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Histaminergic H_1 and H_2 receptors located within the ventromedial hypothalamus regulate food and water intake in rats

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Abstract

The aim of the present study was to investigate the effect of the pharmacological blockade of histamine H_1 and H_2 receptors located within the ventromedial hypothalamus (VMH) on overnight food and water intake and on water intake elicited by two physiological stimuli: hyperosmolarity induced by an acute intragastric salt load and water deprivation. During the overnight period, the pharmacological blockade of both H_1 and H_2 VMH receptors significantly increased food intake and decreased water intake. In hyperosmotic rats, the blockade of H_1 VMH receptors reduced water intake, while the blockade of H_2 receptors in this same region yielded no significant effect. Additionally, in water-deprived rats, the blockade of both H_1 and H_2 receptors located within the VMH induced a significant decrease in water intake. The inhibitory effects on drinking behavior observed in this study do not seem to be a consequence of any "illness-inducing" effect provoked by the central administration of the antihistaminergic agents employed here, because an aversion test indicated that the injection of those compounds into the VMH does not induce any "illness-like" effect. In addition, the central administration of either mepyramine or cimetidine to dehydrated and hyperosmotic rats did not produce any reduction in locomotor activity measured in an open-field arena. Injections of the antihistaminergic agents used here into the regions that circumscribe the VMH produced no significant effects on water or food intake, indicating that the actions observed here may be specifically attributed to the set of histaminergic receptors situated within the VMH.
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1. Introduction

Brain histaminergic pathways comprise a specific neuronal circuitry exclusively originated in the hypothalamic tuberomammilary nucleus that spreads to several brain areas exerting numerous physiological roles (Brown et al., 2001). In recent decades, a growing research effort has established the indisputable role of brain neuronal histamine in the suppressive control of food intake (Lecklin and Tuomisto, 1990), an effect that requires the functional integrity of H_1 receptors in the ventromedial hypothalamus (VMH) and paraventricular nuclei (Sakata et al., 1997; Mercer, 1997).

Less effort has been dedicated to the study of brain neuronal histamine in the control of fluid balance. Yet, histamine elicits a significant dipsogenic effect when injected into the cerebral ventricles (Leibowitz, 1973; Gerald and Maickel, 1972), an action that seems to be physiological, because the synthesis and release of hypothalamic histamine increase following dehydration (Kjaer et al., 1994).

In the great majority of monogastric species, eating is the main impulse that leads to spontaneous water intake (De Castro, 1989; Fitzsimons and Le Magnen, 1969) and histamine has been characterized as one of the key central agents triggering prandial drinking in rats (Kraly, 1983).

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Prandial drinking is inhibited by the pharmacological blockade of peripheral H_1 and H_2 receptors, whose participation is also necessary for the expression of thirst induced by exogenous histamine administration (Kraly, 1990). Few papers have been published on the investigation of the participation of brain histaminergic receptors in the control of water intake induced by distinct physiological stimuli.

In the present study, we investigated whether H_1 and H_2 receptors located within the VMH, a very important structure controlling ingestive behaviors, could have a role in the regulation of water and food intake in rats during the overnight period. We also studied the role of those receptors in water intake in rats submitted to two distinct procedures that lead to hyperosmolarity: intragastric salt load and water deprivation.

2. Methods

2.1. Animals

We used adult Wistar male rats weighing 250±30 g in this study. They were kept under controlled light (lights on from 5 a.m. to 7 p.m.) and temperature (22–24 °C) conditions. In the days preceding the experimental sessions, they had free access to tap water and laboratory chow (Nuvital Nutrientes, Curitiba, Brazil). For each experimental set, a distinct naïve group of rats was used. Procedures used in this study were carried out in accordance with the rules prescribed by the National Institutes of Health Guide for Care and Use of Laboratory Animals (USA).

2.2. Surgical procedure

Following anesthesia with sodium pentobarbital (50 mg/kg), the animals were placed in a Kopf stereotaxic instrument for bilateral implantation of stainless steel cannulas (23 gauge) into the VMH. The stereotaxic coordinates used were: 2.5 mm caudal to bregma, 0.8 lateral to midline and 8.8 mm below the skull (Paxinos and Watson, 1998). The tips of the cannulas were placed 2 mm above the VMH. The cannulas were cemented to the skull bone with dental acrylic resin and jeweler screws and fitted with an obstructer (30 gauge). After surgery, the animals were housed in individual cages for 5 days before the experiments.

2.3. Histology

At the end of the experiments, the animals were anesthetized with ether and submitted to transcardiac perfusion with phosphate-buffered saline (PBS) followed by 10% formalin. The brains were then removed and fixed in 10% formalin. They were frozen and cut into 40-μm sections. To confirm the injection sites in relation to the VMH, the slices were stained with cresyl violet and analyzed by light microscope.

2.4. Drugs and microinjections

The following drugs were used: lithium chloride and the histaminergic antagonists mepyramine maleate (*N*-(4-methoxy-phenylmethyl-*N*′ ,*N*′ -dimethyl-*N*-(2-pyridinyl)-1,2-ethanediamine) and cimetidine were purchased from Sigma (St. Louis, MO). Central injections were performed using a Hamilton microsyringe connected to a Myzzy–Slide–Pak needle through polyethylene tubing. A total volume of 0.5 μl was slowly injected (60–90 s).

The doses of mepyramine used here were based on the work of Lecklin et al. (1998) 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. In another study, the authors (Shimokawa et al., 1996) state that cimetidine, when injected intracerebroventricularly at similar doses, induces convulsion. Therefore, in order to use both drugs in equimolar amounts, and bearing in mind that we were injecting the compounds directly into the cerebral tissue, we decided to test mepyramine and cimetidine in smaller doses (25, 50, 100, 200 and 400 nmol) than those used by Lecklin et al. (1998).

2.5. Plasma sodium concentration and osmolarity measurements

Plasma osmolarity was determined by an Osmette Precision osmometer that measures freezing point depression. Plasma sodium concentration was measured using a digital flame photometer (Micronal, model B62, São Paulo, Brazil).

2.6. Experimental design

2.6.1. Overnight food and water intake

The experiments designed to study the participation of H_1 and H_2 receptors located within the VMH in overnight food and water intake were always initiated at the beginning of the period of darkness, at 7 p.m. (time when central injections were made) when rats normally eat and drink spontaneously. The amounts of water and food intake were recorded for 12 h after the central administration of the histamine antagonists. Control groups received central injections of isotonic saline solution at the same volume and under the same conditions used to administer the histamine H_1 and H_2 antagonists. It should be noted that overnight food and water intake was measured over an initial 10-h period of darkness followed by a final 2-h period of light.

2.6.2. Intragastric salt load

To study the role of H_1 and H_2 receptors located within the VMH on water intake induced by hyperosmolarity, different groups of animals submitted to an acute intra-

gastric salt load received bilateral VMH 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 the administration of 1 ml/100 g of a hypertonic saline solution (1.5 M) via orogastric tubing. The animals were fasted for 14 h (from 6 p.m. to 8 a.m.) the night preceding the experiments. They received an intragastric salt load 10 min after bilateral VMH injections of mepyramine or cimetidine at different doses. The bottles containing water were removed from the cages immediately preceding the intragastric salt load and replaced 30 min later. This 30-min period after intragastric salt load is sufficient to induce significant increases in plasma sodium concentration and osmolarity. These groups of animals were compared to an additional group receiving an intragastric administration of isotonic saline solution followed by bilateral VMH injections of isotonic saline solution.

To evaluate the effect of the intragastric salt load on plasma osmolarity, two groups of animals, one receiving an intragastric salt load and the other receiving intragastric isotonic saline solution, were decapitated 30 min after the experiment and had their trunk blood collected. After centrifugation, plasma was used for the determination of osmolarity and sodium concentration.

2.6.3. Water deprivation

To investigate the role of H_1 and H_2 receptors located within the VMH on drinking behavior of water-deprived animals, water bottles were removed from the individual cages 14 h prior to the onset of the experiments (from 5 p.m. until 7 a.m.). Thirty minutes after the injections of the H_1 or H_2 receptor antagonists into the VMH, graduated bottles were reintroduced into the cages and water intake was monitored for the next 120 min. Control groups received isotonic saline injections into the VMH under the same conditions as to the drug-treated groups.

2.6.4. Aversion tests

To verify that the central administration of either mepyramine or cimetidine is devoid of nonspecific inhibitory "illness-like" effects on water intake, a taste aversion test was performed. We used a protocol based on the experimental design proposed by Nachman (1970). This protocol uses a temporal association between the novel taste of a 0.25% saccharin solution and the distress induced by lithium chloride administration. Five days after the third ventricle cannulation, the animals had their access to water restricted to 15 min/day (between 12 and 12:15 a.m.) for four consecutive days. Under these conditions, they had to achieve a very high water intake during a very short period of time. On the fifth day, they were divided into four different groups that, after being submitted to different pharmacological protocols, had access to bottles containing a saccharin solution (no water was offered on this day). The first group (controls) received two consecutive injections, one immediately following the other, of isotonic saline solution, the first being intraperitoneal and the second into the VMH. In the second group of animals, 0.15 M lithium chloride intraperitoneal injections (0.6% b.w.) were followed by injections of isotonic saline solution into the VMH. This is a second control group in whom the lithium-induced, illness-like effects, a condition that generally disrupts ingestive behaviors in rats, are associated with the novel taste of saccharin. The third and fourth groups of animals received intraperitoneal injections of saline solution in the same volume as that used in the previous group, followed by injections of mepyramine (third group) or cimetidine (fourth group). Both drugs were injected at the dose of 200 nmol. In these groups of animals, we investigated whether the blockade of H_1 and H_2 receptors within the VMH provokes any degree of discomfort that the animals could associate with the novel taste of saccharin and that could lead to a general reduction in ingestive behavior. On the sixth day, at the same time that bottles had been available on the previous days (12 to 12:15 a.m.), bottles containing a saccharin solution were placed in all cages and the amount ingested was recorded. No drugs were injected on this day. It is assumed that the previous administration of any drug producing general, ingestive suppression discomfort at the moment the animal drinks saccharin generates an association that induces a future decrease in saccharin intake.

2.6.5. Open-field test

To test whether the central administration of either mepyramine or cimetidine into the VMH could induce a significant reduction in locomotor activity that could explain the inhibition of water intake observed here, we submitted different groups of dehydrated and hyperosmotic rats receiving VMH injections of both compounds to an open-field test.

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 with the four paws).

The behavioral experiments took place in a sound-attenuated temperature-controlled (24 ± 1 °C) room between 7 and 12 a.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.

2.7. Statistical analysis

A computer software package (SigmaStat for Windows, Jandel Scientific, San Rafael, CA) was used to carry out one-way analysis of variance for each time period. The

post hoc Student–Newman–Keuls test was used for comparison of each treatment with its corresponding time in the control groups. Student's *t*-test was used to analyze the data concerning plasma osmolarity and plasma sodium concentration. The data are presented as mean±S.E.M. The groups were considered significantly different when $p<0.05$.

3. Results

The characteristic site of bilateral injections into the VMH is shown in Fig. 1. A low-powered photomicrograph of the cannula placement into the VMH is shown in Fig. 2.

Fig. 3 describes the effects of central injections of mepyramine and cimetidine (both at the dose of 200 nmol) on food and water intake. Panel A shows that bilateral injections of both histaminergic antagonists into the VMH produced a significant increase in overnight food intake. Data portrayed in Panel B indicate that bilateral injections of either mepyramine or cimetidine yielded a significant decrease in overnight water intake.

Fig. 4 (Panel A) shows that, as expected, animals receiving an intragastric salt load (hyperosmolar intragastric saline) plus VHM injections of isotonic saline showed a significant increase in water intake, when compared to a second group of animals receiving intragastric isotonic saline solution and submitted to the same VMH isotonic saline injections. In this case, the administration of mepyramine into the VMH, at the dose of 100 nmol, was unable to modify the high water intake induced by the intragastric salt load. On the contrary, mepyramine VMH injections, at the

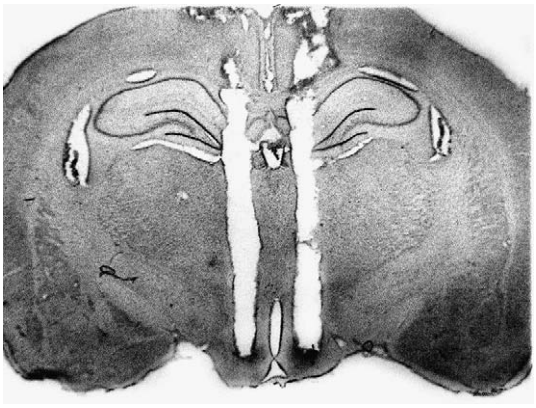

Fig. 2. Low-powered photomicrograph of a frontal section showing the typical position of the cannula within the VMH.

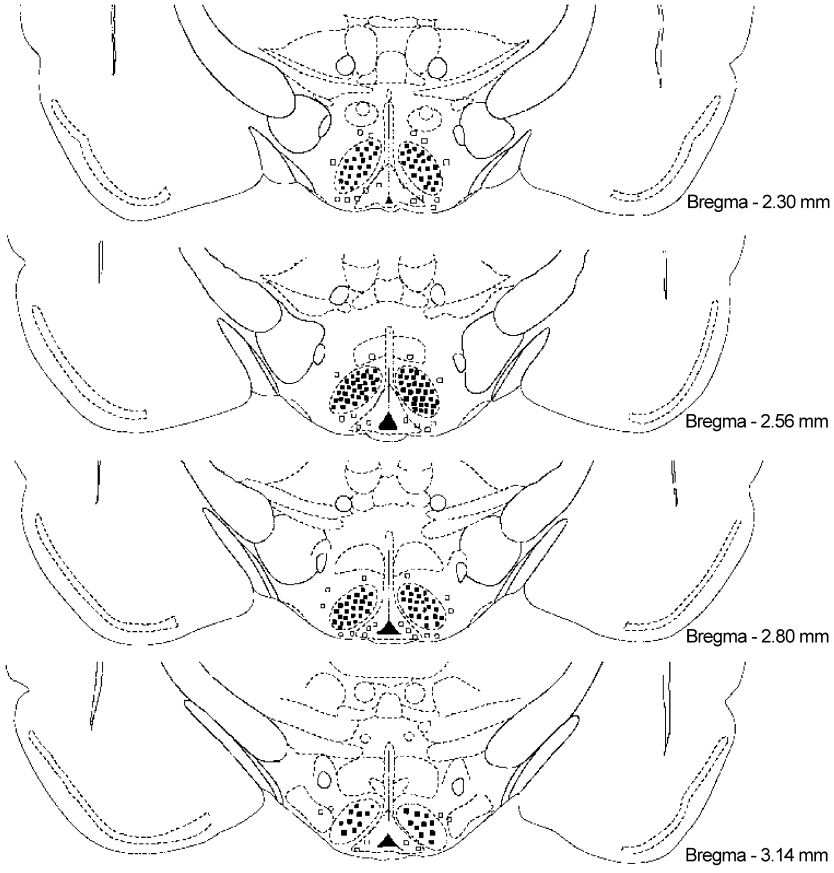

Fig. 1. Schematic representation of the injection sites within (■) and outside (□) the VMH. The coronal sections were adapted from the Atlas of Paxinos and Watson.

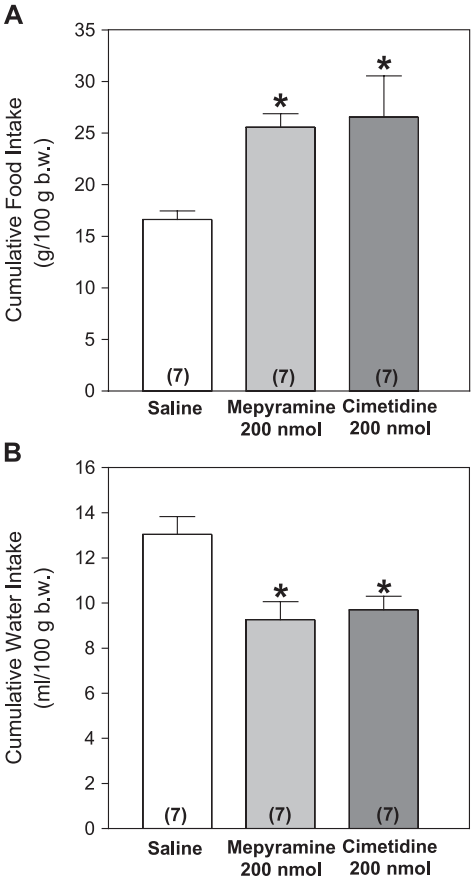

Fig. 3. Overnight cumulative food (Panel A) and water (Panel B) intakes in rats receiving bilateral VMH injections of mepyramine (n=7), cimetidine (n=7) or saline (n=7; controls). Data are presented as mean±S.E.M. Asterisks indicate a statistically significant difference between animals treated with mepyramine or cimetidine when compared to saline-treated controls.

dose of 200 nmol, significantly reduced water intake after the intragastric salt load. Fig. 4 (Panel B) shows that cimetidine VMH injections at both doses used (200 and 400 nmol) were unable to modify the high water intake exhibited by salt-loaded animals. Intragastric salt load effectively increased plasma osmolarity and sodium concentration, as shown in Fig. 5.

Fig. 6 shows that, predictably, water-deprived animals receiving VMH injections of isotonic saline solution displayed a significantly higher water intake, when compared to normohydrated animals also receiving injections of isotonic saline solution into the VMH. Panel A illustrates data revealing that mepyramine injected into the VMH, at the dose of 25 nmol, did not modify water intake in water-deprived rats. At the doses of 50 and 200 nmol, mepyramine VMH injections evoked a significant reduction in water-deprived rats. Panel B of Fig. 6, depicts the effect of cimetidine VMH injections on the water intake of water-deprived rats. Here, it is evident that cimetidine injected into the VMH, at the doses of 25 and 50 nmol was incapable of altering water intake in water-deprived rats. At the highest dose used, however, cimetidine VMH injections significantly blocked water intake in water-deprived animals.

Fig. 7 shows the result of the aversion test performed to evaluate whether any of the histamine antagonists were capable of inducing "illness-like" side effects. As expected, animals making a previous association between lithium chloride and saccharin showed a significant reduction in

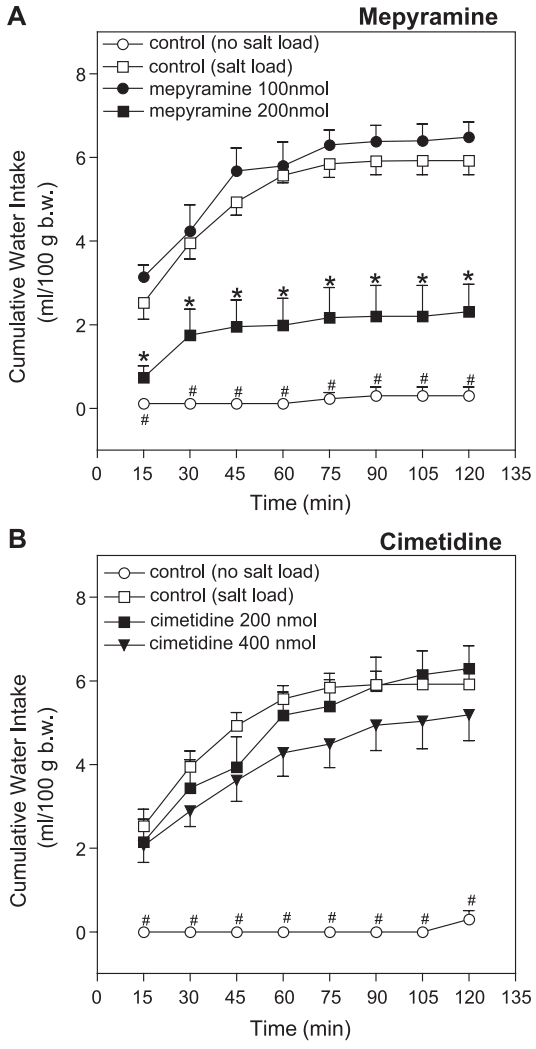

Fig. 4. Panel A: Cumulative water intake in animals receiving an acute intragastric salt load or intragastric isotonic saline treated with VMH bilateral injections of mepyramine. (○, n=13) no salt load+VMH saline; (□, n=7) salt load+VMH saline; (●, n=7) salt load+VMH mepyramine 100 nmol; (■, n=8) salt load+VMH mepyramine 200 nmol. Panel B: Cumulative water intake in animals receiving an acute intragastric salt load or intragastric isotonic saline treated with VMH bilateral injections of cimetidine. (○, n=10) no salt load+VMH saline; (□, n=9) salt load+VMH saline; (■, n=8) salt load+VMH cimetidine 200 nmol; (▼, n=7) salt load+VMH cimetidine 400 nmol. Data are presented as mean±S.E.M. # indicates a statistically significant difference when animals receiving intragastric isotonic saline are compared to animals receiving an intragastric salt load. Asterisks indicate a statistically significant difference (p<0.05) when salt-loaded animals receiving VMH injections of mepyramine are compared to salt-loaded animals receiving VMH injections of saline.

saccharin intake on the following day, as compared to saline-treated controls. In contrast, the previous association of each of the histamine antagonists (mepyramine and cimetidine) with saccharin failed to produce any reduction in saccharin intake the next day, which suggests a reduced probability that illness-like effects could explain the results observed here after the injection of these compounds into the VMH.

Fig. 8 shows that injections of either mepyramine or cimetidine into the VMH, at the highest doses used in the experimental sets already described (400 nmol), were unable to change the pattern of locomotor activity of either dehydrated or hyperosmotic rats, as compared to the locomotor behavior exhibited by dehydrated and hyperosmotic animals receiving VMH injections of saline solution.

Table 1 shows the effect of injections of mepyramine and cimetidine, both at the dose of 200 nmol, on animals whose cannulas were misplaced, being located close but not within the VMH. In this case, overnight food and water intake, as well as water intake following water deprivation and the intragastric salt load, were not modified by any of the histaminergic antagonists used.

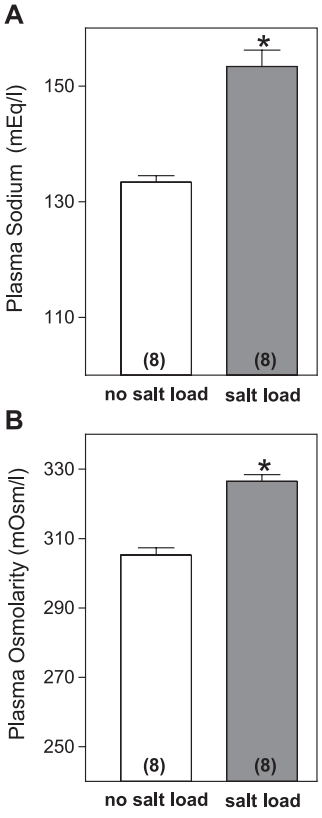

Fig. 5. Plasma sodium concentration (Panel A) and plasma osmolarity (Panel B) in animals submitted or not to an intragastric salt load. Data are presented as mean±S.E.M. Asterisks indicate a statistically significant difference when animals receiving intragastric isotonic saline (no salt load; n=8) are compared to animals receiving an intragastric salt load (n=8).

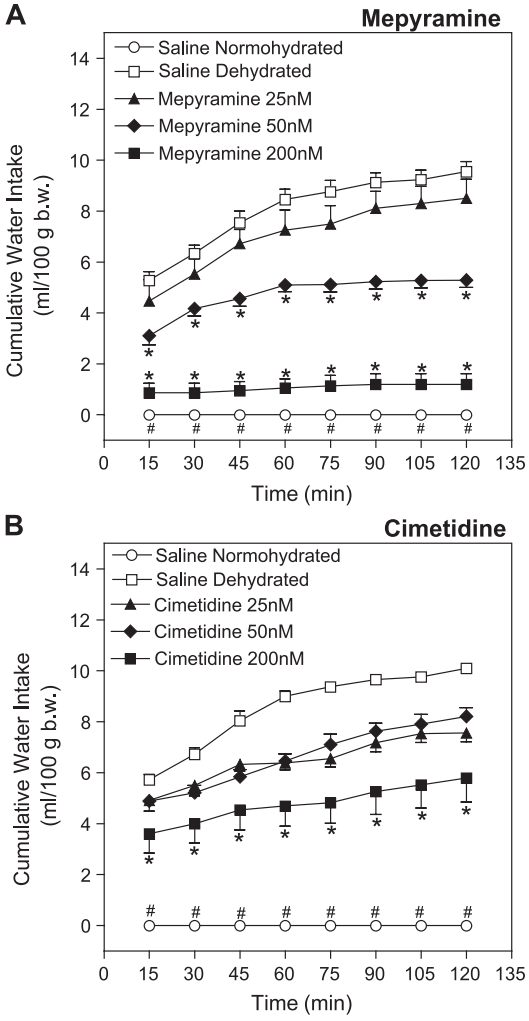

Fig. 6. Panel A: Cumulative water intake in water-deprived or normohydrated animals treated with VMH bilateral injections of mepyramine. (○, n=10) normohydrated+VMH saline; (□, n=11) water-deprived+VMH saline; (▲, n=6) water-deprived+VMH mepyramine 25 nmol; (♦, n=9) water-deprived+VMH mepyramine 50 nmol; (■, n=7) water-deprived+VMH mepyramine 200 nmol. Panel B: Cumulative water intake in water-deprived or normohydrated animals treated with VMH bilateral injections of cimetidine. (○, n=8) normohydrated+VMH saline; (□, n=8) water-deprived+VMH saline; (▲, n=7) water-deprived+VMH cimetidine 25 nmol; (♦, n=6) water-deprived+VMH cimetidine 50 nmol; (■, n=7) water-deprived+VMH cimetidine 200 nmol. Data are presented as mean±S.E.M. # indicate a statistically significant difference when water-deprived and normohydrated animals are compared. Asterisks indicate a statistically significant difference (p<0.05) when water-deprived animals receiving VMH injections of mepyramine or cimetidine are compared to water-deprived controls receiving VMH injections of saline.

4. Discussion

The present data show that the pharmacological blockade of H_1 and H_2 receptors located within the VMH resulted in a significant increase in overnight food intake. Conversely, the pharmacological blockade of VMH H_1 and H_2 receptors reduced overnight water intake. In addition, the data produced here clearly demonstrate that the pharmacological blockade of VMH H_1 receptors by

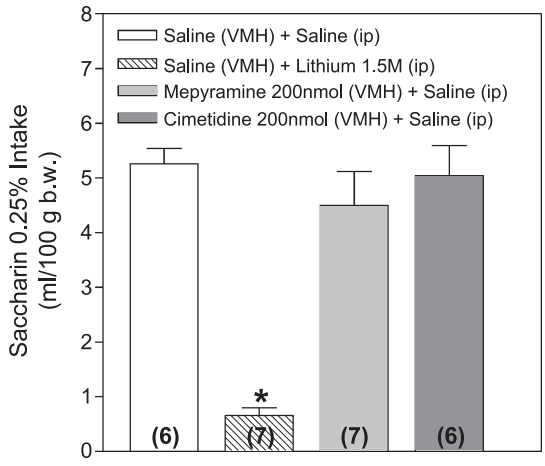

Fig. 7. Saccharin solution (0.25%) consumption (ml/100 g body weight) over 15 min at a second offering in animals receiving VMH injections of mepyramine (200 nmol), cimetidine (200 nmol) or saline (controls). The sequence of injections used during the first saccharin offering and the number of animals used are indicated in the figure. The first injection was into the VMH and the second via intraperitoneal route. The asterisk indicates a statistically significant difference ($p<0.001$) between that particular group and controls (saline+saline). The number of animals used in each experiment is indicated within each respective bar.

mepyramine reduced water intake in salt-loaded animals, while the blockade of H_2 receptors in this same region was unable to modify water intake after intragastric salt load, a procedure that effectively increased plasma osmolarity and plasma sodium concentration, as demonstrated here. The present study also shows that the blockade of both VMH H_1 and H_2 receptors generated a significant reduction in water intake in rats submitted to water deprivation. The histaminergic component identified here seems to be localized within the VMH, because injections of both histaminergic antagonists into the regions surrounding the VMH were unable to induce any significant change in water or food intake.

Injections of both antagonists into the VMH increased food intake. This seems to indicate that this procedure does not yield any "illness-like" effect or any form of motor impairment that could prevent the animals reaching the food or water sources, thus explaining the antidipsogenic actions of mepyramine and cimetidine when injected into the VMH. Indeed, neither dehydrated nor hyperosmotic rats displayed any alteration in locomotor behavior after receiving VMH injections of both antihistaminergic compounds. This was also confirmed by the aversion test performed in this study, indicating that both compounds are devoid of illness-inducing effects.

Brain histaminergic control of food intake is a well-established phenomenon (Morimoto et al., 2000). Indeed, intracerebroventricular injections of histamine suppress food intake in rats (Lecklin and Tuomisto, 1998), cats (Clineschmidt and Lotti, 1973) and goats (Tuomisto and Eriksson, 1979) and feeding releases hypothalamic histamine in rats (Itoh et al., 1991). Both H_1 and H_2 receptors in the brain seem to be involved with food intake regulation. Intracerebroventricular injections of H_1 receptor blockers elicit feeding, a result also obtained when these agents are injected directly into hypothalamic areas, such as the VMH (Fugakawa et al., 1989; Sakata et al., 1988). Therefore, the stimulatory effect of mepyramine injections into the VMH on overnight feeding as observed in the present paper, is in agreement with data previously published. The participation of brain H_2 receptors in the regulation of food intake has been less clearly demonstrated. Intracerebroventricular injections of the H_2 receptor agonist dimaprit did not modify food intake in rats (Lecklin and Tuomisto, 1998). In the present paper, bilateral injections of cimetidine into the VMH provoked a significant increase in overnight food intake, suggesting that H_2 receptors located in this region may exert a tonic inhibitory effect on overnight eating. Some authors have found that central injections of H_2 receptor blockers have no effect on food intake (Lecklin et al., 1998; Doi et al., 1994). However, these authors diluted the antagonists in low pH solutions and it is well known that

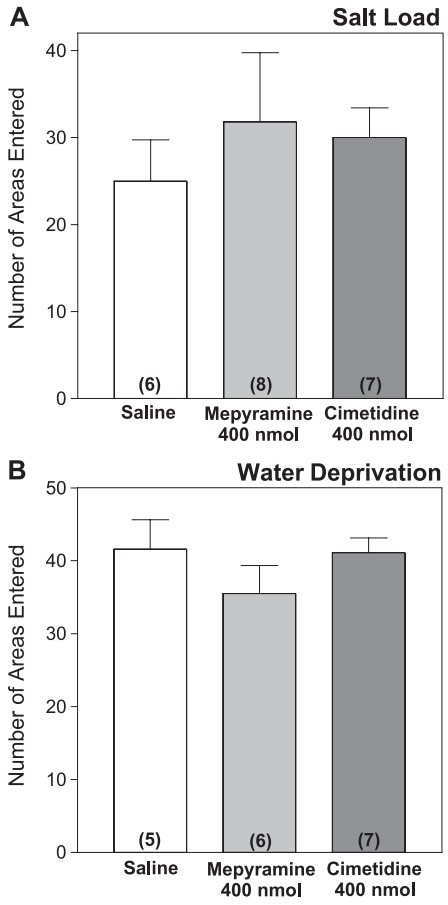

Fig. 8. Effects of VMH injections of mepyramine, cimetidine or saline on exploratory behavior of salt-loaded (Panel A) and water-deprived (Panel B) rats tested in an open-field arena. Bars represent means±S.E.M. The number of animals used in each experiment is indicated within each respective bar.

Table 1
Overnight food and water intake, and water intake after acute intragastric salt load and water deprivation following mepyramine and cimetidine injections in rats bearing cannulas in regions surrounding the VMH

Experimental procedure	Saline	Mepyramine (200 nmol)	Cimetidine (200 nmol)	ANOVA results
Overnight food intake	15.90±3.79 (*n*=4)	23.05±3.58 (*n*=4)	19.95±4.29 (*n*=4)	$F(2,9)=0.85$; $p=0.46$
Overnight water intake	8.44±1.78 (*n*=4)	7.94±1.86 (*n*=4)	10.40±0.88 (*n*=4)	$F(2,9)=0.68$; $p=0.53$
Water intake after deprivation (after 120 min)	8.65±1.89 (*n*=4)	5.81±1.08 (*n*=4)	6.38±1.41 (*n*=4)	$F(2,9)=0.99$; $p=0.40$
Water intake after hyperosmotic saline load (120 min)	5.99±0.46 (*n*=5)	5.44±0.32 (*n*=5)	6.06±0.54 (*n*=5)	$F(2,12)=0.55$; $p=0.58$

Results are shown as mean±S.E.M. There were no statistically significant differences among the groups. The number of animals used is indicated in parenthesis. The values for food intake represent g/100 g b.w. and values for water intake represent ml/100 g b.w.

intracerebroventricular injections of acidic solutions lead to a reduction in food intake in rats (Lecklin et al., 1998). Therefore, the reports in the literature of a lack of effect of H_2 antagonists on food intake, when these compounds are centrally injected may be explained by simple fact that their tendency to enhance eating is counterbalanced by the reduction in food intake generated by the injection of a low pH solution. In the present study, cimetidine was diluted in neutral saline solution. We simply heated the cimetidine-containing solution (37 °C) immediately before the injections in order to obtain homogeneity.

In this study, the pharmacological blockade of VMH H_1 and H_2 receptors elicited a significant decrease in overnight water intake. This clearly indicates that histaminergic H_1 and H_2 receptors located in this structure seem to participate in water intake regulation, exerting a tonic stimulatory drive. These findings appear to be in agreement with other studies in which the dipsogenic effect of central histamine administration has already been demonstrated (Leibowitz, 1973), as has the finding that the antihistamine-induced reduction in water intake may be reversed by the intracerebroventricular administration of histamine in rats (Gerald and Maickel, 1972). In addition, intracerebroventricular injections of selective H_3 receptor agonists, a procedure that reduces brain histaminergic activity, elicit drinking (Lecklin et al., 1998). The present results reveal that the VMH is an important brain structure related not only to the histaminergic control of food but also of water intake.

After demonstrating that both H_1 and H_2 histaminergic receptors located within the VMH seem to exert a stimulatory effect on spontaneous overnight water intake, we decided to investigate whether these receptors could also influence drinking induced by two specific physiological stimuli: water deprivation and hyperosmolarity.

In the present study, we used an acute procedure (intragastric salt load) that effectively leads to hyperosmolarity and hypernatremia, very well-known thirst-inducing stimuli. The blockade of VMH H_1 receptors by mepyramine significantly hampered water intake in hyperosmotic, hypernatremic rats. This seems to indicate that the functional integrity of this set of histaminergic receptors is essential to the triggering of the drinking response in rats under this condition.

Drinking is controlled by redundant mechanisms composed of at least two parallel neural circuits in the brain, the cholinergic and angiotensinergic systems (Fitzsimons, 1980; Saavedra, 1992; Johnson and Thunhorst, 1997). Cholinergic pathways seem to be one of the brain's thirst-inducing circuits activated during hyperosmotic conditions (Hoffman et al., 1978).

A histaminergic/cholinergic interplay seems to occur in the central nervous system. Indeed, it has been demonstrated that histamine H_1 receptor activation induces a clear-cut increase in cholinergic transmission and acetylcholine release in several brain areas, and that acetylcholine release is reduced when histamine H_1 blockers are centrally injected (Bacciottini et al., 2001; Prast et al., 1999). Furthermore, the enhancement in histaminergic transmission by the use of ciproxifan, a selective H_3 antagonist, increases c-*Fos* immunoreactivity in putative hippocampal cholinergic neurons (Bacciottini et al., 2000). The VMH corresponds to a hypothalamic site with a dense expression of cholinergic (Rao et al., 1987; Dohanich and McEwen, 1985) and histaminergic (Palacios et al., 1981; Schwartz et al., 1991) neurons. In addition, the VMH seems to be an important site related to the control of drinking, exerting a stimulatory drive on water intake, because VMH electrolytic and chemical lesions reduce drinking induced by angiotensin II central injections (Bastos et al., 1997; Do Vale et al., 1997). Thus, it is reasonable to suggest that H_1 histaminergic receptors in the VMH may exert a stimulatory role on acetylcholine release by cholinergic neurons in this region, yielding a positive drive that elicits drinking behavior in hyperosmotic, hypernatremic rats.

In this study, the administration of cimetidine into the VMH failed to produce any change in water intake induced by hyperosmolarity and hypernatremia in rats, suggesting that histaminergic H_2 receptors located in the VMH do not participate in the mechanisms that regulate drinking during hyperosmolarity. This seems to be in agreement with the findings of other research groups, demonstrating that intracerebroventricular injections of the H_2 receptor blocker cimetidine were unable to modify water intake after an intragastric salt load in rats (Kraly et al., 1995).

In this study, we have demonstrated that the pharmacological blockade of both H_1 and H_2 histaminergic receptors

in the VMH significantly reduces water intake in water-deprived rats. It would appear that thirst-triggering mechanisms activated during water deprivation require functionally active VMH H_1 and H_2 receptors. These results are in agreement with data previously published in the literature, showing that the activation of brain histaminergic pathways induces a dipsogenic response. We simply add here that a specific histaminergic component located within the VMH is essential to the generation of this dipsogenic response.

Dehydration-induced water intake is mainly regulated by the brain angiotensinergic system (Saavedra, 1992). After an extensive literature review, we were unable to find information regarding a brain histamine/angiotensin morphofunctional interplay that could easily explain the results obtained here. Yet, the VMH is a structure that sends multiple projections to several areas strongly related to the control of drinking behavior, such as the septal area, the bed nuclei of the stria terminalis, several subsets of the amygdala, the zona incerta and the medial preoptic area. It is possible that the pharmacological blockade of VMH H_1 and H_2 receptors disrupts some essential connection to areas associated with thirst-triggering mechanisms. The undeniable specificity of VMH H_1 and H_2 receptors in the control of water intake is additionally confirmed by the fact that injections of both histaminergic antagonists into areas surrounding the VMH were unable to interfere with overnight drinking or water intake induced by the two physiological stimuli used in the present study.

As previously stated, water intake induced by hyperosmolarity and by water deprivation is centrally regulated by distinct operational systems. Figs. 3 and 5 clearly show that the inhibitory effect of mepyramine VMH injections in water-deprived rats is achieved with doses lower than those required to hamper water intake in salt-loaded rats. This may suggest that the VMH H_1 component is more strongly activated after salt loads than during water deprivation.

This study was not designed to evaluate how important parameters, such as meal size, latency to initiate drinking after a meal or the temporal relationship between drinking bouts and meals, may be modified by the blockade of histamine VMH H_1 and H_2 receptors and further studies will be necessary to clarify these issues.

Brain histamine is a neurochemical system exhibiting endogenous anticonvulsant and antidepressant effects. It modulates pain, as well as ingestive behaviors, and participates in cognitive processes. It also exerts regulatory actions on thermoregulation and on sleep/arousal cycling, and influences the cardiovascular and neuroendocrine systems (Brown et al., 2001). This, associated with the broad clinical use of antihistaminergic agents still used as antiallergic or antiacid agents that easily cross the blood–brain barrier, makes the brain histamine circuitry a collateral target for a vast list of regularly prescribed drugs in today's medical practice. Elucidation of the role of a selective subset of histaminergic receptors in the central nervous system is, therefore, of both physiological and clinical importance.

In summary, the present study clearly establishes that the pharmacological blockade of H_1 and H_2 histaminergic receptors located within the VMH significantly increases overnight food intake and decreases water intake during this same period. We have also shown that the pharmacological blockade of VMH H_1 receptors reduces water intake in animals rendered hyperosmotic and hypernatremic after an intragastric salt load, while the blockade of H_2 receptors in this same region has no significant effect. Furthermore, the blockade of both H_1 and H_2 receptors located in the VMH yields a significant decrease in water intake in water-deprived rats. The inhibitory effects on water intake demonstrated here do not seem to be a consequence of any "illness-like" effects produced by the central administration of the antihistaminergic agents used because an aversion test showed that both compounds are devoid of illness-inducing effects when injected into the VMH. The effects observed here seem to point to the selective importance of H_1 and H_2 receptors anatomically located within the VMH, in view of the fact that the blockade of those receptors situated in the regions that circumscribe that structure induces no significant effect.

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References

Bacciottini L, Giovannelli L, Passani MB, Scunack W, Mannaioni PF, Blandina P. Ciproxifan and cimetidine modulates c-*fos* expression in septal neurons, and acetylcholine release from hippocampus of freely moving rats. Inflamm Res 2000;49:S41–2.

Bacciottini L, Passani MB, Mannaioni PF, Blandina P. Interactions between histaminergic and cholinergic systems in learning and memory. Behav Brain Res 2001;124:183–94.

Bastos R, Menani JV, Saad WA, Renzi A, Silveira JEN, Camargo LAA. Ventromedial hypothalamus lesions increase the dipsogenic responses and reduce the pressor response to median preoptic area activation. Physiol Behav 1997;62:311–6.

Brown RE, Stevens DR, Haas HL. The physiology of brain histamine. Prog Neurobiol 2001;63:637–72.

Clineschmidt BV, Lotti VJ. Histamine: intraventricular injections suppress ingestive behavior in the cat. Arch Int Pharmacodyn 1973;206:288–98.

De Castro JM. The interactions of fluid and food intake in the spontaneous feeding and drinking pattern of rats. Physiol Behav 1989;45:861–70.

Dohanich GP, McEwen BS. Cholineigic limbic projections and behavioral role of basal forebrain nuclei in the rat. Brain Res Bull 1985;16:477–82.

Doi T, Sakata T, Yoshimatsu H, Machidiri H, Kurokawa M, Jayasekara LALW, et al. Hypothalamic neuronal histamine regulates feeding circadian rhythm in rats. Brain Res 1994;641:311–8.

Do Vale CF, Camargo GMPA, Saad WA, Manai JV, Renzi A, Luiz AC, et al. Effect of ibotenate lesions of the ventromedial hypothalamus

on the water and salt intake induced by activation of the median preoptic nucleus in sodium-depleted rats. J Auton Nerv Syst 1997;66:19–25.
Fitzsimons JT. Peptides, thirst and sodium appetite. Proc R Soc Lond, B Biol Sci 1980;210:165–82.
Fitzsimons JT, Le Magnen J. Eating as a regulatory control of drinking in the rat. J Comp Physiol Psychol 1969;67:273–83.
Fugakawa K, Sakata T, Shiraishi T, Yoshimatsu H, Fujimoto K, Ookuma K, et al. Neuronal histamine modulates feeding behavior through H_1-receptor in rat hypothalamus. Am J Physiol 1989;256:R605–11.
Gerald MC, Maickel RP. Studies on the possible role of brain histamine in behaviour. Br J Pharmacol 1972;44:462–71.
Hoffman WE, Ganten U, Phillips MI, Schmid PG, Schelling P, Ganten D. Inhibition of drinking on water-deprived rats by combined central angiotensin II and cholinergic receptor blockade. Am J Physiol 1978;234:F41–7.
Itoh Y, Oishi R, Saeki K. Feeding-induce increase in the extracellular concentration of histamine in rat hypothalamus as measured by in vivo microdialysis. Neurosci Lett 1991;125:235–7.
Johnson AK, Thunhorst RL. The neuroendocrinology of thirst and salt appetite: visceral sensory signals and mechanisms of central integration. Front Neuroendocrinol 1997;18:292–353.
Kjaer A, Knigge U, Rouleau A, Garbarg M, Warberg J. Dehydration-induced release of vasopressin involves activation of hypothalamic histaminergic neurons. Endocrinology 1994;135:675–81.
Kraly FS. Histamine plays a part in induction of drinking by food intake. Nature 1983;302:65–6.
Kraly FS. Drinking elicited by eating. In: Epstein AN, Morrison A, editors. Progress in Psychobiology and Physiological Psychology, vol. 14. New York: Academic Press; 1990. p. 67–133.
Kraly FS, Kim Y-M, Tribuzio RA. Renal nerve transection inhibits drinking elicited by eating and by intragastric osmotic loads in rats. Physiol Behav 1995;58:1129–36.
Lecklin A, Tuomisto L. Feed intake after inhibition of histamine catabolism. Agents Actions 1990;30:216–9.
Lecklin A, Tuomisto L. The blockade of H_1 receptors attenuates the suppression of feeding and diuresis induced by inhibition of histamine catabolism. Pharmacol Biochem Behav 1998;59:753–8.
Lecklin A, Etu-Seppälä P, Stark H, Tuomisto L. Effects of intracerebroventricular infused histamine and selective H_1, H_2 and H_3 agonists on food and water intake and urine flow in Wistar rats. Brain Res 1998;793:279–88.
Leibowitz SF. Histamine: a stimulatory effect on drinking behavior in the rat. Brain Res 1973;63:440–4.
Mercer LP. Histamine and the neuroregulation of food intake. Nutrition 1997;13:581–2.
Morimoto T, Yamamoto Y, Yamatodani A. Brain histamine and feeding behavior. Behav Brain Res 2000;124:145–50.
Nachman M. Learned taste and temperature aversions due to lithium chloride sickness after temporal delays. J Comp Physiol Psychol 1970;73:22–30.
Palacios JM, Wamsley JK, Kuhar MJ. The distribution of histamine H_1-receptor in the rat brain: an autoradiographic study. Neuroscience 1981;6:15–37.
Paxinos G, Watson C. The rat brain in stereotaxic coordinates. 4th ed. Academic Press; 1998.
Prast H, Tran MH, Lamberti C, Fischer H, Kraus M, Grass K, et al. Histaminergic neurons modulate acetylcholine release in the ventral striatum: role of H_1 and H_2 histamine receptors. Naunyn Schmiedebergs Arch Pharmacol 1999;360:552–7.
Rao ZR, Yamano M, Wanaka A, Tatehata T, Shiosaka S, Tohyama M. Distribution of cholinergic neurons and fibers in the hypothalamus of the rat using choline acetyltransferase as marker. Neuroscience 1987;20:923–34.
Saavedra JM. Brain and pituitary angiotensin. Endocr Rev 1992;13:329–80.
Sakata T, Ookuma K, Fugakawa K, Fujimoto K, Yoshimatsu H, Shiraishi T, et al. Blockade of histamine H_1-receptor in the rat ventromedial hypothalamus and feeding elicitation. Brain Res 1988;441:403–7.
Sakata T, Yoshimatsu H, Kurokawa M. Hypothalamic neuronal histamine: implications of its homeostatic control of energy metabolism. Nutrition 1997;13:403–11.
Sakata T, Fugakawa K, Fujimoto K, Yoshimatsu H, Shiraishi T, Wada H. Feeding induce by blockade of histamine H_1-receptors in rat brain. Experientia 1998;44:2316–218.
Schwartz J-C, Arrang J-M, Garbarg M, Pollard H, Ruat M. Histaminergic transmission in the mammalian brain. Physiol Rev 1991;71:1–511.
Shimokawa M, Yamamoto K, Kawakami J, Sawada Y, Iga T. Neurotoxic convulsions induced by histamine H_2 receptors antagonists in mice. Toxicol Appl Pharmacol 1996;136:317–23.
Tuomisto L, Eriksson L. Antidiuresis induced by infusions of histamine into the brain ventricles of conscious hydrated goats. Eur J Pharmacol 1979;54:191–201.