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Cholinergic agonist reverses H1-induced memory deficit in mice

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ABSTRACT

This study investigated the effects of bilateral intraamygdalar microinjections of PNU-282987, a nicotinic cholinergic agonist, on anxiety and the reversal of amnesia induced by chlorpheniramine (CPA), an H1 histaminergic antagonist, in mice subjected to the elevated plusmaze (EPM). Two experiments were performed with seventy-nine adult male Swiss mice. The isolated microinjections of PNU-282987 did not produce effects on emotional memory; however, the combined microinjections of PNU-282987 and CPA were able to reverse the deficit in memory induced by CPA (ANOVA, $p < 0.05$). Taken together, these results suggest that intraamygdalar injections of PNU-282987 did not induce effects on anxiety and emotional memory per se; however, concurrent microinjections of PNU-282987 and CPA-reverse amnesia induced-CPA which is suggestive of an interaction between the histaminergic and cholinergic systems in the modulation of emotion memory acquisition in mice.

1. Introduction

Emotions play a crucial role in human behavior since there is a strong motivational component in memorizing an experience that presents an emotional aspect with the propose of instruct the brain how to modulates its behavioral strategy in similar future situations (Strata, 2015). The amygdala has been known to play an important regulatory role in the acquisition of emotionally based learning and memory (Galvis-Alonso et al., 2010; Serafim et al., 2012). Additionally, moderate densities of histaminergic fibers supply the amygdaloid complex (Haas et al., 2008; Watanabe et al., 1984), which is a set of nuclei with functional and anatomical distinction (Ehrlich et al., 2009).

Histamine functions are mediated through the stimulation of four different G-protein receptors subtypes: H1, H2, H3 an H4 (Leurs et al., 2009). High density of postsynaptically located. Histamine H1 receptors are significantly expressed in the amygdala and these receptors are related to cognitive functions since their activation leads to the mobilization of intracellular Ca^{2+} by activating the Gq family of G-proteins (Hill, 1990), regulating intracellular signaling pathways that modulate neuroplasticity (Sadek and Stark, 2016). Studies have suggested the involvement of these receptors in anxiety states (Zarrindast et al., 2005), learning and memory (Serafim et al., 2013). The study by Zarrindast et al. (2005) showed that an H1 antagonist (pyrilamine) could significantly reverse the anxiogenic effect of histamine in rats using the elevated plus-maze test. In relation to participation of H1 receptor in emotional memory, a study showed that H1 receptor antagonist (chlorpheniramine - CPA) induced memory deficit in mice re-exposed to elevated plus-maze testing (Serafim et al., 2013). Furthermore, currently, several groups of researchers have studied H3 histaminergic receptors to better elucidated the role of these receptors in learning and emotional memory (Bahi et al., 2014; Serafim et al., 2013). It has been shown that the H3 receptors also participated in the emotional memory acquisition since H3Rs acts as an autoreceptor inhibiting the synthesis and release of histamine upon its activation; further, these receptors occurs also as heteroreceptors modulating the release other neurotransmitters including acetylcholine (Sadek and Stark, 2016).

Besides, the participation of the histaminergic system in the processes of learning and memory, experimental and clinical evidence has showed to the hypothesis that cerebral acetylcholine plays a crucial role in mnemonic processes since there are a strong correlation between cholinergic transmission and cognitive function improvement (McLean et al., 2016; Kitagawa et al., 2003). The two main classes of cholinergic receptors are recognized by acetylcholine, muscarinic receptors (a G protein coupled receptor) and nicotinic receptors (ligand-

Abbreviations: HA, histamine; HNS, histaminergic neural system; ACh, acetylcholine; EPM, elevated plus maze; CPA, chlorpheniramine maleate; SAL, saline; T1, Trial 1; T2, Trial 2; %OAE, percentage of open arm entries; %OAT, percentage of open arm time; OAE, open arm entries; EAE, enclosed arm entries; OAT, open arm time; EAT, enclosed arm time; TE, total arm entries; CT, central area time; NBM, nucleus *basalis magnocellularis*; BLA, basolateral complex of the amygdala; IA, inhibitory avoidance.

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gated ion channels) (Albuquerque et al., 2009). The most prevalent subtypes of the nicotinic acetylcholine receptors (nAChRs) in the brain are comprised of α4, β2 and α7 subunits (Dani and Bertrand, 2007) and it has been suggested that they play an important role in cognition (Levin et al., 2006; Boccia et al., 2010). A study conducted by Vicens et al. (2016) showed that administration of PNU, an alfa nicotinic cholinergic receptor agonist did not show effect on acquisition of a spatial learning task but a reversal of stress effects on retention in the Morris water maze was observed in mice with susceptibility to Alzheimer's disease. Acute and chronic administration of nicotinic cholinergic agonists into the hippocampus and frontal cortex facilitates learning and memory in rats in the inhibitory avoidance task (Levin and Simon, 1998), while the administration of the nicotinic cholinergic agonist GTS-21 over 5 days facilitates performance related to memory and attention tasks in humans (Kitagawa et al., 2003).

A study in our laboratory (Serafim et al., 2012) demonstrated that microinjection of CPA (0.16 nmol) into the amygdala impairs emotional memory in mice that are re-exposed to the EPM, which suggests that the inhibitory effect of this dose might be caused by the actions of this antagonist at the H1 receptors that are present in the amygdala. This inhibitory effect could induce a decrease in cholinergic activity in this structure and could thus compromise the expression of emotional memory. Based on these results, the present study is the first to investigate whether an agonist of the alpha 7 nicotinic cholinergic receptor (PNU-282987) reverses the emotional memory deficits induced by an H1 receptor antagonist (CPA) in mice that are re-exposed to the EPM.

2. Material and methods

2.1. Animals

Male Swiss mice weighing 25–40 g at testing were used. The mice were housed in groups of 5 per cage (28 × 18 × 11 cm) and maintained under a 12-h light cycle (lights on at 7 a.m.) in a controlled environment at a temperature of 23 ± 1 °C and a relative humidity of 50 ± 5%. Food and drinking water were provided ad libitum. All mice were experimentally naïve at the beginning of the study. The experimental sessions were conducted during the light period of the cycle (9 a.m. – 2 p.m.). All of the tests were performed under illumination nearly 100 lx, as measured on the central platform of the EPM.

All procedures were approved by the Ethics Committee on Animal Experimentation of the Federal University of Sao Carlos (Process #009/13) and were compliant with the US National Institutes of Health Guide for the Care and Use of Laboratory Animals.

2.2. Drugs

Chlorpheniramine maleate salt (Sigma Chemical Co., St. Louis, MO), an H1 receptor antagonist, was dissolved in sterile 0.9% saline solution (SAL). The CPA solution was microinjected at a dose of 0.16 nmol in a volume of 0.1 μl. The dose used was based on a previous study conducted in our laboratory (Serafim et al., 2012).

PNU-282987[*N*-(3R)-1-Azabicyclo[2.2.2]oct-3-yl-4-chlorobenzamide], a selective agonist of the alpha 7 nicotinic cholinergic receptor (nAChRα7; Sigma-Aldrich), was microinjected at dose of 0.1 nmol in a volume of 0.1 μl (Ishida et al., 2011).

2.3. Surgery and microinjection

The mice were implanted with a 7-mm stainless steel guide cannula (25-gauge) under ketamine chlorhydrate and xylazine solution anesthesia (100 mg/kg and 10 mg/kg, respectively, delivered via i.p. injection). The surgical procedure was performed in a stereotaxic frame (Stoelting Co., Illinois, USA). The stereotaxic coordinates (Franklin and Paxinos, 2001) for the amygdala were the following: antero-posterior (AP) = −0.8 mm; lateral (L) = ±3.0 mm; and ventral (V) = −3.5 mm from the skull surface. The guide cannula was fixed to the skull using dental acrylic and jeweler's screws. During implantation, the guide cannula was aimed to terminate 1 mm above the target site. A dummy cannula (33-gauge stainless steel wire) was inserted into each guide cannula immediately after surgery to reduce the incidence of occlusion. Post-operative analgesia was provided via subcutaneous injection of flunixin meglumine (Banamine; Veterinary Therapeutic Guide).

After five days of recovery from the surgery, the experimental solutions were bilaterally microinjected into the amygdala using microinjection units (33-gauge) that extended 1 mm beyond the tip of the guide cannula. Each microinjection unit was attached to a 5-μl Hamilton microsyringe via polyethylene tubing (PE-10), and the administration was controlled by an infusion pump (BI2000, Insight Equipamentos Cientificos Ltda.) programmed to deliver a volume of 0.1 μl over a period of 60 s. The microinjection procedure consisted of gently restraining the animal, removing the dummy cannula, inserting the injection unit, infusing the solution and keeping the injection unit in situ for an additional 60 s.

2.4. Apparatus

The apparatus used for EPM testing was similar to those developed by Lister (1987). The acrylic maze was elevated to a height of 38.5 cm and consisted of four arms, two of which were open (30 × 6 × 0.6 cm) and two of which were enclosed (30 × 6 × 15.5 cm). The arms extended from a common central platform (6 × 6 cm). Classically, the EPM evaluates anxiety because the animals behavior associated with the emotional components during the test, expressed the conflict between the motivation to explore the maze and the natural tendency to avoid open spaces, behavior inherent in rodents (Lister, 1990).

Additionally, this model was also proposed to evaluate memory. Carobrez and Bertoglio (2005) suggested that the use of the EPM has been extended to the understanding of the biological basis of emotional components related to learning and memory. Therefore, anxiety can be inferred by the activity in the open arms during the exposure and emotional learning it can be inferred by reducing the exploration of open arms (open arm entries and time spent in the open arms) during re-exposure (File, 1993; Dal-Col et al., 2003). Galvis-Alonso et al. (2010), suggested that during the test, the animals acquired information about safe and dangerous areas of the maze and the use of re-exposure allows inferring memory acquisition and retention.

2.5. Experimental procedure and behavioral analysis

Experiments 1 and 2 were performed on two consecutive days termed Trial 1 (T1) and Trial 2 (T2). In Experiment 1, the mice received bilateral intra-amygdalar microinjections of SAL or PNU (0.1 nmol) administered before T1 and T2. After 5 min of these procedures, the animals were exposed (T1) and reexposed (T2) to the EPM (Fig. 1a). In Experiment 2, on T1, the mice received bilateral intra-amygdalar combined microinjections of SAL, CPA (0.16 nmol) or PNU (0.1 nmol). After 5 min, the animals were exposed to the EPM. T2 involved only re-exposure to the EPM (Fig. 1b). The procedure for the amygdaloid complex microinjections was performed as described by Baptista et al. (2009) and Barbalho et al. (2009).

Five days after the surgical procedures, the experiment was initiated with the transfer of the mice to the test room, where they were allowed to rest for 1 h before being placed individually in the center of the plus-maze facing an open arm and allowed 5 min of free exploration. The maze was cleaned with ethanol (20%) between tests to avoid possible biases due to odors and/or residues left by the previously tested mice. All sessions were video-recorded using a camera positioned above the maze that was linked to a computer in an adjacent room. The behavioral parameters were defined according to previous studies (Lister, 1987; Rodgers and Johnson, 1995) and included the following: the frequencies