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Involvement of histamine receptors in the acquisition of inhibitory avoidance in *Carassius auratus*

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Abstract

This study investigated the involvement of H_1 and H_2 histaminegic receptors on the acquisition of a new task in *Carassius auratus* by using an inhibitory avoidance paradigm in which the animals had to learn to avoid an aversive stimulus. Before training, the fish received injections of H_2 antagonist zolantidine at a dose of 20 mg/kg, or H_1 antagonist chlorpheniramine at a dose of 4 or 16 mg/kg. Control animals were injected with distilled water. A facilitatory effect of chlorpheniramine was observed at the dose of 16 mg/kg. On the other hand, the administration of 20 mg/kg of zolantidine inhibited acquisition. Place preference conditioning was used to observe the aversive or reinforcing effects of the drugs, which could interfere with the inhibitory avoidance procedure; however, no effects were observed. Thus, it can be suggested that both receptors, H_1 and H_2, are involved in the acquisition of a new task in this species.

Keywords: Acquisition; *Carassius auratus*; Chlorpheniramine; Inhibitory avoidance; Zolantidine

1. Introduction

The tuberomammillary nucleus (TM) in the posterior hypothalamus is the main source of neuronal histamine in the brain, from where fibers are projected to practically all brain areas (Panula et al., 1984; Watanabe et al., 1984). Histamine (HA) acts centrally on 3 types of receptors, H_1, H_2 and H_3 receptors (Leurs et al., 1995). Many studies have shown the involvement of HA in different physiological and cognitive functions, including learning and memory (for a review see, Brown et al., 2001).

Some studies point to a facilitatory effect of HA on learning and memory. An in-vitro study showed a strong increase in excitability, facilitation of *N*-methyl-D-aspartate receptors and long term potentiation after bathing hippocampus slices in HA, suggesting a memory-facilitating role for HA (Haas et al., 1995). Intracerebroventricular injection of HA improved learning in rats (De Almeida and Izquierdo, 1986). H_3 antagonist thioperamide, which increases HA release, improved learning in senescence-accelerated rats (Meguro et al., 1995). Administration of histamine-synthesis blocker α-fluoromethilhistidine suppressed avoidance learning in rats (Kamei et al., 1993) and histidine, a precursor of HA reduced the learning deficit caused by scopolamine (Chen and Kamei, 2000; Miyazaki et al., 1995).

However, studies suggesting an inhibitory role of HA in learning and memory are also cumulating. Facilitation of retention was observed in adult and aged rats with bilateral TM lesion in a variety of learning tests, including aversively motivated tests, a discrimination task and a habituation paradigm (Huston et al., 1997). The administration of H_3 agonist (*R*)-alpha-methylhistamine had a memory enhancing action in the recall of spatial information (Smith et al., 1994).

The reason for these discrepancies may be elucidated by the knowledge of the distinct and opposing modulating actions that histaminergic TM neurons might take by activating different receptor subtypes in the different systems involved in learning processes (Passani et al., 2000).

There is evidence of the involvement of histaminergic H_1 and H_2 receptors in memory consolidation. Chlorpheniramine (CPA), an H_1 receptor antagonist, improved reversal learning

Abbreviations: CPA, chlorpheniramine; HA, histamine; TM, tuberomammillary nucleus; ZOL, zolantidine.

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and memory in *Carassius auratus* (Spieler et al., 1999). CPA also facilitated memory retention of inhibitory avoidance in the same species (Medalha et al., 2000). Administration of CPA rather than H_2-receptor antagonist ranitidine presented reinforcing effects and improved learning in the nucleus basalis magnocellularis and nucleus accumbens, tuberomammillary projection areas known to be crucial for learning and memory (Huston et al., 1997). The injection of H_2 antagonist cimetidine impaired retention in rats (Flood et al., 1998).

Regardless many studies have reported the role of these receptors on memory consolidation, there is not much information about their involvement in acquisition. So, the aim of this study was investigate the involvement of H_1 and H_2 receptors in acquisition, using an inhibitory avoidance paradigm for *C. auratus* (goldfish), since this experimental model had not yet been used to this date in order to observe the effects of histaminergic drugs on acquisition in this species.

2. Methods

2.1. Animals

One hundred and twenty-one experimentally naive goldfish of unknown sex, weighing between 2.0 and 6.0 g were used (67 for the Inhibitory avoidance procedure and 54 for the Place preference conditioning procedure). A 2-week acclimatization interval was allowed from the purchasing of the fish to the beginning of the experiment.

One week prior to the experiment, the animals were placed in 30-l aquariums (15 animals per aquarium), at 18–22 °C with constant filtering and aeration, and fed five times a week with flake food (Wardly Corporation, NJ, USA). The animals were identified by their characteristics, such as color and tail type.

2.2. Drugs

The H_1 receptor antagonist, chlorphemiramine maleat salt (Sigma, USA) was dissolved in distilled water to the concentrations of 2 and 8 mg/ml. The H_2 receptor antagonist, zolantidine (ZOL) maleat salt (Sigma, USA) was dissolved in distilled water to the concentration of 10 mg/ml. The drugs were administered intraperitoneally (i.p.) at a volume of 2 ml/kg of body weight, so that the final doses used were ZOL 20 mg/kg and CPA 4 and 16 mg/kg of body weight. These doses were chosen based on earlier experiments (Faganello et al., 2003; Mattioli et al., 1998; Miyazaki et al., 1997).

Distilled water was used as control. Both Drugs and vehicle were placed in coded eppendorf tubes under refrigeration. This coding was unknown to the experimenter at the moment of the tests.

2.3. Inhibitory avoidance procedure

A rectangular aquarium (30 cm long, 15 cm high and 15 cm wide) was used, divided by a sliding door into two equal compartments, one black and one white. On the black side of the aquarium was a pulley system from where a weight of 45 g could be dropped. All experiments were carried out with only one animal at a time.

The experiment was performed on two consecutive days (habituation and training), allowing the fish to habituate to the aquarium 1 day before the training. The animal was placed in the white compartment and after 30 s the sliding door was opened and the fish had 10 min to explore the aquarium.

The fish received the injection 20 min before the training (vehicle group: $n=14$, CPA4: $n=18$, CPA16: $n=17$, ZOL: $n=18$). For the training, the animal was placed on the white side of the aquarium. After 30 s, the sliding door was opened and the fish was allowed 5 min to move to the black compartment. Animals that did not do so in 5 min were excluded from the experiment, since they would not experience the aversive stimulus. Two animals were excluded from the vehicle group, two from the CPA 4 mg/kg, six from the CPA 16 mg/kg and three from the group treated with ZOL.

Once the fish had entered the black compartment, a 45-g weight was dropped in front of it (T1). The animal would then turn back or if this did not happen, it was gently relocated to the starting compartment, the sliding door was closed and the procedure was repeated 4 more times, with 5-s intervals between each trial (T2, T3, T4 an T5). The time the fish took to enter the black compartment was recorded for each training trial. Images of the training were recorded with a video camera positioned 50 cm above the aquarium and a crossing was established when the dorsal fin passed the line between black and white compartments.

In order to verify if the results of the inhibitory avoidance procedure were due to motor activation, the motor activity of the animals during the training trials was observed (vehicle group: $n=11$; CPA4: $n=15$; CPA16: $n=15$; ZOL: $n=17$). This analysis was made after the end of the experiment through the use of the recorded images of the training. The white compartment was divided in three equal compartments on the monitor screen. The number of crossings between compartments divided by the total time spent in the white compartment during the training was calculated and considered as indicative of motor activity. The swimming activity was analyzed only in terms of horizontal swimming. The vertical movements could not be registered since the aquarium walls were covered by an opaque film.

2.4. Place preference conditioning procedure

A square aquarium (30 cm sides, 15 cm high) was used for the place preference conditioning procedure. At the medium point of each side of the aquarium a perpendicular plastic barrier (4 cm long and 10 cm high) was fixed, dividing it in 4 equal compartments. The barriers did not cross and allowed the free passage between the compartments (hence forward denominated “open aquarium”). A transparent cylinder could be adapted between the barriers, blocking the passage between compartments (“closed aquarium”) (Fig. 1).

This experiment was performed for six consecutive days. On the first day, the animal was placed in the open aquarium for 10 min to explore the area. On the second day, this procedure