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Research article

Cerebellar vermis H_2 receptors mediate fear memory consolidation in mice

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HIGHLIGHTS

- The H_2 receptor agonist dimaprit facilitates memory retention of inhibitory avoidance.
- The H_2 receptor antagonist ranitidine inhibits inhibitory avoidance consolidation.
- Cerebellar vermis H_2 receptors mediate fear memory consolidation in mice.

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ABSTRACT

Histaminergic fibers are present in the molecular and granular layers of the cerebellum and have a high density in the vermis and flocullus. Evidence supports that the cerebellar histaminergic system is involved in memory consolidation. Our recent study showed that histamine injections facilitate the retention of an inhibitory avoidance task, which was abolished by pretreatment with an H_2 receptor antagonist. In the present study, we investigated the effects of intracerebellar post training injections of H_1 and H_2 receptor antagonists as well as the selective H_2 receptor agonist on fear memory consolidation. The cerebellar vermi of male mice were implanted with guide cannulae, and after three days of recovery, the inhibitory avoidance test was performed. Immediately after a training session, animals received a microinjection of the following histaminergic drugs: experiment 1, saline or chlorpheniramine (0.016, 0.052 or 0.16 nmol); experiment 2, saline or ranitidine (0.57, 2.85 or 5.07 nmol); and experiment 3, saline or dimaprit (1, 2 or 4 nmol).

Twenty-four hours later, a retention test was performed. The data were analyzed using one-way analysis of variance (ANOVA) and Duncan's tests. Animals microinjected with chlorpheniramine did not show any behavioral effects at the doses that we used. Intra-cerebellar injection of the H_2 receptor antagonist ranitidine inhibited, while the selective H_2 receptor agonist dimaprit facilitated, memory consolidation, suggesting that H_2 receptors mediate memory consolidation in the inhibitory avoidance task in mice.

1. Introduction

Histamine is a biogenic amine and an important neurotransmitter–neuromodulator in the central nervous system [1,2]. Histaminergic neurons are located in the tuberomammillary nucleus of the posterior hypothalamus, and their efferent fibers project to nearly the entire brain [3]. The following four histamine receptors have been identified: H_1–H_4 subtypes in the brain [4,1,2]. The H_1 and H_2 receptors potentiate excitatory inputs, while H_3 receptors down-regulate histamine synthesis and release histamine as well as other neurotransmitters [2]; also, the H_4 receptors have recently been reported within the neurons of the central nervous system, and they have a poorly understood role [4].

The neural histaminergic system has been involved in several physiological functions or responses, including arousal, body temperature, sleep-wake cycle, and cognition [1,5,6]. Additionally, there is consistent evidence that neuronal histamine plays an important role in learning and memory processes [1,2,7], but the actual contribution of the histaminergic system to these processes is still controversial.

Abbreviations: CPA, chlorpheniramine; SAL, saline; RA, ranitidine; DM, dimaprit; (LTP), long term potentiation.

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The cerebellum has traditionally been implicated in controlling movement. However, the cerebellum's role in non-motor functions, including cognitive and emotional processes, has received increasing attention [8,9,10]. Recent results from studying how Purkinje cells encode movement signals suggest that the cerebellar cortex circuitry can support associative learning, sequencing, working memory, and forward internal models in non-motor domains [8]. Strata et al. [11] suggest that the cerebellum is involved in the regulation of affective reactions as well as in forming the association between sensory stimuli and their emotional values. Some studies that image patients with cerebellar lesions have been used to elucidate the role of the cerebellum in processing emotion [12,13,10]. Reports using functional magnetic resonance have shown that cerebellar areas around the vermis are activated during associative learning and mental recall of emotional personal episodes in humans [14,13]. In both human and animal models, lesions of the cerebellar vermis may affect the retention of a fear memory without altering the baseline motor/autonomic responses to the frightening stimuli [12,9]. Other findings indicate that the cerebellar vermis is involved in long-term memory formation in certain types of defense behavior after training [9,15].

Studies have demonstrated a relationship between the histaminergic system and cerebellum [16,17,18]. Histamine-immunoreactive fibers are seen in the molecular and granular layers of the cerebellum in several species, including humans, and they have a high density in the vermis and floculus [2,3,17]. Autoradiographic mapping and in situ hybridization experiments have demonstrated the presence of H_1, H_2 and H_3 receptors in the rat cerebellar cortex and deep cerebellar nuclei [19,20], and they suggest that histamine may play an important role in modulating the excitability of cerebellar neurons. We recently demonstrated that the cerebellar histaminergic system is involved in emotional memory consolidation [16,21]. We recently found that histamine injections facilitated the retention of an inhibitory avoidance task, which was abolished by pretreatment with an H_2 receptor antagonist [21].

Therefore, in the present study, we investigated the effects of intra cerebellar post-training injections of H_1 and H_2 receptor antagonists as well as selective H_2 receptors agonist on fear memory consolidation.

2. Materials and methods

2.1. Animals

Male Swiss mice (Federal University of Sao Carlos, UFSCar SP, Brazil), weighing 25–35 g, were maintained under a 12 h light cycle (lights on at 7:00 a.m.) in a controlled environment at a temperature of 23 ± 1 °C and humidity of 50 ± 5%. All mice were experimentally naive, and the experimental sessions were conducted during the light period of the cycle (9:00–15:00 h).

2.2. Drugs

The H_1 receptor antagonist chlorpheniramine maleat salt, H_2 receptor antagonist ranitidine hydrochloride and the selective H_2 receptor agonist dimaprit (Sigma Chemical Co., USA) were prepared in a vehicle of physiological saline. Saline solution was used as an experimental control. The doses were based on previous research [16,22,21] and on pilot work in our own laboratory.

2.3. Surgery and microinjection

Mice were intraperitoneally anesthetized using ketamine hydrochloride (100 mg/kg) and xylazine (10 mg/kg) solution, administered local anesthesia (3% lidocaine with norepinephrine 1:50,000), and placed in a Stoelting stereotaxic instrument. A single 7-mm stainless steel guide cannula (25 gauge) was implanted in the cerebellar vermis according to the following coordinates from the mouse brain atlas [23]: 6.5 mm posterior to the bregma, 0 mm lateral to the midline, and 2.0 mm ventral to the skull surface. The guide cannula was fixed to the skull using dental acrylic and jeweler's screws. A dummy cannula (33 gauge stainless steel wire) was inserted into the guide cannula to reduce the incidence of occlusion. Postoperative analgesia was provided for 3 days by adding acetaminophen (200 mg/ml) to the drinking water at a ratio of 0.2 ml of acetaminophen to 250 ml of water (the final concentration was 0.16 mg/ml).

Saline and drug solutions were infused into the cerebellar vermis using a microinjection unit (33 gauge cannula), which extended 2.0 mm beyond the tip of the guide cannula. The microinjection unit was attached to a 5-μl Hamilton microsyringe via polyethylene tubing (PE-10), and an infusion pump that was programmed to deliver a volume of 0.1 μl over a period of 60 s controlled the administration [16].

2.4. Inhibitory avoidance task

Rodents have an aversion to brightly illuminated areas, and they have a preference for dark compartments, which represent secure areas [24]. In the inhibitory avoidance task, during the acquisition trial, an instinctive response is punished by a foot shock in the dark compartment, and in the retention trial, the animal is returned to the area, and avoidance of the punished context is observed.

The apparatus consisted of an acrylic box ($48 \times 24.5 \times 25$ cm) with two compartments that have the same size, one light (under illumination 400 l×) and one dark (with black acrylic), separated by a guillotine door (9×10 cm). The floor was made of stainless-steel rods (2.5 mm in diameter), spaced 1 cm apart, that delivered electric shocks at an intensity of 0.5 mA for 3 s. The box was connected to a computer with software (Insight Equipamentos Científicos Ltd., Brazil) that triggered the apparatus, and a camera recorded the experiment.

Animals were placed in the apparatus after 1 h of habituation in the experimental room. Each animal was gently placed in the light compartment for 5 s, after which the guillotine door was lifted, and the latency of the animal crossing to the dark (shock) compartment was timed. Animals that waited for more than 100 s to cross to the other side were excluded ($n = 3$). Once the animal crossed to the next compartment with all four paws, the door was closed and the mouse was moved to its home cage. The habituation trial was repeated after 30 min, and it was followed, after the same interval, by the acquisition trial, during which the guillotine door was closed and a foot shock (0.5 mA, 3 s) was delivered immediately after the animal entered the dark compartment. After 20 s, the mouse was removed from the apparatus and placed temporarily in the home cage. Two minutes later, the animal was retested in the same way as before; if the mouse did not enter the dark compartment during the 120-s period, a successful acquisition of inhibitory avoidance response was recorded. Otherwise, when the mouse entered the dark compartment a second time, the door was closed and the mouse received the same shock as before. After retesting, if the animal successfully achieved inhibitory avoidance, it was removed from the apparatus and injected via the guide cannula as follows: experiment 1, saline (SAL) or chlorpheniramine (CPA 0.016, 0.052 or 0.16 nmol); experiment 2, SAL or ranitidine (RA 0.57, 2.85 or 5.07 nmol); and experiment 3, SAL or dimaprit (DM 1, 2 or 4 nmol). If not, the animal was excluded ($n = 2$).

Twenty-four hours after training, a retention test was performed to determine memory consolidation. Each animal was placed in the light compartment for 5 s, the door was opened, and the latency was measured for entering the dark compartment. The test session