surrounded by 50 cm high walls made of brown plywood with a frontal glass wall. The floor of the open field was divided by black lines into 12 equal rectangles. In the session of training, the animal is gently placed in front of the left posterior corner of the box and left to explore the arena during 5 min. After 24 h, the animals were submitted to a similar open field session, which corresponds to the test session. In both sessions, the rearings and crossings of the black lines were recorded. The decrease in the number of crossings and rearings between the two sessions was taken as a measure of the retention of habituation [33,34,36]. To analyze the effect of training session on the ecto-nucleotidases activities, the animals were submitted to the behavioral procedure described above and were sacrificed immediately (0 min) after the training session. In order to investigate the effect of test sessions on the ecto-nucleotidases activities, the animals were submitted to the training session and 24 h later exposed again to the behavioral apparatus to perform the test session. The animals were sacrificed 0, 60 or 120 min after the test session. All behavioral procedures were conducted at 8:00–11:00 am.

Synaptosomes from hippocampus were isolated [23] and ATP, ADP and AMP hydrolysis were assayed as described previously [4,7,17]. Released inorganic phosphate was determined according to Chan et al. [13]. Protein was measured by the Coomassie Blue method [9] using bovine serum albumin as standard. The data obtained for the enzyme activities are presented as mean ± S.E.M. of a number of animals studied in each condition. The statistical analysis used in these experiments was one-way ANOVA, followed by the Duncan multiple test. The differences between training and test performance were evaluated by Student *t*-test. $P < 0.05$ was considered to represent a significant difference in both statistical analysis used.

In the present study, we have demonstrated the effect of habituation in open field on NTPDases and ecto-5′-nucleotidase activities in rat hippocampal synaptosomes. The number of crossings was 93.3 ± 4.12 for training session and 45.3 ± 4.69 for test session in open field with a 24 h interval between the sessions (mean ± S.E.M.; $n = 18$; $P < 0.05$). Rearings of training and test session performances were 17.2 ± 1.35 and 7.33 ± 0.99, respectively (mean ± S.E.M.; $n = 18$; $P < 0.05$). There was a good evidence for habituation in all animals tested. There were no significant alterations on ATP ($n = 6$), ADP ($n = 6$) and AMP ($n = 6$) hydrolysis immediately after the training session (Figs. 1A, B and 2). However, immediately after test session (0 min), it has been observed a significant increase on ATP hydrolysis (208.5 ± 17.8 nmol Pi min^{-1}. mg $protein^{-1}$, $n = 6$), when compared to control group (129.2 ± 7.1 nmol Pi min^{-1} mg $protein^{-1}$), but none on ADP and AMP hydrolysis ($n = 6$). At 60 min after test session, our results have shown a significant increase of NTPDase activity (227 ± 14.6 and 56.4 ± 7.6 nmol Pi min^{-1} mg $protein^{-1}$ for ATP and ADP hydrolysis, respectively; $n = 6$), when compared to control group (129.2 ± 7.1 and 35.1

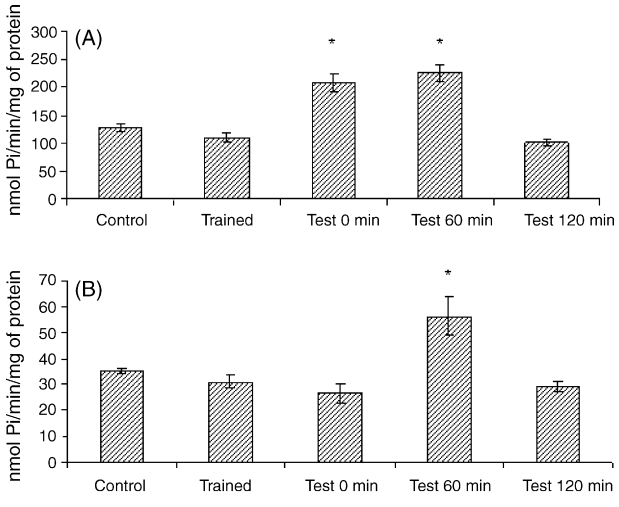

Fig. 1. Effect of training (trained group) and test sessions at 0 min (Test 0 min), 60 min (Test 60 min), 120 min (Test 120 min) in the habituation to an open field on the ATP (A) and ADP (B) hydrolysis in rat hippocampal synaptosomes. Control group represents naive rats. Bars indicate the means ± S.E.M for six different experiments. Data are expressed in specific activity (nmol Pi min^{-1} mg^{-1} protein). (*) Significantly different from the control group ($P < 0.05$; Duncan test).

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