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Involvement of central H_1 and H_2 receptors in water intake induced by hyperosmolarity, hypovolemia and central cholinergic stimulation

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Abstract

In the present study we investigated the participation of central H_1 and H_2 histaminergic receptors in water intake induced by hyperosmolarity (evoked by intragastric salt load), by hypovolemia (promoted by the subcutaneous administration of polyethyleneglycol) and by the pharmacological stimulation of central cholinergic pathways by the muscarinic agonist carbachol in male Wistar rats. The data presented here show that the pharmacological blockade of central H_1 histaminergic receptors by third ventricle injections of mepyramine significantly decreased water intake induced by hyperosmolarity, hypovolemia and by the intracerebroventricular injections of carbachol. On the other hand, the pharmacological blockade of central H_2 histaminergic receptors by third ventricle injections of cimetidine significantly reduced water intake in hypovolemic and hyperosmotic animals, but failed to alter water intake induced by central cholinergic stimulation by carbachol. We conclude that H_1 and H_2 brain histaminergic receptors are involved in inducing thirst during hyperosmolarity and hypovolemia and that H_1 histaminergic receptors located post-synaptically in relation to cholinergic pathways seem to be important in triggering drinking following central pharmacological cholinergic stimulation.

Endocrine and autonomic regulation; Osmotic and thermal regulation
Keywords: Histamine; Water intake; Cimetidine; Mepyramine; Hypovolemia; Hyperosmolarity; Carbachol

1. Introduction

Central histaminergic pathways are involved in the control of numerous visceral and behavioral responses. Indeed, brain histamine participates in the control of body temperature, modulates pain perception and the sleep/wake cycle, affects the synthesis and release of hypothalamic products and pituitary hormones and strongly influences food intake [1,2].

Less attention has been given to the role of brain histaminergic circuitries in the control of fluid balance. Central injections of histamine have been shown to induce water intake [3,4] and brain histamine has also been reported to influence urine output by modulating vasopressin release through its action on the paraventricular nucleus [5,6].

We have been investigating the role of brain histaminergic pathways and histaminergic receptor subtypes in the control of water and salt intake, and recently reported that the pharmacological blockade of central H_1 and H_2 histaminergic receptors, induced by third ventricle injections of histamine antagonists, inhibits water and salt intake induced by central angiotensinergic stimulation, while this same pharmacological procedure fails to modify water intake induced by water deprivation [7]. In another study, we showed that the pharmacological blockade of H_1 and H_2 histaminergic receptors located within the ventromedial hypothalamus (VMH) significantly decreases water intake during the overnight period. In this same study, we also demonstrated that the pharmacological blockade of central H_1 receptors attenuates water intake elicited by hyperosmolarity, while the blockade of central H_2 receptors has no effect on this condition. Additionally, we showed that the pharmacological blockade of central H_1 and H_2 receptors impairs water intake produced by water deprivation [8].

In the present study, we investigated the role of central H_1 and H_2 receptors in the control of water intake elicited by two different

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thirst-inducing physiological stimuli: hyperosmolarity (induced by intragastric salt load) and hypovolemia (produced by subcutaneous polyethyleneglycol administration). Additionally, the existence of a well-documented histamine/cholinergic interplay in the central nervous system [9–12], in which histamine seems to modulate cholinergic transmission, prompted us to investigate the participation of histaminergic receptors in water intake induced by central cholinergic stimulation, a classical thirst-inducing pharmacological approach.

2. Material and methods

2.1. Animals

In the present study, we used male Wistar rats weighing 240± 20 g. They were housed in individual cages and kept under controlled light (lights on from 7 A.M. to 7 P.M.) and temperature (22–24 °C) conditions. In all experimental protocols central injections of saline (controls) and each individual dose of the histaminergic agents were tested in a naïve group of animals. All experiments were conducted between 7 A.M. and 12 P.M. The experimental protocols were conducted according to the rules suggested by the National Institutes of Health (USA) and were approved by a local committee that analyzes ethical aspects of research with laboratory animals.

2.2. Surgical procedures

The cannulation of the third ventricle was performed under pentobarbital anesthesia (50 mg/kg i.p.) 5 days before the experimental sessions. A stereotaxic apparatus (David Kopf Instruments, Tujunga, CA) was used to implant a 15 mm, 22-gauge, stainless steel cannula. The following coordinates were used: anteroposterior=0.5 mm behind the bregma; lateral=0.0 mm; vertical 8.5 mm below the skull. The animals were placed in the stereotaxic apparatus with the head inclined 2.0 mm upwards to avoid lesions to the midline structures related to body fluid and electrolyte control. A microphotograph showing third ventricle cannulation using these procedures does not produce any damage to the brain structures involved in water and salt intake regulation has been previously published by our group [13]. The cannulas were cemented to the skull bone with dental acrylic and an obturator (28-gauge) was provided to avoid obstruction. After sacrifice by CO_2 inhalation, we verified whether the tip of the cannula was correctly positioned by injecting Blue Evans dye (2.0 μl) into the third ventricle. Only data from animals in which the cannulas were strictly inside the third ventricle were analyzed. In order to minimize the stress of the experimental maneuvers, the animals were handled every day.

2.3. Drugs and microinjections

The following drugs were used: mepyramine maleate (*N*-(4-methoxy-phenylmethyl-*N*′,*N*′-dimethyl-*N*-(2-pyridinyl)-1,2-ethanediamine), an H_1 histaminergic receptor antagonist, cimetidine, an H_2 histaminergic receptor antagonist, and polyethylene glycol (m.w. 15.000–20.000; PEG) were purchased from Sigma Co., St. Louis, MO. Central injections were performed using a Hamilton microsyringe connected to a Myzzy-Slide-Pak needle through polyethylene tubing. All drugs were dissolved in isotonic saline solution. The final volume injected was 2 μl over a period of 90 s.

The pharmacological agents used in the present study are selective at the doses at which they were administered. Mepyramine, which has a high affinity for H_1 receptors (pK_d=9.4) may interact with cholinergic receptors at micromolar concentrations [14,15], however, in the present experiment, the compound was used at nanomolar doses. Cimetidine exhibits agonistic properties in $GABA_A$ receptors only when used at doses significantly higher than those used in the present study [16]. The doses of mepyramine used here were based on a previous work carried out by another group [17] in which intracerebroventricular infusions of this compound were used to study the role of central H_1 receptors on food and water intake. In that paper, the authors used a fixed dose of 800 nmol of mepyramine. Another study from a different group states that cimetidine, when injected intracerebroventricularly at similar doses, induces convulsion [18]. Therefore, in order to use both drugs in equimolar amounts, we decided to test mepyramine and cimetidine at smaller doses (50, 100, 200 and 400 nmol) than those used by the group of Lecklin [17].

2.4. Intragastric salt load

To study the role of central H_1 and H_2 receptors in water intake induced by hyperosmolarity, different groups of animals submitted to an acute intragastric salt load received third ventricle injections of H_1 or H_2 receptor antagonists (mepyramine and cimetidine, respectively), and had their water intake monitored during 120 min. Intragastric salt load was achieved by administering 1 ml/100 g of a hypertonic saline solution (1.5 M) via orogastric tubing. In this case, the animals were fasted for 14 h (from 6 P.M. to 8 A.M.) the night preceding the experiments, in order to obtain a uniform electrolyte absorption in all individuals. They received an intragastric salt load 10 min after third ventricle injections of mepyramine or cimetidine at different doses. These groups of animals were compared to an additional group receiving intragastric administration of isotonic saline solution followed by third ventricle injections of isotonic saline solution.

2.5. Polyethylene glycol administration

A 30% PEG solution was prepared in 0.15 M sodium chloride by heating the mixture to approximately 50 °C while stirring constantly. This solution was administered subcutaneously (2 ml/100 g) 4 h before the third ventricle injections of the histaminergic antagonists (mepyramine and cimetidine) or the isotonic saline solution (controls). Graduated bottles were removed from the cages immediately before PEG administration and reintroduced 30 min after the icv injections. Cumulative water intake was measured over the following 120 min. These groups of animals were also compared to an additional group receiving subcutaneous injections of isotonic saline solution in the same volume as the PEG solution followed by third ventricle injections of saline. The dose of PEG used in the present study is identical to that successfully used in previous experiments carried out at this laboratory [19].