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Role of the ecto-nucleotidases in the cooperative effect of adenosine and neuropeptide-S on locomotor activity in mice

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ABSTRACT

Activation of adenosine receptors modifies the action of classic neurotransmitters (i.e. dopamine, glutamate and acetylcholine) and other neuromodulators, like vasoactive intestinal peptide (VIP), calcitonin gene-related peptide (CGRP) and neuropeptide S (NPS). Similarly to adenosine, NPS is involved in the regulation of stimulus and response to fear and arousal. Thus, the present study investigates the effects of NPS on locomotor activity in mice treated with or without α,β-methylene adenosine 5′-diphosphate (AOPCP), the inhibitor of ecto-5′-nucleotidase. Additionally, we evaluate the activity of ecto-5′-nucleotidase in brain slices of mice treated with or without NPS. Male adult CF-1 mice received i.c.v. NPS as 0.1 nmol injection with or without pre-treatment with 1 nmol α,β-methylene adenosine 5′-diphosphate (AOPCP), the selective inhibitor of ecto-5′-nucleotidase, to evaluate locomotor activity. In another set of experiments, mice received i.c.v. infusion of 0.1 nmol NPS to assay enzymatic activity in brain slices. The results demonstrated that the pre-treatment with AOPCP, which was inactive per se, prevented NPS-induced hyperlocomotion in mice. The dose of 0.1 nmol NPS was efficient to induce hyperlocomotion in animals during the observation period in the activity cage. Regarding enzymatic activity, i.c.v. NPS injection did not induce any significant alterations in ATP and AMP hydrolysis in striatum and hippocampus brain slices of mice. The present study shows that the hyperlocomotor effect of NPS depends on the ecto-5′-nucleotidase activity.

1. Introduction

Neuropeptide S (NPS) is a 20-amino acid peptide recently identified in the brain and peripheral tissues of distinct species of vertebrates. This peptide is the endogenous ligand of a G-protein coupled receptor named NPSR receptor (Xu et al., 2004). In cells expressing the recombinant NPS receptor, NPS increases Ca^{2+} mobilization, intracellular cAMP formation and phosphorylation of extracellular signal regulated-kinase (ERK1/2) (Xu et al., 2004; Reinscheid et al., 2005). NPS receptor (NPSR) is expressed in the medial amygdala, substantia nigra pars compacta, subiculum, dorsal raphe, hypothalamus, thalamus, in the pyramidal cell layer of the ventral hippocampus, and was widely distributed in the cortex (Leonard and Ring, 2011). Higher NPS levels were found in cortex, hypothalamus, amygdala, endopiriform nucleus, subiculum, and nuclei of the thalamic midline, while moderate levels were found in substantia nigra (Xu et al., 2007). Conversely, NPS precursor mRNA is found highly expressed only in a cluster of neurons located between the locus coeruleus and Barrington's nucleus (Xu et al., 2004).

The neuroanatomical expression of NPS and its receptor NPSR supports the role played by this peptidergic system in physiological functions such as anxiety (Xu et al., 2004; Jungling et al., 2008; Leonard et al., 2008; Rizzi et al., 2008; Vitale et al., 2008), arousal (Xu et al., 2004; Rizzi et al., 2008), food intake (Beck et al., 2005; Smith et al., 2006), locomotion (Xu et al., 2004; Roth et al., 2006; Smith et al., 2006; Leonard et al., 2008; Okamura et al., 2008; Rizzi et al., 2008; Castro et al., 2009b), nociception (Li et al., 2009), memory (Han et al., 2009; Jungling et al., 2008) and drug addiction (Cannella et al., 2009,

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Pañeda et al., 2009). Additionally, distinct laboratories around the world have revealed that NPS promotes hyperlocomotion in mice (Xu et al., 2004; Roth et al., 2006; Leonard et al., 2008; Rizzi et al., 2008; Castro et al., 2009a, 2009b; Boeck et al., 2010), and rats (Smith et al., 2006).

Recently, a study demonstrated that the effects evoked by NPS on mouse locomotion are similar to those observed after the administration of stimulating caffeine doses (Rizzi et al., 2008). Also, NPS and caffeine act in the regulation of wakefulness states (Xu et al., 2004; Rizzi et al., 2008), and food intake (Beck et al., 2005). Probably, there is an interaction between the adenosinergic and NPS–NPSR receptor systems, because Lage et al. (2006), using the PCR technique, have shown alterations in expression of mRNA NPS and NPSR receptor in rat hypothalamus and brainstem after acute and repeated caffeine treatments. Very recently, our research group has demonstrated the inhibitory effect induced by caffeine and ZM 241385, a selective adenosine A_{2A} receptor antagonist, on hyperlocomotion induced by NPS in mice (Boeck et al., 2010).

Adenosine, operating via inhibitory adenosine A_1 receptors or excitatory adenosine A_{2A} receptors, has widespread modulatory actions in the nervous system and may interfere with the action of other classic neurotransmitters and neuromodulators (Ribeiro, 1999). Interestingly enough, in a synaptic cleft, adenosine available could have two distinct sources: (1) it may come from the release via bi-directional nucleoside transporter; (2) and/or from adenine nucleotides released, which are degraded by a chain of ecto-nucleotidases (Hoehn and White, 1990; Craig and White, 1993; Cunha et al., 1996). The most relevant ecto-enzymes involved in this chain are those of the ecto-nucleoside triphosphate diphosphohydrolases family (E-NTPDases), which hydrolyze nucleoside tri- and di-phosphates. At least eight different members of the NTPDase family have been discovered, cloned and studied over the last few years. NTPDases1, 2, and 3 are expressed in nervous tissue and mediate the termination of ATP signaling in the synaptic cleft (Zimmermann et al., 1998; Wink et al., 2006). The produced nucleoside monophosphates in the cleft are hydrolyzed by ecto-5′-nucleotidase (E.C. 3.1.3.5) (Fredholm et al., 2005; Robson et al., 2006), that is a pivotal step in extracellular adenosine production from the enzymatic chain (James and Richardson, 1993). It has been suggested that adenosine release leads to preferential A_1 receptor activation, while adenosine formed from the ecto-nucleotidase pathway leads to favored adenosine A_{2A} receptor activation (Cunha et al., 1996; Boeck et al., 2005). In this context, our group recently demonstrated the inhibitory effect induced by caffeine or ZM 241385, a selective A_{2A} receptor antagonist, on hyperlocomotion induced by NPS in mice (Boeck et al., 2010).

Thus, considering that adenosine plays a modulatory effect in the hyperlocomotion evoked by NPS, the present study aimed at investigating whether the involvement of adenosine in the hyperlocomotor effect of NPS is due to its production via ecto-nucleotidases pathway. To test this hypothesis we pre-treated mice with α,β-methylene-adenosine 5′-diphosphate (AOPCP), the specific ecto-5′-nucleotidase inhibitor, before NPS challenge. The present study also investigated whether NPS treatment is able to modify the E-NTPDase and ecto-5′-nucleotidase activities in hippocampal and striatal mouse brain slices.

2. Materials and methods

2.1. Materials

Adenosine 5′-triphosphate (ATP), adenosine 5′-monophosphate (AMP) and α,β-methylene adenosine 5′-diphosphate (AOPCP) were obtained from Sigma (St Louis, MO, USA). Human NPS was synthesized by Dr R. Guerrini, Department of Pharmaceutical Science and Biotechnology Center, University of Ferrara, according to published methods (Roth et al., 2006). NPS was dissolved in saline solution (NaCl 0.9 g%, w/v) and intracerebroventricularly (i.c.v.) administered in mice. AOPCP had its pH adjusted with NaOH 0.1 M and was diluted in saline solution. All other chemicals were of analytical reagent grade and purchased from local suppliers.

2.2. Animals and surgical procedure

Male albino CF-1 mice (2–3 months of age, 30–35 g) were obtained from our breeding colony (UNESC). Six animals were housed per cage with food and water freely available and were maintained on a 12-h light/dark cycle (lights on at 7:00 a.m.). All experimental procedures involving animals were performed in accordance with the National Institute of Heath's Guide for the Care and Use of Laboratory Animals and the recommendations of the Sociedade Brasileira de Ciências em Animais de Laboratório (SBCAL) for animal care, designed to minimize suffering and limit the number of animals used. To avoid circadian variations all experiments were carried out between 8:00 a.m. and 1:00 p.m. This study was approved by the local ethics committee (Comitê de Ética no Uso de Animais da Universidade do Extremo Sul Catarinense, no. 045/2009).

Surgery and i.c.v. infusion techniques were conducted according to Schmidt et al. (2000). Naïve mice were anesthetized with 7% chloral hydrate (w/v, 10 mL/kg body weight, i.p.). In a stereotaxic apparatus, the mouse skull skin was removed and an i.c.v. guide cannula (27-gauge) was unilaterally implanted 1.0 mm posterior to bregma, 1.0 mm to the right of the midline and 1.0 mm above the right lateral brain ventricle. The guide cannula was implanted 1.5 mm ventral to the superior surface of the skull and fixed with jeweler's acrylic cement. In the experiments, performed 48 h after surgery, an i.c.v. infusion was performed using a 30-gauge cannula that was fitted into the implanted guide cannula and connected by a polyethylene tube to a Hamilton microsyringe. The tip of the infusion cannula protruded 1 mm beyond the guide cannula, aiming at the lateral brain ventricle. After experiments, methylene blue (4 μL) was injected through the cannula and animals without dye in the lateral brain ventricle were discarded.

2.3. Treatments

Mice received i.c.v. vehicle injection (saline solution; 5 μL) or AOPCP (1 nmol; 5 μL) just before testing locomotor activity. Five minutes following AOPCP administration, mice received i.c.v. vehicle or NPS injection (0.1 nmol; 1 μL) and their locomotor activity was evaluated for 25 min. Previous studies performed in our laboratory showed that this dose of NPS produces a higher stimulatory effect on locomotion (Castro et al., 2009a; Castro et al., 2009b; Boeck et al., 2010). The dose of AOPCP employed in the present study was chosen based on the previous study in mice (Saute et al., 2006).

In another set of experiments, mice received vehicle or NPS (0.1 nmol) in the lateral ventricle as a constant volume of 1 μL, 5 min before the enzymatic assay.

2.4. Locomotor activity assay

An infrared beam array cage (Insight Equipments, Ribeirão Preto, Brazil) connected to a PC was used to assess locomotor activity in mice. The infrared beam array cage consists of a cubicle made of clear Perspex (48×50 cm) surrounded by 50-cm-high walls. Two blocks facing each other and containing an infrared array recorded horizontal activity, and a similar system assessed vertical activity. Just after i.c.v. vehicle or AOPCP injection non-habituated animals were gently placed on the center of the arena and were individually allowed to explore the apparatus for 5 min. Mice were gently removed from the cage, received an i.c.v. vehicle or NPS injection and were returned to the apparatus to explore it for another 25-min period (totaling 30 min of observation). All behavioral experiments were conducted in a well