Intraventricular melanin-concentrating hormone stimulates water intake independent of food intake

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Clegg, Deborah J., Ellen L. Air, Stephen C. Benoit, Randall S. Sakai, Randy J. Seeley, and Stephen C. Woods. Intraventricular melanin-concentrating hormone stimulates water intake independent of food intake. Am J Physiol Regul Integr Comp Physiol 284: R494–R499, 2003.—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 third ventricular (i3vt) administration of MCH and AgRP. MCH (2.5, 5, and 10 μg i3vt) significantly increased food as well as water intake over 4 h 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 after 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.

agouti-related protein; orexin-A; lateral hypothalamus

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 (27, 29, 44), and lesions of the LH cause severe anorexia and adipsia (1, 51–53). Consistent with the role of the LH 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 (23, 38, 43, 54, 55). Intracerebroventricular administration of MCH elicits eating (9, 38). Consistent with this, mice that overexpress MCH in the LH are obese and insulin resistant (24). The converse is also true, with mice lacking MCH having reduced food intake, body weight, and body fat (43). 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 (38). The LH also contains neurons that synthesize orexins (also termed hypocretins) (6, 10, 20, 35, 42, 57), neuropeptides that are also implicated in the control of food intake. Intraventricular administration of orexin-A or orexin-B increases food intake (42), and fasting increases the levels of prepro-orexin mRNA in the LH (10, 35, 42).

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 (6, 15, 17, 31). NPY and AgRP gene expression in the ARC are increased during food restriction (3, 15, 21, 28, 31, 56), and projections from the ARC synapse on MCH- and orexin-producing neurons (2, 25, 37). 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 (5, 8, 14, 30, 45, 47).

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 interconnected. 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 (34, 46, 47) that is not secondary to food intake because it occurs in the absence of food (48). Orexin A stimulates water intake as well (22, 42), and this also reportedly occurs whether or not food is available to the animals. Consistent with this, expression of prepro-orexin mRNA in the LH is upregulated by dehydration (22). We have found no reports of water intake after AgRP or MCH administration. However, when rats...
have only a hypertonic saline solution to drink, a situation that leads to plasma hyperosmolarity and that normally elicits increased water intake, there is a marked increase in MCH-like immunoreactivity (MCH-LI) in the LH (36, 58). In the present series of experiments, we evaluated the hypothesis that MCH or AgRP increases water consumption independent of its 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 American Association for Accreditation of Laboratory Animal Care-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-h (light-dark) schedule at constant temperature (20°C). They had free access to pelleted rat chow (Harlan-Teklad, 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 third cerebral ventricle as described previously (7). In the procedure, rats were anesthetized with ketamine-xylazine (10:6.5 g/rat) and AgRP (7 H9262 l. 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 after the administration of MCH (2.5, 5, and 10 H9262 g/rat) and AgRP (0.7, 2.1, 7 H9262 g/rat) were assessed during the middle of the light phase, 5 h before lights out. For these assessments, food, and water was removed for 1 h before the injections and returned immediately after the injections. Food and water intake were assessed hourly for 4 h. Subsequent experiments used doses of MCH (5 H9262 g/rat) and AgRP (7 H9262 g/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 after the administration of MCH (5 H9262 g/rat) or AgRP (7 H9262 g/rat) at the beginning of the dark. The injections were given 30 min before lights out, and food and water were then removed for 30 min and returned at lights out. Food and water were assessed hourly for 4 h.

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 2 h after the injections. Animals were injected with either 5 H9262 g MCH, 7 H9262 g AgRP in 2 H9262 ml saline, or saline 5 h before the onset of dark. Animals were allowed access to water only, and intake was assessed every 30 min over 2 h. Food was then returned, and intake was monitored over the next 2 h.

A separate cohort of rats was habituated to hanging wire metabolic cages for 1 wk before experimentation. On the experimental day, food and water were removed from the cages 1 h before an i3vt injection of 5 H9262 g MCH in 2 H9262 ml saline or saline alone. Urine was collected 1 h 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 1 h before an i3vt injection of 5 H9262 g MCH in 2 H9262 ml saline or saline alone. One hour after the injection, the rats were killed 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 multivariate ANOVA with time as the repeated measure, followed by planned t-tests). Significance was set at P < 0.05 (2-tailed).

RESULTS

In general, these data were comparable at every time point assessed. The figures and the analyses presented reflect the 2-h 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 after their administration; i.e., MCH stimulates food/water intake within 10 min of injection, whereas AgRP stimulates food intake beginning 45–60 min after injection. The latency to drink water after an MCH injection is 2.6 ± 0.5 min, whereas the latency to consume food is 9.5 ± 2.5 min (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 min and to consume food is 78.0 ± 10.2 min. 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 min, and a latency to drink is 55 ± 0.8 min), 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-h time point.

MCH and AgRP stimulate food intake. Food intake data for MCH and AgRP are presented in Figs. 1–4. During the light, all doses of MCH (2.5, 5, 10 H9262 g) were significantly orexigenic relative to saline (Fig. 1), whereas only the highest dose of AgRP (7 H9262 g) was orexigenic (Fig. 2). Food intake was significantly higher in the saline condition during the dark than in the light (P < 0.01, Figs. 3 and 4), and 5 H9262 g MCH (Fig. 3) and 7 H9262 g AgRP (Fig. 4) were comparably orexigenic.

MCH, but not AgRP, stimulates water intake. In the control (saline only) condition during the light, rats

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consumed <2 g each of food and water (Figs. 1 and 2). At this time, i3vt MCH but not AgRP stimulated water intake (Figs. 1 and 2). After 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 (Fig. 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 (Figs. 3 and 4). Even though the effect of MCH on water intake when food was present attained statistical significance whereas that after AgRP did not, water intake was nonetheless slightly elevated after 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 (5 µg), or AgRP (7 µg) and allowed access to water but no food. As depicted in Figs. 5 and 6, MCH significantly increased water intake whereas AgRP did not. All rats receiving MCH urinated in the hour after 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 after i3vt MCH (0.18 ± 0.13 ml) was not significantly different from that which occurred in the control condition (0.33 ± 0.20 ml, P > 0.05). Furthermore, 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 mosmol/kgH2O, n = 5; and 310 ± 2.1 mosmol/kgH2O, 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 (9, 14) and others (12, 16, 19, 38–40, 42, 57) 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 compared with 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 after MCH but not after AgRP. Therefore, the drinking response after MCH cannot be a consequence of increased food consumption. When food was returned in that experiment 2 h after the injections, rats that had received either MCH or AgRP had elevated food intake in the 60 min after 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 after MCH but before having access to water. Urine output did increase after 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 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, because lesions of these regions of the brain result in both aphagia and adipsia (26, 32). 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 (4). LH-lesioned rats drink as much water as controls after intracellular or extracellular dehydration but restrict their ad libitum daily water intake to the minimal requirement for fluid balance, suggesting a specific impairment of secondary drinking (11).

Consistent with these observations, stimulation of the LH and immediately surrounding regions, either electrically or chemically, increases water intake (13, 29). Oomura et al. (33) 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. (41). Because 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 (41). Additionally, Zamir et al. (58) found that an osmotic stimulus (2% NaCl as drinking water for 120 h) caused a marked increase in MCH-LI concentrations in the LH and posterior lobe of the pituitary. These data are consist-

![Fig. 5. Effect of i3vt MCH on water intake in the absence of food: mean (±SE) 2-h water intake after i3vt administration of MCH (5 μg/rat) or vehicle (n = 8). *P < 0.05 compared with vehicle.](http://ajpregu.physiology.org/)

![Fig. 6. Effect of i3vt AgRP on water intake in the absence of food: mean (±SE) 2-h water intake after i3vt administration of AgRP (7 μg/rat) or vehicle (n = 8).](http://ajpregu.physiology.org/)
tent 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 more than half a century, and numerous conceptualizations of the nature of that control have been expressed over that span. Stellar (49) was particularly influential when suggesting that functional centers exist in the hypothalamus, each center responsible for one or another aspect of ingestion. 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 (18). Analogously, the LH was considered an eating center because stimulation there led to increased food intake and lesions of the LH caused severe aphagia (1, 51). Animals with stimulating electrodes in the LH increase food and/or water intake, depending on 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 Refs. 50, 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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