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.
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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].

2.6. Central cholinergic stimulation

To induce pharmacological stimulation of central cholinergic pathways, animals received third ventricle injections of carbachol at the dose of 2 μg, a dose that has been previously used in other studies [20–22]. Control animals received third ventricle injections of isotonic saline solution. To study the participation of central H_1 and H_2 receptors in water after central cholinergic stimulation, different groups of rats received third ventricle injections of different doses of mepyramine, a selective H_1 antagonist, or the H_2 receptor antagonist cimetidine 15 min before receiving carbachol (2 μg). As in the previous experimental sets, fluid intake was first measured 15 min later and thereafter for the following 120 min. All groups were compared to a group of rats receiving central administration of saline instead of carbachol.

2.7. Hematocrit measurement

Two separate groups of animals, one receiving subcutaneous injections of polyethyleneglycol and the other treated subcutaneously with isotonic saline solution, were used to estimate the efficacy of PEG in inducing hypovolemia. In these groups blood samples were collected from the tip of the tail into microhematocrit tubes 4 h and 30 min after these subcutaneous injections. The blood samples were centrifuged and read immediately following collection.

2.8. Open field test

To test whether the third ventricle injections of either mepyramine or cimetidine were able to induce any significant reduction in locomotor activity that could explain the inhibition of water intake observed here, we submitted different groups of rats to an open field test 30 min after receiving third ventricle injections of either one of the two compounds or saline.

The apparatus consisted of a circular wooden box (60 cm in diameter and 60 cm high) with an open top. The floor was divided into eight areas of equal size with a circle at the center (42.43 cm). Hand-operated counters and stopwatches were used to score locomotion (measured as the number of floor units entered by the animal with all four paws) for 10 min.

The behavioral experiments took place in a sound-attenuated, temperature-controlled (24±1 °C) room between 7 A.M. and 12 P.M. Two 40 W fluorescent lights placed 1.50 m away from the apparatus illuminated the environment. A white-noise generator provided constant background noise and the apparatus was cleaned with 70% ethanol and dried before each session to minimize olfactory cues.

2.9. Statistical analysis

A computer software package (SigmaStat for Windows, Jandel Scientific, San Rafael-CA) was used to carry out the one-way analysis of variance for each time point. The post-hoc Student–Newman–Keuls test was used for comparison among the distinct treatments. The data are presented as the mean±SEM. The effects were considered significantly different when $p<0.05$.
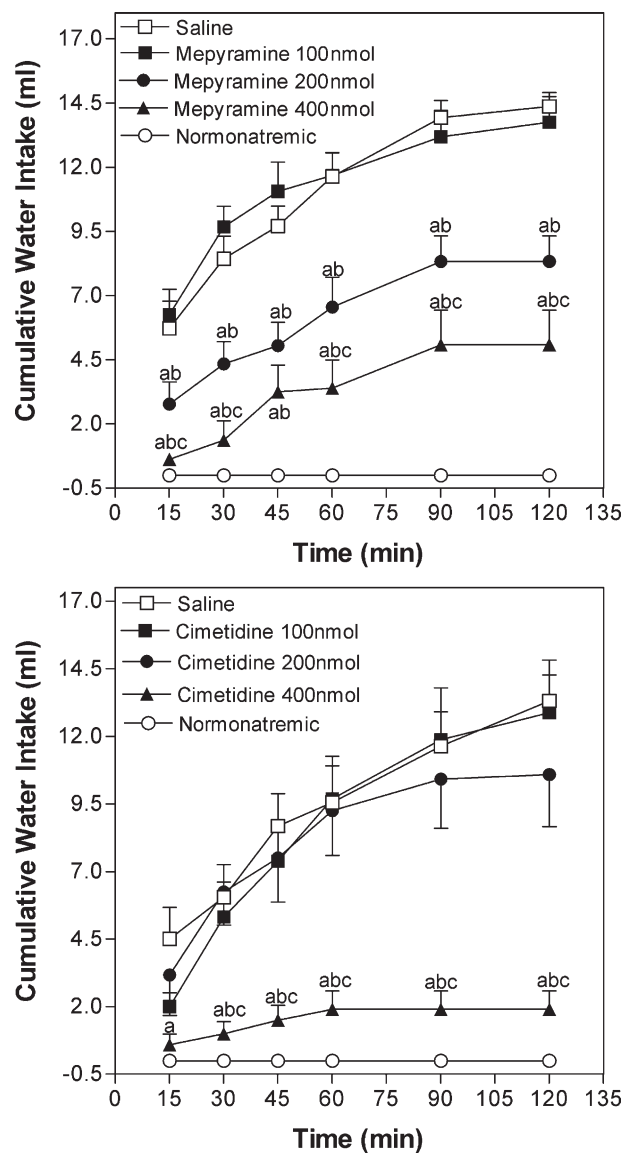

Fig. 1. Panel A: Cumulative water intake in animals receiving an acute intragastric salt load (hyperosmotic animals) or intragastric isotonic saline treated with third ventricle injections of mepyramine. (O, $n=8$) no salt load+icv saline; (□, $n=7$) hyperosmotic+icv saline; (■, $n=8$) salt load+icv mepyramine 100 nmol; (●, $n=9$) hyperosmotic+icv mepyramine 200 nmol; (▲, $n=8$) hyperosmotic+icv mepyramine 400 nmol; Panel B: Cumulative water intake in animals receiving an acute intragastric salt load or intragastric isotonic saline treated with third ventricle injections of cimetidine. (O, $n=7$) no salt load+icv saline; (□, $n=11$) hyperosmotic+icv saline; (■, $n=8$) hyperosmotic+icv cimetidine 100 nmol; (●, $n=6$) hyperosmotic+icv cimetidine 200 nmol; (▲, $n=11$) hyperosmotic+icv cimetidine 400 nmol. Data are presented as the mean±SEM. "a" indicates a statistically significant difference ($p<0.05$) when salt-loaded animals receiving third ventricle injections of mepyramine or cimetidine are compared to salt-loaded animals receiving third ventricle injections of saline. "b" indicates a statistically significant difference ($p<0.05$) when salt-loaded animals receiving third ventricle injections of mepyramine or cimetidine at the doses of 200 and 400 nmol are compared to salt-loaded animals receiving mepyramine or cimetidine at the dose 100 nmol. "c" indicates a statistically significant difference ($p<0.05$) when animals receiving third ventricle injections of mepyramine or cimetidine at the dose of 400 nmol are compared to animals receiving cimetidine at the dose 200 nmol. Each curve in the graph has been obtained from a naïve group of animals.

3. Results

Fig. 1 (panel A) shows the effect of the central administration of the H_1 histaminergic antagonist mepyramine, at different doses, on water intake in rats submitted to an intragastric salt load. As expected, a significant increase in water intake was observed in salt-loaded animals (hyperosmolar intragastric saline) receiving third ventricle injections of saline solution when compared to the group of animals not submitted to salt load (intragastric isotonic saline) also receiving third ventricle injections of isotonic saline solution. At the lowest dose used (100 nmol), the central administration of mepyramine failed to modify the high water intake observed in the group of salt-loaded animals. At the other doses used (200 and 400 nmol), mepyramine significantly inhibited the dipsogenic response induced by intragastric salt load.

Fig. 1 (panel B) shows the effect of the central administration of the H_2 histaminergic antagonist, cimetidine, at different doses, on water intake in rats submitted to an intragastric salt load. As in the previous experiment, intragastric salt load produced a significant increase in water intake. At doses of 100 and 200 nmol, third ventricle injections of cimetidine had no effect on the increase in

water intake observed in salt-loaded animals. Conversely, at the highest dose used (400 nmol), the central administration of cimetidine significantly blunted the increase in water intake seen in salt-loaded rats.

Fig. 2 (panel A) shows the effect of the central administration of the H_1 histaminergic antagonist, mepyramine, at different doses, on water intake in hypovolemic rats. There was a significant increase in water intake in hypovolemic animals (treated with subcutaneous PEG) receiving third ventricle injections of isotonic saline solution when compared to normovolemic rats (those treated with subcutaneous isotonic saline solution) also receiving third ventricle injections of isotonic saline solution. At the lowest dose used (100 nmol), mepyramine failed to induce any change in the dipsogenic effect of hypovolemia. At the intermediate dose of 200 nmol, the central administration of mepyramine was able to attenuate the increase in water intake in hypovolemic animals only during the first 60 min of the experiment. At the highest dose used (400 nmol), mepyramine significantly blunted the increase in drinking seen in hypovolemic animals for the entire duration of the experiment.

Fig. 2 (panel B) shows the effect of the central administration of the H_2 histaminergic antagonist, cimetidine, at different doses, on water intake in hypovolemic rats. As in the previous experimental set, a significant increase in water intake was observed in hypovolemic animals receiving third ventricle injections of isotonic saline solution when compared to normovolemic rats also receiving third ventricle injections of isotonic saline solution. At all doses used (25, 50 and 100 nmol), the central administration of cimetidine resulted in a significant decrease in the dipsogenic effect produced by hypovolemia.

Fig. 3 (panel A) shows the effect of the central administration of the H_1 histaminergic antagonist, mepyramine, at different
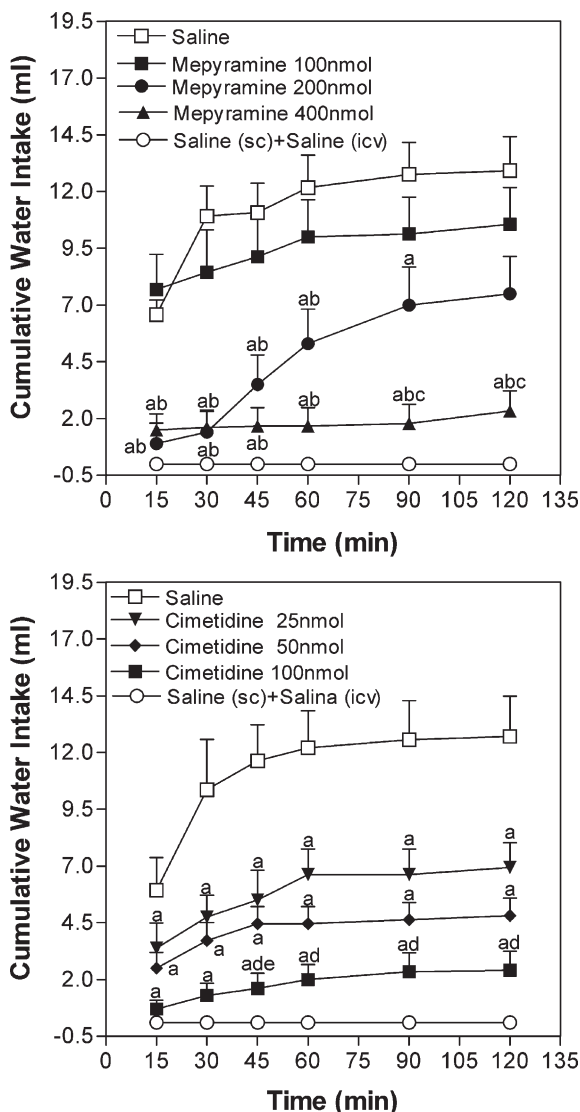

Fig. 2. Panel A: Cumulative water intake in animals receiving subcutaneous administration of PEG or isotonic saline solution treated with third ventricle injections of mepyramine. (O, $n=7$) sc saline solution+icv saline; ($\square$, $n=7$) sc PEG+icv saline; ($\blacksquare$, $n=9$) sc PEG+icv mepyramine 100 nmol; ($\bullet$, $n=6$) sc PEG+icv mepyramine 200 nmol; ($\blacktriangle$, $n=9$) sc PEG+icv mepyramine 400 nmol; Data are presented as the mean±SEM. "a" indicates a statistically significant difference ($p<0.05$) when animals receiving third ventricle injections of mepyramine are compared to animals receiving third ventricle injections of saline. "b" indicates a statistically significant difference ($p<0.05$) when animals receiving third ventricle injections of mepyramine at the doses of 200 and 400 nmol are compared to animals receiving mepyramine at the dose 100 nmol. "c" indicates a statistically significant difference ($p<0.05$) when animals receiving third ventricle injections of mepyramine at the dose of 400 nmol are compared to animals receiving mepyramine at the dose 200 nmol. Panel B: Cumulative water intake in animals receiving subcutaneous administration of PEG or isotonic saline solution treated with third ventricle injections of cimetidine. (O, $n=10$) sc isotonic saline solution+icv saline; ($\square$, $n=9$) sc PEG+icv saline; ($\blacksquare$, $n=10$) sc PEG+icv cimetidine 100 nmol; ($\blacklozenge$, $n=11$) sc PEG+icv cimetidine 50 nmol; ($\blacktriangledown$, $n=9$) sc PEG+icv cimetidine 25 nmol. Data are presented as mean±SEM. "a" indicates a statistically significant difference ($p<0.05$) when animals receiving third ventricle injections of cimetidine are compared to salt-loaded animals receiving third ventricle injections of saline. "d" indicates a statistically significant difference ($p<0.05$) when animals receiving third ventricle injections of cimetidine at the doses of 100 and 50 nmol are compared to salt-loaded animals receiving cimetidine at the dose 25 nmol. "e" indicates a statistically significant difference ($p<0.05$) when animals receiving third ventricle injections of cimetidine at the dose of 100 nmol are compared to animals receiving cimetidine at the dose 50 nmol. Each curve in the graph has been obtained from a naïve group of animals.

doses, on water intake induced by third ventricle injections of carbachol (2 ug). In the group of animals receiving central administration of the cholinergic agonist, carbachol, a significant increase in water intake was seen compared to animals receiving third ventricle injections of isotonic saline solution. At the lowest dose used (100 nmol) mepyramine failed to modify carbachol-induced water intake. At the other doses used (200 and 400 nmol),
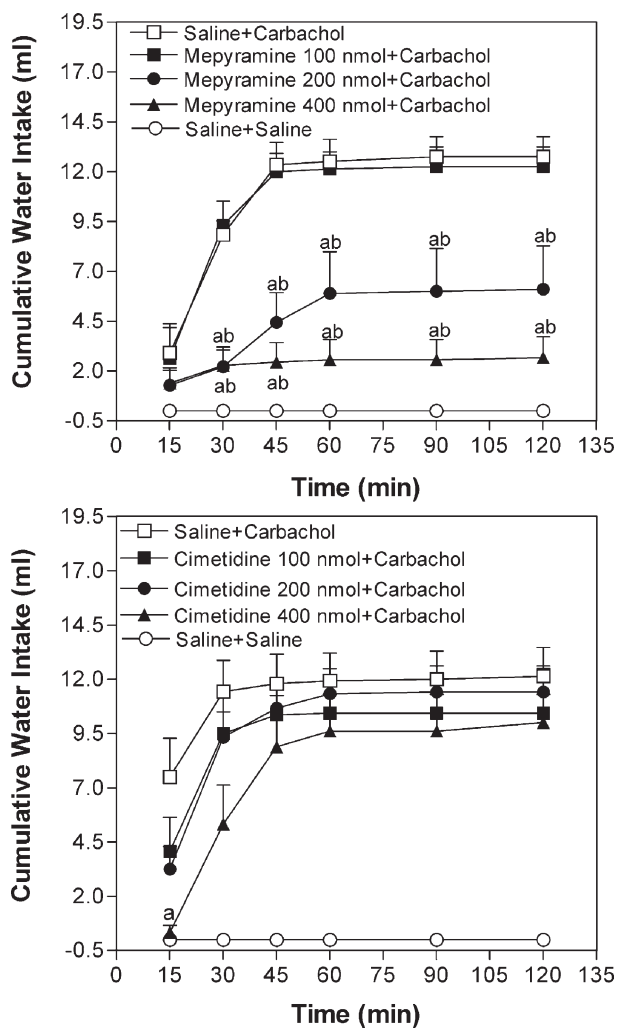

Fig. 3. Panel A — Cumulative water intake 15 min following third ventricle injections of mepyramine in rats receiving third ventricle injections of carbachol (2 μg). (□; $n=6$); saline+carbachol; (■; $n=8$) mepyramine 100 nmol+carbachol; (●; $n=9$) mepyramine 200 nmol+carbachol; (▲; $n=9$) mepyramine 400 nmol+ carbachol. An additional group of animals not submitted to central cholinergic stimulation (saline+saline) is also shown (○; $n=12$). "a" indicates a statistically significant difference ($p<0.05$) when animals receiving third ventricle injections of mepyramine are compared to animals receiving third ventricle injections of saline. "b" indicates a statistically significant difference ($p<0.05$) when animals receiving third ventricle injections of mepyramine at the doses of 200 and 400 nmol are compared to animals receiving mepyramine at the dose 100 nmol. Panel B — Cumulative water intake 15 min following third ventricle injections of cimetidine in rats receiving third ventricle injections of carbachol (2 μg). (□; $n=7$) saline+carbachol; (■; $n=7$) cimetidine 100 nmol+ carbachol; (●; $n=6$) cimetidine 200 nmol+carbachol; (▲; $n=9$) cimetidine 400 nmol+carbachol. An additional group of animals not submitted to central cholinergic stimulation (saline+saline) is also shown (○; $n=10$). Data are presented as mean±SEM. Each curve in the graph has been obtained from a naïve group of animals.

Table 1
Effects of third ventricle injections of mepyramine (400 nmol/rat), cimetine (400 nmol/rat), or saline on the behavioral parameters in the open-field test

Behavior	Saline (6)	Mepyramine (6)	Cimetidine (6)	One-way ANOVA
Areas entered	33.67±4.36	33.33±4.63	34.00±4.11	$F_{(2,15)}=0.00582$; $p=0.9942$
Times in the peripheral areas	23.50±2.43	26.00±3.25	26.67±3.77	$F_{(2,15)}=0.271$; $p=0.7665$
Times in the center areas	10.17±2.75	7.33±3.08	7.33±1.41	$F_{(2,15)}=0.421$; $p=0.6637$
Stops	10.67±1.12	10.50±1.23	11.17±2.21	$F_{(2,15)}=0.0472$; $p=0.9541$
Rearing	6.33±0.88	5.17±1.11	7.00±1.98	$F_{(2,15)}=0.435$; $p=0.6552$
Grooming	2.00±0.58	1.33±0.49	1.67±0.33	$F_{(2,15)}=0.484$; $p=0.6257$
Diuresis	0.67±0.21	1.17±0.31	1.33±0.49	$F_{(2,15)}=0.942$; $p=0.4117$
Defecation	1.67±0.56	2.50±0.43	1.33±0.42	$F_{(2,15)}=1.61$; $p=0.2323$

Data is presented as mean±SEM of the number of occurrences of each listed behavior in a 10-minute long open-field test. The number of animals in each treatment group is in parenthesis.

mepyramine significantly decreased the dipsogenic response seen following central administration of carbachol.

Fig. 3 (panel B) shows the effect of the central administration of the H₂ histaminergic antagonist, cimetidine, at different doses, on water intake induced by third ventricle injections of carbachol (2 μg). As in the previous experimental set, the central administration of carbachol induced a significant increase in drinking. Nonetheless, in this case, cimetidine failed to modify the dipsogenic response induced by third ventricle injections of carbachol at any of the doses used (100, 200 or 400 nmol).

A significant increase ($p<0.05$) in hematocrit levels (49.3± 0.23%) was observed 4 h and 30 min after the administration of PEG when compared to a control group receiving subcutaneous injections of isotonic saline solution (43.2±0.72%).

As shown in Table 1, neither third ventricle injections of mepyramine nor cimetidine were able to alter the animals' locomotor activity pattern and the behavior of rats compared to the pattern observed in rats receiving third ventricle injections of saline solution, even at the highest dose used in the experimental sets already described (400 nmol) in an open field.

Table 2, condenses the results of the overall analysis of the effects of third ventricle injections of H₁ and H₂ receptor antagonists or saline on water intake at each time point.

4. Discussion

The present data clearly demonstrate that third ventricle injections of mepyramine, an H₁ antagonist, induced a significant, dose-dependent decrease in water intake induced by hyperosmolarity, hypovolemia or by the pharmacological stimulation of central cholinergic pathways by intracerebroventricular injections of carbachol, a muscarinic agonist. On the other hand, the central administration of cimetidine, an H₂ antagonist, significantly reduced water intake in hypovolemic animals in a dose-dependent

Table 2
Overall analysis of the effects of third ventricle injections of H_1 and H_2 receptors antagonists or saline on water intake at each period of time

	Time (min)	Mepyramine	Cimetidine
Hyperosmolarity	15	$F_{(3,28)} = 11.4$; $p < 0.0001$	$F_{(3,32)} = 3.76$; $p = 0.0203$
	30	$F_{(3,28)} = 21.8$; $p < 0.0001$	$F_{(3,32)} = 6.0$; $p = 0.0023$
	45	$F_{(3,28)} = 13.9$; $p < 0.0001$	$F_{(3,32)} = 10.4$; $p < 0.0001$
	60	$F_{(3,28)} = 14.8$; $p < 0.0001$	$F_{(3,32)} = 9.96$; $p < 0.0001$
	90	$F_{(3,28)} = 15.0$; $p < 0.0001$	$F_{(3,32)} = 13.9$; $p < 0.0001$
	120	$F_{(3,28)} = 17.8$; $p < 0.0001$	$F_{(3,32)} = 20.0$; $p < 0.0001$
Hypovolemia	15	$F_{(3,24)} = 10.1$; $p = 0.0002$	$F_{(3,32)} = 5.52$; $p = 0.0036$
	30	$F_{(3,24)} = 12.0$; $p < 0.0001$	$F_{(3,32)} = 10.6$; $p < 0.0001$
	45	$F_{(3,24)} = 11.9$; $p < 0.0001$	$F_{(3,32)} = 14.9$; $p < 0.0001$
	60	$F_{(3,24)} = 13.3$; $p < 0.0001$	$F_{(3,32)} = 17.2$; $p < 0.0001$
	90	$F_{(3,24)} = 13.1$; $p < 0.0001$	$F_{(3,32)} = 15.4$; $p < 0.0001$
	120	$F_{(3,24)} = 11.9$; $p < 0.0001$	$F_{(3,32)} = 15.2$; $p < 0.0001$
Carbachol	15	$F_{(3,28)} = 0.535$; $p = 0.6618$	$F_{(3,25)} = 6.14$; $p = 0.0028$
	30	$F_{(3,28)} = 16.8$; $p < 0.0001$	$F_{(3,25)} = 2.65$; $p = 0.0706$
	45	$F_{(3,28)} = 15.9$; $p < 0.0001$	$F_{(3,25)} = 0.497$; $p = 0.6871$
	60	$F_{(3,28)} = 10.1$; $p = 0.0001$	$F_{(3,25)} = 0.336$; $p = 0.7992$
	90	$F_{(3,28)} = 10.1$; $p = 0.0001$	$F_{(3,25)} = 0.360$; $p = 0.7822$
	120	$F_{(3,28)} = 9.71$; $p = 0.0001$	$F_{(3,25)} = 0.296$; $p = 0.8280$

The data were analyzed using the one-way ANOVA for each time point. For follow-up statistical tests to contrast specific groups additional post-hoc Student–Newman–Keuls tests were conducted. The group means for the various parameters analyzed were considered to be significantly different when $p < 0.05$. These results are shown in Figs. 1, 2 and 3.

way while in hyperosmotic animals this compound was able to inhibit water intake only at the highest dose used. Third ventricle injections of cimetidine failed to alter water intake induced by central cholinergic stimulation by carbachol.

Plasma osmolarity, blood volume and blood pressure are constantly regulated by the central nervous system. Indeed, a complex central circuitry involving many brain areas and neuro-transmitters receives a continuous flow of information related to these parameters and operates intricate mechanisms controlling corrective visceral and behavioral responses that include stimu-lation or inhibition of water and salt intake. Several central neurotransmitters play a significant role in the control of thirst. The cholinergic, adrenergic and serotonergic systems in the brain strongly influence water intake, exerting both positive and negative drives on drinking behavior, depending on the area in

which each particular subset of neurons is located, the subtype of receptor activated and the animal's state of hydration [23,24].

The central histaminergic pathways participate in the control of water and salt intake, but the nature of this participation is not yet fully understood [25]. Central administration of histamine into several hypothalamic sites induces a significant increase in water intake [4,26,27], and the decrease in drinking behavior promoted by antihistaminics is reversed by intracerebroven-tricular injections of histamine [3,28,29].

By modulating not only histamine synthesis but the synthesis and release of several other neurotransmitters, central H_3 recept-ors may exhibit complex effects on water intake. Indeed, the central administration of H_3 receptor agonists, which decreases brain histaminergic activity, elicits drinking [17], thereby indicating that this effect cannot be attributed to H_3 receptor-dependent modifications in histaminergic activity. In another paper [30] the authors demonstrated that intracerebroventricular injections of a selective H_3 receptor antagonist attenuates drinking elicited by intragastric salt-load. The activation of post-synaptic H_3 receptors or H_3 receptors functioning as heteroceptors modulating the synthesis and release of other neurotransmitters may explain this apparent paradox.

We have previously shown that, in hyperosmotic rats, the activation of histaminergic H_1 receptors located in the ventromedial hypothalamus (VMH) stimulates water intake, while histaminergic H_2 receptors in this same region do not participate in the control of drinking behavior in this same group of animals. In addition, we have also shown that when activated both H_1 and H_2 histaminergic receptors located in the VMH increase overnight water intake and drinking behavior induced by a 14-hour period of water deprivation [8].

In another study, we showed that 1) the activation of central H_1 and H_2 histaminergic receptors stimulates salt intake induced by a 24-hour period of water deprivation, 2) the functional integrity of central H_1 histaminergic receptors is required to trigger salt intake in sodium-depleted rats, while central H_2 histaminergic receptors play no significant role in this mechanism and 3) both H_1 and H_2 histaminergic receptors participate in the mechanisms leading to water and salt intake in rats following central angiotensinergic stimulation [7]. It is important to note that several central structures and neurotransmitters normally linked to the stimula-tion of salt intake also increase water intake.

In the present study, third ventricle injections of mepyramine and cimetidine significantly blunted water intake induced by hypovolemia, indicating that the functional integrity of these receptors is essential for inducing thirst when blood volume is decreased. Water intake induced by hypovolemia is predomi-nantly triggered by the activation of peripheral and central angiotensinergic components [23,24,31]. However, it should be noted that hypovolemia may induce thirst even in the absence of any increase in angiotensin II levels, as occurs in nephrectomized rats [32]. We have previously shown that the pharmacological blockade of H_1 and H_2 central histaminergic receptors attenuates water intake induced by central angiotensinergic stimulation [7]. Taken together, the data produced by our laboratory indicate that brain H_1 and H_2 histaminergic receptors are required to induce drinking in the presence of a direct pharmacological stimulation

of brain angiotensin II receptors or following hypovolemia, a physiological condition associated with the endogenous activation of central angiotensinergic pathways.

In the present study, third ventricle injections of mepyramine, an H_1 antagonist, significantly reduced water intake in animals receiving an intragastric salt load. This finding is in agreement with previous data from our laboratory showing that this same type of receptor located in the VMH is also necessary for the full expression of water intake in hyperosmotic animals. In addition, in the present study, third ventricle injections of cimetidine, an H_2 antagonist, significantly impaired the dipsogenic response induced by hyperosmolarity, while in the previously mentioned study, injections of cimetidine into the VMH failed to modify drinking behavior induced by this condition. The accumulated data from our previous and current studies indicate that 1) H_1 histaminergic receptors located both in the VMH and in other brain regions reached by injections of histaminergic drugs into the third ventricle participate in the thirst-inducing mechanisms triggered by hyperosmolarity and that 2) the activation of brain H_2 histaminergic receptors located in regions reached by injections of histaminergic drugs into the third ventricle is necessary to induce drinking in hyperosmotic animals, while these receptors located in the VMH play no significant role in this response.

Water intake induced by hyperosmolarity depends strongly on the activation of central cholinergic pathways and different brain areas may be involved [23,24,31,33,34] In this study, the pharmacological blockade of brain H_1 histaminergic receptors significantly decreased water intake both in animals submitted to an intragastric salt load and in animals receiving central cholinergic stimulation. Conversely, the pharmacological blockade of brain H_2 histaminergic receptors attenuated water intake in hyperosmotic animals, but failed to modify water intake in the group of rats receiving central cholinergic stimulation by third ventricle injections of carbachol. This suggests that hyperosmolarity, a physiological condition that certainly induces a myriad of complex alterations in the diverse central nervous system circuitries related to thirst regulation, triggers water intake through a mechanism that requires both H_1 and H_2 receptor involvements, whereas drinking behavior resulting from the pharmacological activation of central cholinergic pathways does not require H_2 histaminergic receptor activation.

A clear interaction has been demonstrated between the central histaminergic and cholinergic pathways in the central nervous system [9,10]. The activation of H_1 histaminergic receptors induces a significant increase in cholinergic transmission and acetylcholine release at many brain sites, and the central administration of histamine H_1 antagonists decreases acetylcholine release [9,12]. In addition, the central administration of selective H_3 histaminergic receptor antagonists, a procedure that increases histaminergic neurotransmission, augments c-*fos* immunoreactivity in cholinergic neurons [11]. In a previous paper, we suggested that H_1 histaminergic receptors located in the VMH may exert a stimulatory drive on acetylcholine release by cholinergic neurons, resulting in an increase in water intake induced by hyperosmolarity. A similar hypothesis may be applied here, linking brain H_1 histaminergic receptors to the release of

acetylcholine in some thirst-triggering region of the brain. However, third ventricle injections of mepyramine were also able to attenuate water intake in animals receiving central administration of carbachol. This may indicate that H_1 histaminergic receptors located in circuitries post-synaptically situated in relation to the cholinergic pathways may also be involved in the thirst-inducing mechanisms triggered by central pharmacological cholinergic activation. These mechanisms leading to water intake following central cholinergic activation probably do not depend on histaminergic H_2 receptors located post-synaptically in relation to the cholinergic pathways, since central cimetidine administration failed to modify carbachol-induced drinking behavior.

The thirst-inducing procedures used in the present study generate physiological and pharmacological stimuli that normally trigger water intake. Indeed, we have demonstrated that intragastric salt load, using the same methodology applied in this study, produces a significant increase in plasma osmolarity and in plasma sodium concentration [8]. Furthermore, subcutaneous administration of PEG certainly produced hypovolemia, as indicated by the significant increase in hematocrit in the group submitted to this procedure, as compared to the group of control animals receiving isotonic saline solution subcutaneously. The induction of water intake by central cholinergic stimulation has been largely demonstrated and the use of intracerebroventricular administration of carbachol at the doses used in the present study to induce thirst is in accordance with data produced by other groups of investigators [35].

The inhibitory effects of mepyramine on water intake induced by hyperosmolarity, hypovolemia or by the pharmacological stimulation of central cholinergic pathways by intracerebroventricular injections of carbachol, as well as the inhibitory effect of cimetidine on water intake induced by hypovolemia were typically dose-dependent. This indicates a selective interaction of those compounds with central histaminergic receptors. Analysis of the effects of the various doses of the compounds used in the present study indicates that H_2 histaminergic inhibition of water intake in hyperosmotic animals requires a greater amount of pharmacological stimulation in contrast with the inhibitory influence exerted by central H_1 receptors that is obtained after significantly lower pharmacological stimulation. It is also important to note that, in hypovolemic animals, the magnitude of central H_1 pharmacological stimulation required to inhibit water intake is significantly greater than the central H_2 pharmacological stimulation necessary to produce an antidipsogenic effect.

The inhibition of water intake induced by the central administration of the histamine receptor antagonists seems to result from a specific action of the compounds on the brain circuitries that regulate drinking behavior and does not indicate a general impairment of the central nervous system. Indeed, in previous studies we have used an aversion test to show that third ventricle injections of either mepyramine or cimetidine fail to generate any "illness-like" effects [7]. Moreover, we have previously shown that third ventricle injections of these compounds selectively impair water intake but fail to alter the hedonic behavior represented by the consumption of a tasty saccharin solution [7]. We have also shown that the inhibition of

water intake induced by the central administration of mepyramine and cimetidine is not due to a deficit in locomotor activity since animals receiving the highest dose of the compounds used in this study showed no modification in locomotor activity and behavior as measured by an open field test.

The brain histaminergic system participates in the control of a large number of visceral and behavioral homeostatic processes [1] such as pain perception and the intake of food, water and salt. Central histaminergic circuitries are also involved in thermoregulation and in the control of the sleep/wake cycle. They also influence cardiovascular and neuroendocrine effectors. Furthermore, the central histaminergic system may be involved in important pathological conditions. Indeed, subcortical histaminergic projections have shown significant degeneration in Alzheimer's disease [36,37]; histamine levels and histidine decarboxylase activity are lower in Alzheimer disease and Down's syndrome [38,39]; histamine levels in the brains of patients with Parkinson's disease are selectively higher in the putamen, substantia nigra and globus pallidus [40]; and levels of t-methylhistamine, a histamine metabolite, are higher in the spinal fluid of schizophrenic patients [41]. All these facts, associated with the large clinical application of anti-histaminergic agents such as antiallergic or antacid agents that cross the blood–brain barrier, as well as some antipsychotics and recently developed antidepressants, make the brain histaminergic system a target for an extensive list of drugs used in current medical practice. Therefore, the investigation of the physiological roles played by central histaminergic receptors is opportune and relevant.

Acknowledgments

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