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The role of histamine receptors in the consolidation of object recognition memory

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ABSTRACT

Findings have shown that histamine receptors in the hippocampus modulate the acquisition and extinction of fear motivated learning. In order to determine the role of hippocampal histaminergic receptors on recognition memory, adult male *Wistar* rats with indwelling infusion cannulae stereotaxically placed in the CA1 region of dorsal hippocampus were trained in an object recognition learning task involving exposure to two different stimulus objects in an enclosed environment. In the test session, one of the objects presented during training was replaced by a novel one. Recognition memory retention was assessed 24 h after training by comparing the time spent in exploration (sniffing and touching) of the known object with that of the novel one. When infused in the CA1 region immediately, 30, 120 or 360 min posttraining, the H1-receptor antagonist, pyrilamine, the H2-receptor antagonist, ranitidine, and the H3-receptor agonist, imetit, blocked long-term memory retention in a time dependent manner (30–120 min) without affecting general exploratory behavior, anxiety state or hippocampal function. Our data indicate that histaminergic system modulates consolidation of object recognition memory through H1, H2 and H3 receptors.

1. Introduction

Memory consolidation is the post-training process that makes recently acquired information stable over a period of time after training (McGaugh, 2000). Abundant evidence shows it takes place mainly in the hippocampus (Alberini, 2005; Izquierdo & Medina, 1997; Izquierdo et al., 2006; McGaugh, 2000), although other brain structures and a variety of neurotransmitters play a role depending on the nature of the memory being processed (Alvarez & Banzan, 2008; Ambrogi Lorenzini, Baldi, Buccherelli, Sacchetti, & Tassoni, 1999; Izquierdo et al., 2006; Poldrack & Packard, 2002).

The object recognition paradigm (OR) is a memory test based on the natural propensity of rodents to explore novelty, which confers to the animals the ability to discriminate between novel and familiar entities (De Lima et al., 2005; Ennaceur & Delacour, 1988; Romero-Granados, Fontán-Lozano, Delgado-Garcia, & Carrión, 2010). Recent evidence suggests that it is dependent, at least in great part, on the hippocampus (Broadbent, Gaskin, Squire, & Clark, 2009), in particular on the CA1 region (Clarke, Cammarota, Gruart, Izquierdo, & Delgado-Garcia, 2010). This paradigm is especially suited to test the effects of pharmacological interventions on learning and memory (Dere, Huston, & De Souza Silva, 2007). Novelty induces arousal and has motivational effects that are used as a reinforcement to induce learning (Bevins & Besheer, 2005). Multiple brain structures, as well as multiple neurotransmitter/neuromodulators like histamine are involved in the induction and regulation of arousal and its behavioral consequences (Miller & O'Callaghan, 2006; Passani & Blandina, 2011; Passani, Giannoni, Bucherelli, Baldi, & Blandina, 2007). A difficulty to recognize familiar items or to discriminate them from novel ones is one of the early symptoms of cognitive decline in Alzheimer's disease (AD; Budson, Dodson, Daffner, & Schacter, 2005; Dudas, Clague, Thompson, Graham, & Hodges, 2005). Agents capable of increasing brain concentrations of acetylcholine (ACh), like acetylcholinesterase inhibitors are well-known therapeutic tools to improve cognitive deficits associated with neurodegenerative disorders (Esbenshade et al., 2008). Recent pre-clinical studies have demonstrated that ACh can be regulated by the activation of H3 receptor in brain structures associated with cognition, suggesting histaminergic system like a target for the treatment of cognitive deficits (Bacciottini, Passani, Mannaioni, & Blandina, 2001; Brioni, Esbenshade, Garrison, Bitner, & Cowart, 2011; Esbenshade et al., 2008; Giannoni et al., 2010; Komater et al., 2005; Medhurst, Atkins et al., 2007; Medhurst, Briggs et al., 2007).

Histamine is synthesized by neurons in the tuberomammillary nucleus (TMN) whose axons ramify throughout the brain. Of the

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four histamine receptors identified so far only H1, H2 and H3 subtypes are expressed in the brain. The H4 subtype occurs mainly in peripheral tissues. H1 and H2-receptors potentiate excitatory inputs while H3-receptors down-regulate histamine synthesis and release as well as the releases of other neurotransmitters (Brown, Stevens, & Haas, 2001; Hass, Sergeeva, & SelbACh, 2008; Passani & Blandina, 2011; Pillot et al., 2002). Histamine controls the sleep–wake cycle, eating behavior, nociception and modulates the activity of the hippocampus (Hass et al., 2008; Passani et al., 2007). It has been recently shown to play a role in fear memory consolidation (Bonini et al., 2011; Da Silva, Bonini, Bevilaqua, & Izquierdo, 2006; Benetti, Baldi, Bucherelli, Blandina, & Passani, 2012b; Benetti, da Silveira, da Silva, Cammarota, & Izquierdo, 2012a) and to strongly modulate the extinction of two different aversive tasks through action in the hippocampus, amygdala and ventromedial prefrontal cortex (Fiorenza, Rosa, Izquierdo, & Myskiw, 2012).

Although it is well known that sedative antihistaminic compounds induce cognitive decline in human through blockade of H1-receptor (Yanai et al., 1995), both facilitatory and inhibitory effects of neuronal histamine on learning and memory have been described in animal behavior studies. De Almeida and Izquierdo (1986) found that intracerebroventricular (i.c.v.) administration of histamine facilitates the retention of step-down inhibitory avoidance behavior. However, Huston, Wagner, and Hasenöhrl (1997) reported that bilateral lesion of the TMN improves performance in several learning and memory paradigms.

Here we investigated the role of the histaminergic system on consolidation of OR memory, a well-established learning paradigm for studying declarative memory in rats, analyzing the possible interplay between the different subtypes of histaminergic receptors.

2. Materials and methods

2.1. Animals, surgery and drugs infusion procedure

Male *Wistar* rats (3-month-old, 300–330 g) purchased from our regular provider, the Fundação Estadual de Produção e Pesquisa em Saúde do Rio Grande do Sul, Porto Alegre were used. The animals were housed 4 to a cage and kept with free access to food and water under a 12/12-light/dark cycle, with lights on at 7:00 AM. The temperature of the animal's room was maintained at 22–24 °C. Indwelling stainless steel 27-g guide cannulae were implanted under deep anesthesia (75 mg/kg ketamine [König, Sao Paulo] plus 10 mg/kg/xylazine [Coopers, Sao Paulo]). The cannulae were aimed 1.0 mm above the CA1 region of the dorsal hippocampus; at the time of infusion a tight fitting 30-g inner probe was introduced into the guides and protruded 1 mm, to reach the coordinates AP −4.2, LL ±3.0, VD −2.0 from the atlas of Paxinos and Watson (1986). Animals were allowed to recover from surgery for 4 days before submitting them to any other procedure.

Infusions (1 μl/side) were carried out over 60 s and the cannulae were left in place for 60 additional seconds to minimize backflow. The placement of the cannulae was verified postmortem: 2–4 h after the last behavioral test, by infusing 1 μl/side of a 4% methylene blue solution as described above and the extension of the dye 30 min thereafter was taken as an indication of the presumable diffusion of the vehicle or drug previously given to each animal (Clarke et al., 2010; Fiorenza et al., 2012). Only data from animals; with correct cannulae implants (i.e., within 1 mm of the intended site) were analyzed. All experimental procedures followed the guidelines of the USA National Institutes of Health Guide for the Care and Use of Laboratory Animals (DHEW Publications, NIH 80-23) and were approved by the Animal Care and Use Committees of the Pontifical Catholic University of Rio Grande do Sul. Every effort was made to reduce the number of animals used and to minimize their suffering.

2.2. Drugs

Drugs were purchased from Sigma–Aldrich (St. Louis, MO, USA; pyrilamine, pyridylethylamine, ranitidine and imetit); or Tocris Cookson Ltd. (Avonmouth, Bristol, UK; dimaprit and thioperamide). They were dissolved in saline and stored at −20 °C. Before use, aliquots were diluted to working concentration with vehicle (saline). The doses used were based on pilot experiments and previous work in which their effect and efficacy was established (Benetti & Izquierdo, 2013; Bonini et al., 2011; Da Silva et al., 2006; Fiorenza et al., 2012).

2.3. Behavioral procedures

2.3.1. Object recognition paradigm

The object recognition task was based on the original description by Ennaceur and Delacour (1988) (see also Clarke et al., 2010; De Lima et al., 2005). It was conducted in an open field arena (60 × 40 × 50 cm) built of polyvinyl chloride plastic, plywood and transparent acrylic as described by Myskiw et al. (2008) and Furini et al. (2010). Before training, animals were habituated to the experimental arena by allowing them to freely explore it during 20 min per day for 4 days in the absence of any other behaviorally relevant stimulus. The stimulus objects were made of metal, glass or glazed ceramic. Glued to the base of each object was a round piece of velcro, which was used to fix the object to the arena's floor. The role (familiar or novel) as well as the relative position of the two stimulus objects were counterbalanced and randomly permuted for each experimental animal. The open field arena and the stimulus objects were cleaned thoroughly between trials to ensure the absence of olfactory cues. Exploration was defined as sniffing or touching the stimulus object with the nose and/or forepaws. Sitting on or turning around the objects was not considered exploratory behavior. A video camera was positioned over the arena and the rats' behavior was recorded using a video tracking and analysis system for later evaluation. The experiments were performed by an observer blind to the treatment condition of the animals. For training, rats were placed in the open field containing two different objects and left to freely explore them for 5 min. The test session was performed 24 h after training. In the test sessions one of the familiar objects was randomly replaced by a novel one, and rats were reintroduced into the open field for five additional minutes. The compounds to be tested were bilaterally infused into the CA1 region of the dorsal hippocampus (1 μl/side) immediately, 30, 120 or 360 min after training.

2.3.2. Open field and plus maze

To analyze exploratory and locomotor activities, the animals were placed on the left rear quadrant of a 60 × 40 × 50 cm open field with white plywood walls and a floor divided into 12 equal squares. The number of line crossings and the number of rearings were measured over 5 min and taken as an indicator of locomotor and exploratory activities, respectively. To evaluate anxiety state, rats were exposed to an elevated plus maze as detailed in Pellow, Chopin, File, and Briley (1985). The total number of entries into the four arms, the number of entries and the time spent into the open arms were recorded over a 5 min session. In the open field and plus maze paradigms the animals received a bilaterally infusion of drugs into the CA1 region of dorsal hippocampus (1 μl/side) 24 h before tests.