Intraventricular Melanin Concentrating Hormone Stimulates Water Intake Independent of Food Intake

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MCH stimulates water intake
ABSTRACT

The lateral hypothalamus (LH) has a critical role in the control of feeding and drinking. Melanin concentrating hormone (MCH) is an orexigenic peptidergic neurotransmitter produced primarily in the LH, and Agouti-Related Protein (AgRP) is an orexigenic peptidergic neurotransmitter produced exclusively in the arcuate (ARC), an area that innervates the LH. We assessed drinking and eating after the 3rd ventricular (i3vt) administration of MCH and AgRP. MCH (2.5, 5, 10 µg, i3vt) significantly increased food as well as water intake over 4 hrs when administered during either the light or the dark portion of the day/night cycle. When MCH (5 µg) was administered to rats with access to water but no food, they drank significantly more water than when given the vehicle. AgRP (7 µg, i3vt), on the other hand, increased water intake, but only in proportion to food intake during the dark and the light, and water intake was not increased following i3vt AgRP in the absence of food. Hence, in contrast to AgRP, MCH elicits increased water intake independent of food intake. These results are consistent with historical data linking activity of the LH with water as well as food intake.
Introduction

The lateral hypothalamus (LH) is comprised of diffuse nerve cell bodies and has been linked to the control of both food and water intake. Electrical stimulation of the LH elicits increased food and water intake (1-3), and lesions of the LH cause severe anorexia and adipsia (4-7). Consistent with the LH’s role in the control of food intake, two peptide systems have been identified in the LH that stimulate food intake. Melanin-concentrating hormone (MCH) is produced almost exclusively in the LH, and several lines of evidence implicate MCH in the regulation of food intake and body weight in mammals (8-12). Intracerebroventricular administration of MCH elicits eating (9, 13). Consistent with this, mice that over-express MCH in the LH are obese and insulin resistant (14). The converse is also true, with mice lacking MCH having reduced food intake, body weight and body fat (10). Consistent with MCH being involved in the regulation of energy homeostasis, fasting increases expression of MCH mRNA in the LH of both normal and obese animals (9). The LH also contains neurons that synthesize orexins (also termed hypocretins) (15-20), neuropeptides that are also implicated in the control of food intake. Intraventricular administration of orexin-A or orexin-B increases food intake (20), and fasting increases the levels of preprorexin mRNA in the LH (18-20).

The LH receives prominent projections from the hypothalamic arcuate nucleus (ARC), a site of synthesis of other orexigenic peptides. The best known of these are neuropeptide Y (NPY) and agouti-related protein (AgRP), peptides that are synthesized within the same ARC neurons (15, 21-23). NPY and AgRP gene expression in the ARC are increased during food restriction (21, 23-27), and projections from the ARC synapse on MCH- and orexin-producing neurons (28-30). NPY and AgRP elicit robust eating when administered either into the ventricular system or into specific
hypothalamic nuclei, with the most potent response for NPY seen in the periventricular region of the hypothalamus (31-36).

The overlap of circuitry among all of these orexigenic peptides in the LH supports the hypothesis that their roles in stimulating food intake may be functionally inter-connected. While ample evidence links each of these peptide systems to the control of food intake, several of them have also been implicated in the control of fluid intake. Intraventricular administration of NPY elicits a dose-dependent increase of water intake (31, 37, 38) that is not secondary to food intake since it occurs in the absence of food (39). Orexin A stimulates water intake as well (20, 40), and this also reportedly occurs whether food is available to the animals or not. Consistent with this, expression of prepro-orexin mRNA in the LH is up-regulated by dehydration (40). We have found no reports of water intake following AgRP or MCH administration. However, when rats have only a hypertonic saline solution to drink, a situation which leads to plasma hyperosmolarity and which normally elicits increased water intake, there is a marked increase in MCH-like immunoreactivity (MCH-LI) in the LH (41, 42). In the present series of experiments, we evaluated the hypothesis that MCH or AgRP increase water consumption independent of their ability to increase food intake.

Materials and Methods

Animals. All procedures were approved by the Institutional Animal Care and Use Committee of the University of Cincinnati and were conducted in AAALAC-accredited facilities. Male Long-Evans rats (350-500 g; Harlan, Indianapolis, IN) were housed individually in plastic tub cages and maintained on a 12:12 (light:dark) schedule at constant temperature (20° C). They had free access to pelleted rat chow (Harlan-Teklab, Indianapolis, IN) and water except as noted below. One week
after arrival in the vivarium, each rat was implanted with a cannula stereotaxically placed in the 3rd cerebral ventricle as described previously (43). In the procedure, rats were anesthetized with ketamine/xylazine (10:6.5 solution; 0.1 mL/kg) and placed into a stereotaxic frame. A 22-gauge guide sleeve was lowered along the midsagittal plane into the 3rd ventricle according to the coordinates of Paxinos and Watson (2.2 mm caudal to bregma and 7.5 mm ventral to the sinus, with lambda and bregma at the same vertical coordinate) and then secured to the skull with screws and dental acrylic. Following recovery of body weight, cannula placement was confirmed via injection of 10 ng angiotensin II through a cannula extending 1 mm beyond the tip of the guide sleeve (i3vt injection). Only those animals that consumed at least 5 mL of water within 30 minutes of the injection were deemed to have correct placement and were used in the subsequent studies. All animals were then habituated to daily handling, and to receive i3vt injections of physiological saline (2 µl) at the same time each day (see below). Rats in each experiment were assigned to groups matched by body weight.

Procedure. Experimental drugs were physiological saline, MCH (Phoenix Pharmaceuticals, Inc., Mountain View, CA) in saline, or AgRP (Phoenix Pharmaceuticals) in saline, each in 2 µl. Individual rats received saline, MCH and AgRP on different experimental days, the order of the doses of MCH and AgRP being random for each rat, with at least 3 days between successive treatments.

Food and water intake following the administration of MCH (2.5, 5, and 10 µg/rat) and AgRP (0.7, 2.1, 7 µg/rat) were assessed during the middle of the light phase, 5 hours prior to lights out. For these assessments, food but not water was removed for one hour prior to the injections and returned
immediately after the injections. Food and water intake were assessed hourly for 4 hours.

Subsequent experiments used doses of MCH (5 µg/rat) and AgRP (7 µg/rat) found to be orexigenic in the first experiment.

To determine the effect of time of day, a separate cohort of weight-matched rats was assessed for food and water intake following the administration of MCH (5 µg/rat) or AgRP (7 µg/rat) at the beginning of the dark. The injections were given 30 minutes prior to lights out, and food and water were then removed for 30 minutes and returned at lights out. Food and water were assessed hourly for 4 hours.

In a novel cohort of rats, water intake was assessed in the absence of food during the light phase. In this situation, the same general paradigm as above was followed except that food was not available until two hours following the injections. Animals were injected with either 5 µg MCH, 7 µg AgRP in 2 µl saline, or saline 5 hours prior to the onset of dark. Animals were allowed access to water only and intake was assessed every 30 minutes over two hours. Food was then returned, and intake was monitored over the next two hours.

A separate cohort of rats was habituated to hanging wire metabolic cages for one week prior to experimentation. On the experimental day, food and water were removed from the cages one hour prior to an i3vt injection of 5 µg MCH in 2 µl saline or saline alone. Urine was collected one hour later. The rats had no food or water access during the hour.
Food and water were removed from the cages of another cohort of rats one hour prior to an i3vt injection of 5 μg MCH in 2 μl saline or saline alone. One hour following the injection, the rats were sacrificed by CO₂ anesthesia and decapitation. Trunk blood was collected in EDTA-coated tubes. Plasma was isolated from whole blood by centrifugation and stored at −20°C until analysis for osmolality by the clinical laboratories of the Health Alliance of Cincinnati.

Data Analysis. The data were analyzed by parametric statistics (repeated measures MANOVA with time as the repeated measure, followed by planned t-tests). Significance was set at P < 0.05, 2-tailed.

Results

In general, the data were comparable at every time point assessed. The figures and the analyses presented reflect the 2-hr values, but they are representative of the other times as well.

MCH, but not AgRP, stimulates water intake with a shorter latency than food intake.

MCH and AgRP reportedly stimulate food intake over different intervals following their administration; i.e., MCH stimulates food/water intake within 10 minutes of injection, whereas AgRP stimulates food intake beginning 45 to 60 minutes after injection. The latency to drink water following an MCH injection is 2.6 ± 0.5 minutes, whereas the latency to consume food is 9.5 ± 2.5 minutes (P < 0.05). These are in contrast to what occurs for a saline injected animal in which the latency to drink water is 78.0 ± 12.6 minutes, and to consume food is 78.0 ± 10.2 minutes. As depicted in the latency numbers, every MCH-treated animal consumed water first, followed by
food. AgRP-treated animals, on the other hand, consumed food first, followed by ingestion of water (latency to consume chow is 40 ± 0.4 minutes, and a latency to drink is 55 ± 0.8 minutes), both of which are significantly different from the MCH-treated animals (P < 0.05). Therefore, to make the most appropriate comparisons, data are presented for the 2-hr time point.

**MCH and AgRP stimulate food intake.**

Food intake data for MCH and AgRP are presented in Figures 1-4. During the light, all doses of MCH (2.5, 5, 10 µg) were significantly orexigenic relative to saline (Figure 1) whereas only the highest dose of AgRP (7 µg) was orexigenic (Figure 2). Food intake was significantly higher in the saline condition during the dark than in the light (P < 0.01, Figures 3 and 4), and 5 µg of MCH (Figure 3) and 7 µg of AgRP (Figure 4) were comparably orexigenic.

**MCH, but not AgRP, stimulates water intake.**

In the control (saline only) condition during the light, rats consumed less than 2 g each of food and water (Figures 1 and 2). At this time, i3vt MCH but not AgRP stimulated water intake (Figures 1 and 2). Following the lowest dose of MCH (2.5 µg) the increase of water intake did not attain significance, but it did after the two higher doses (Figure 1). In contrast, water intake did not reliably increase after any dose of i3vt AgRP during the light. Likewise, MCH but not AgRP significantly increased water intake during the dark (Figures 3 and 4). Even though the effect of MCH on water intake when food was present attained statistical significance whereas that following AgRP did not, water intake was nonetheless slightly elevated following AgRP.

**MCH stimulates water intake in the absence of food.**
To assess water intake without the confound of food intake, animals were administered saline, MCH (5µg) or AgRP (7µg) and allowed access to water but no food. As depicted in Figures 5 and 6, MCH significantly increased water intake whereas AgRP did not. All rats receiving MCH urinated in the hour following administration and water consumption whereas no rat receiving saline was observed to urinate.

**MCH neither creates a diuresis nor increases plasma osmolality.**

The volume of urine excreted, in the absence of water access, during the hour following i3vt MCH (0.18 ± 0.13 mL) was not significantly different than that which occurred in the control condition (0.33 ± 0.20 mL, p > 0.05). Further, the same number of rats in each group excreted urine (2 of 7 per group, and the data represent averages for all animals treated). There was no difference in plasma osmolality between saline and MCH-treated groups (316 ± 2.4 mOs/kg, n = 5; and 310 ± 2.1 mOs/kg, n = 6, respectively).

**Discussion**

The relative abilities of MCH and AgRP to stimulate food and water intake were determined in male Long Evans rats. As we (13, 36), and others (9, 17, 20, 44-48), have observed, selected doses of these peptides increased acute food intake when water was simultaneously available. When food was simultaneously available, MCH but not AgRP also caused a significant increase of water intake, and this was true during the light as well as the dark. Consistent with this, the latency to consume water in the MCH-treated animals was significantly shorter than the latency to consume food. Additionally, the AgRP-treated animals consumed food first, followed by water with
significantly longer latencies when compared to MCH-treated animals. In contrast, the latency to consume water or food in saline treated animals was much longer.

While these data imply that MCH stimulates water intake independent of food consumption, such a conclusion dictates that water intake be assessed when food is not available. We therefore repeated the experiment in the absence of food. Again, water intake was increased following MCH but not following AgRP. Therefore, the drinking response after MCH cannot be a consequence of increased food consumption. When food was returned in that experiment two hours after the injections, rats which had received either MCH or AgRP had elevated food intake in the 60 min following the return of chow (data not depicted). All of these observations are consistent with the conclusion that MCH stimulates both food and water intake.

Increased water intake could result from stimulation of central circuits directly influencing the behavior, or, alternatively, could be secondary to water loss (diuresis) or hypertonicity (increased osmolality). We therefore assessed both urine output and plasma osmolality after MCH administration. Plasma osmolality was comparable between groups, and urine output was unchanged following MCH but prior to having access to water. Urine output did increase following water consumption in MCH-treated animals, however. The present data therefore support a central role for MCH to elicit water consumption that is not dependent on overt changes in fluid balance. Specifically, our data suggest that the effect of MCH on water consumption is centrally mediated, and is independent of any effects on water excretion or osmolality. One possibility, therefore, is that food and water intake may be mediated by anatomically separate populations of MCH.
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receptors. Future studies may focus on the specific sites of actions to further our understanding of
the mechanism and location by which MCH stimulates water intake.

The LH and the adjacent Zona Incerta (ZI) are thought to have an important role in regulating
drinking as well as feeding behavior, since lesions of these regions of the brain result in both
aphagia and adipsia (49, 50). LH lesions typically result in an adipsia that outlasts the more
transient period of aphagia as these animals approach a lower body weight. In fact, animals with
LH lesions become principally prandial drinkers in that they only drink when they eat, and then
drink just enough to facilitate mastication (51). LH-lesioned rats drink as much water as controls
following intracellular or extracellular dehydration, but restrict their ad lib daily water intake to the
minimal requirement for fluid balance, suggesting a specific impairment of secondary drinking (52).

Consistent with these observations, stimulation of the LH and immediately surrounding regions,
either electrically or chemically, increases water intake (1, 53). Oomura et al. (54) found that some
LH neurons are osmosensitive; activity of these neurons is increased by administration of a high
concentration of NaCl solution. All of these observations suggest that the neurons in the LH and ZI
have important roles in drinking behavior. The expression of MCH receptor mRNA has been
reported in the LH and ZI by Saito et al. (55). Since MCH neurons densely innervate the cerebral
cortex and limbic system, it is possible that MCH may also have a role in cognitive, emotional and
motivational aspects of drinking behavior (55). Additionally, Zamir et al. (41) found that an
osmotic stimulus (2% NaCl as drinking water for 120-hours) caused a marked increase in MCH-LI
concentrations in the LH and posterior lobe of the pituitary. These data are consistent with the
hypothesis that both the food and water intake induced by electrical stimulation of the LH are mediated by the MCH system.

Neurobiologists have been investigating the hypothalamic control over ingestive behavior for over half a century, and numerous conceptualizations of the nature of that control have been expressed over that span. Stellar was particularly influential when he suggested that functional centers exist in the hypothalamus, each center responsible for one or another aspect of ingestion (56). In this schema, the ventromedial hypothalamus (VMH) was considered a satiety center because stimulation there caused animals to stop eating and because a lesion of the VMH caused animals to eat large meals and become obese (57). Analogously, the lateral hypothalamus (LH) was considered an eating center because stimulation there led to increased food intake and lesions of the LH caused severe aphagia (4-7). Animals with stimulating electrodes in the LH increase food and/or water intake, depending upon what is available to them, suggesting that the same or proximally close neurons in the LH are responsible for both behaviors. While the tenet that the hypothalamus is organized according to functional centers has been challenged many times over the years (see reviews in (58, 59)), the LH remains an important center involved in the control of ingestion. Furthermore, our data suggest that MCH, a neuropeptide secreted from the LH, is involved in both water and food intake.
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Figure 1. Effect of Intraventricular (i3vt) MCH on Food and Water Intake During the Light.
Mean (+ SEM) 2-h food (open bars) and water intake (filled bars) after i3vt administration of MCH (2.5, 5, and 10 µg/rat) or vehicle. Rats (n = 40) received the 4 injections in random order, * p < 0.05 as compared to vehicle.

Figure 2. Effect of Intraventricular (i3vt) AgRP on Food and Water Intake During the Light.
Mean (+ SEM) 2-h food (open bars) and water intake (filled bars) after i3vt administration of AgRP (0.7, 2.1, 7 µg/rat) or vehicle. Rats (n = 30) received the 4 injections in random order, * p < 0.05 as compared to vehicle.

Figure 3. Effect of Intraventricular (i3vt) MCH on Food and Water Intake During the Dark.
Mean (+ SEM) 2-h food (open bars) and water intake (filled bars) after i3vt administration of MCH (5 µg/rat) or vehicle. Rats (n = 20) received both injections in random order, * p < 0.05 as compared to vehicle.

Figure 4. Effect of Intraventricular (i3vt) AgRP on Food and Water Intake During the Dark.
Mean (+ SEM) 2-h food (open bars) and water intake (filled bars) after i3vt administration of AgRP (7 µg/rat) or vehicle. Rats (n = 28) received both injections in random order, * p < 0.05 as compared to vehicle.
Figure 5. Effect of Intraventricular (i3vt) MCH on Water Intake in the Absence of Food.

Mean (+ SEM) 2-h water intake after i3vt administration of MCH (5 µg/rat) or vehicle (n = 8), * p < 0.05 as compared to vehicle.

Figure 6. Effect of Intraventricular (i3vt) AgRP on Water Intake in the Absence of Food.

Mean (+ SEM) 2-h water intake after i3vt administration of AgRP (7 µg/rat) or vehicle (n = 8), * p < 0.05 as compared to vehicle.
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Figure 1

![Bar chart showing water intake (g) for saline and different doses of MCH.](chart1)

- **saline**
- **2.5 µg**
- **5.0 µg**
- **10.0 µg**

![Graph showing effect of different doses of MCH on 2-h water intake.](chart2)

- **2-h food**
- **2-h water**

Figure 2

Intake (g)

- * indicates a significant difference.
Figure 3

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Figure 4
Figure 5

![Bar graph showing MCH Stimulates Water Intake](image1)

Figure 6

![Bar graph showing AgRP](image2)