Effect of intracerebroventricular alpha-MSH on food intake, adiposity, c-Fos induction, and neuropeptide expression.

Permalink
https://escholarship.org/uc/item/5rq4c359

Journal
American journal of physiology. Regulatory, integrative and comparative physiology, 279(2)

ISSN
0363-6119

Authors
McMinn, JE
Wilkinson, CW
Havel, PJ
et al.

Publication Date
2000-08-01

DOI
10.1152/ajpregu.2000.279.2.r695

Peer reviewed
Effect of intracerebroventricular α-MSH on food intake, adiposity, c-Fos induction, and neuropeptide expression

JULIE E. McMINTN, CHARLES W. WILKINSON, STEPHEN C. WOODS, AND MICHAEL W. SCHWARTZ

Program in Nutritional Sciences, Departments of Psychiatry and Behavioral Sciences and Medicine, University of Washington, Seattle 98195; Puget Sound Veterans Affairs Health Care System, Seattle 98108; Harborview Medical Center, Seattle, Washington 98104; Department of Nutrition, University of California at Davis, Davis, California 95616; and Department of Psychiatry, University of Cincinnati, Cincinnati, Ohio 45267

Received 22 October 1999; accepted in final form 20 March 2000

McMinn, Julie E., Charles W. Wilkinson, Peter J. Havel, Stephen C. Woods, and Michael W. Schwartz. Effect of intracerebroventricular α-MSH on food intake, adiposity, c-Fos induction, and neuropeptide expression. Am J Physiol Regulatory Integrative Comp Physiol 279: R695–R703, 2000.—α-Melanocyte-stimulating hormone (α-MSH) is a hypothalamic neuropeptide proposed to play a key role in energy homeostasis. To investigate the behavioral, metabolic, and hypothalamic responses to chronic central α-MSH administration, α-MSH was infused continuously into the third cerebral ventricle of rats for 6 days. Chronic α-MSH infusion reduced cumulative food intake by 10.7% (P < 0.05 vs. saline) and body weight by 4.3% (P < 0.01 vs. saline), which in turn lowered plasma insulin levels by 29.3% (P < 0.05 vs. saline). However, α-MSH did not cause adipose-specific wasting nor did it alter hypothalamic neuropeptide mRNA levels. Central α-MSH infusion acutely activated neurons in forebrain areas such as the hypothalamic paraventricular nucleus, as measured by a 254% increase in c-Fos-like immunoreactivity (P < 0.01 vs. saline), as well as satiety pathways in the hindbrain. Our findings suggest that, although an increase of central melanocortin receptor signaling acutely reduces food intake and body weight, its anorectic potency wanes during chronic infusion and causes only a modest decrease of body weight.

α-Melanocyte-stimulating hormone (α-MSH) and its analogs acutely suppress food intake after intracerebroventricular administration in rats and mice (6, 11, 14, 23, 29, 31, 40, 41). Several observations suggest a physiological role for this anorectic response to α-MSH. For example, conditions associated with an increased drive to consume food, such as fasting (2, 5) and genetic leptin deficiency (26, 36), are accompanied by reduced expression of the gene encoding proopiomelanocortin (POMC; the precursor molecule for α-MSH) in the hypothalamic arcuate nucleus (Arc). Conversely, POMC mRNA is upregulated in the overfed state (15) and by acute injections of leptin (26, 36). In addition, rodents and humans with mutations of the gene encoding POMC, or POMC processing enzymes, are obese and hyperphagic (20, 30).

After its release from axon terminals in hypothalamic areas such as the paraventricular nucleus (PVN), α-MSH binds to and activates neuronal melanocortin-4 (MC-4) receptors. Intracerebroventricularly administered MC-4 receptor antagonists acutely stimulate food intake (11, 33), and MC-4 receptor-deficient mice (19) and humans (42) have an obese phenotype, suggesting that this receptor subtype plays a major role in the anorectic action of α-MSH. The agouti mouse is also obese, presumably due to ectopic production of the melanocortin receptor antagonist agouti in the brain (11). Agouti-related peptide (AgRP) is a naturally occurring endogenous antagonist to the MC-4 receptor that is synthesized in a subset of Arc neurons adjacent to POMC cells. Centrally administered AgRP blocks the catabolic action of α-MSH and increases food intake when given intracerebroventricularly, suggesting that melanocortin signaling is essential to constrain food intake. AgRP is colocalized with neuropeptide Y (NPY) in Arc neurons, and, similar to NPY, AgRP is upregulated in response to fasting (16, 27), leptin deficiency (27, 43), and streptozotocin-induced diabetes (18), suggesting that AgRP also plays a physiological role in body weight regulation. We therefore hypothesized that chronic intracerebroventricular infusion of α-MSH would promote a sustained state of negative energy balance, leading to a reduction in body weight.

Candidate targets of first-order neurons releasing α-MSH include second-order neurons that express the catabolic peptide corticotropin-releasing hormone (CRH), contained in the parvocellular region of the PVN, as well as second-order neurons expressing the orexigenic peptide melanin-concentrating hormone (MCH). Central delivery of MCH also promotes a state of increased food intake (17). AgRP, on the other hand, is expressed in neurons that inhibit food intake by suppressing the release of orexigenic peptides, such as NPY (16, 50). Ala-MSH increases MCH mRNA in neuronal populations, but the effect of α-MSH on CRH mRNA is unknown (50).

Developmental studies have shown that the melanocortin signaling pathway is a key player in the development of the hypothalamic satiety system. In mice, conditional ablation of the MC-4 receptor in neuronal populations results in obesity and hyperphagia (45). However, mice with mutations in the agouti gene, which encode the endogenous MC-4 receptor antagonist agouti, are also obese and hyperphagic (20, 30). This suggests that both melanocortin agonists and antagonists play a critical role in energy homeostasis.

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

http://www.ajpregu.org 0363-6119/00 $5.00 Copyright © 2000 the American Physiological Society
(MCH) in the lateral hypothalamic area (LHA). In support of this model, both the PVN and LHA are supplied by melanocortin-containing projections from the Arc (8, 9) and express MC-4 receptor mRNA (7a, 28). In addition, expression of CRH and MCH is altered by changes in energy balance (5, 32). We hypothesized, therefore, that CRH and MCH neurons are targets of \( \alpha \)-MSH signaling, whereas POMC and AgRP gene expression appear to be regulated by a direct action of leptin, binding to leptin receptors located on Arc neurons transcribing these peptides (1, 7, 10). Thus we also hypothesized that intracerebroventricular \( \alpha \)-MSH infusion would alter CRH and MCH gene expression via melanocortin receptors located in areas of the brain containing these neuropeptides, but would not alter POMC or AgRP expression.

To test these hypotheses, we administered \( \alpha \)-MSH into the third ventricle of rats over 6 days to ascertain its chronic effects on food intake, body weight, plasma hormone levels, body composition, and neuropeptide expression in the hypothalamus. In addition, we hypothesized that a single injection of \( \alpha \)-MSH would acutely activate neuronal substrates in energy regulation pathways. To identify these sites, we injected rats in the third ventricle with \( \alpha \)-MSH and quantified c-Fos-like immunoreactive nuclei in the forebrain and hindbrain.

MATERIALS AND METHODS

Animals

All studies used male Long-Evans rats (300–350 g) from the breeding colony maintained by the Department of Psychology at the University of Washington housed individually in wire-mesh hanging cages (experiments 1 and 3) or male Wistar rats weighing 300–350 g (Simonsen Laboratories, Gilroy, CA) were housed in polycarbonate cages (experiment 2) in a temperature-controlled vivarium on a 12:12-h light-dark schedule. Unless otherwise specified, animals were given free access to pelleted rat chow (Harlan Teklad, Madison, WI, experiments 1 and 3; Ralston Purina, St. Louis, MO, experiment 2) and water at all times. All procedures were performed according to institutional guidelines of the Animal Care and Use Committee at the Seattle Veterans Affairs Medical Center and University of Washington.

Cannula Placement

Rats were habituated with daily handling for 1 wk before surgery. After anesthesia induced by intraperitoneal injection of ketamine-xylazine (60 mg/kg ketamine and 8 mg/kg xylazine), a 21-gauge cannula (Plastics One, Roanoke, VA) was placed stereotaxically into the third ventricle using a previously described method (37, 39). Cannula placement was verified 1 wk after surgery by intracerebroventricular injection of 10 ng angiotensin II (American Peptide, Sunnyvale, CA) diluted in 1 \( \mu \)l saline. Animals not consuming at least 5 ml water 30 min postinjection were excluded as cannulation failures (4% of all rats). Experiments were performed at least 2–3 wk after surgery.

Experimental Protocols

**Experiment 1: acute effect of \( \alpha \)-MSH on food intake.** Cannulas were surgically implanted in the third ventricle of Long-Evans rats, as described above. Food hoppers were removed from the animal cages at 1500 and weighed. At 1600, animals were injected in the third ventricle with either 5 \( \mu \)l of sterile, 0.9% preservative-free saline (Fujisawa USA, Deerfield, IL) or an equal volume of human \( \alpha \)-MSH (Peninsula Laboratories, Belmont, CA) in saline using an injector (Plastics One) attached by polyethylene tubing to a 25-\( \mu \)l glass syringe (Hamilton, Reno, NV). A study comparing doses of 0, 2.5, 25, and 50 \( \mu \)g \( \alpha \)-MSH (\( n = 5–7 \)) was performed. For each trial, infusate was delivered manually over a period of 2 min, after which time animals received an intramuscular injection of 8 mg gentamicin sulfate to prevent infection and were immediately returned to their home cages. Food hoppers were replaced on the cages at 1730, and lights out occurred at 1800. Food-intake data were collected at 1-, 2-, 3-, 4-, 16-, and 24-h time points. A recovery period of at least 6 days was allowed between trials.

**Experiment 2: chronic intracerebroventricular \( \alpha \)-MSH infusion.** On the basis of preliminary results showing that a single intracerebroventricular injection of 10 \( \mu \)g \( \alpha \)-MSH reduced food intake in Wistar rats and that a chronic intracerebroventricular infusion of 96 \( \mu \)g/day is no more effective than 24 \( \mu \)g/day at reducing food intake and body weight after 6 days (data not shown), we selected a dose of 24 \( \mu \)g/day for this study. Three weeks after cannula placement, each rat was anesthetized by intraperitoneal injection of ketamine-xylazine and received a subcutaneous osmotic minipump (Azelt model 2001, Palo Alto, CA) delivering either 1 \( \mu \)g/\( \mu \)l human \( \alpha \)-MSH in 0.9% saline vehicle at a rate of 1 \( \mu \)l/h (24 \( \mu \)g/day; \( n = 9 \)) or vehicle alone (\( n = 7 \)) via a polyethylene catheter that was connected to the ventricular cannula. A saline-infused group pair-fed to the food intake of the \( \alpha \)-MSH group (\( n = 9 \)) was also included to determine if some responses to intracerebroventricular \( \alpha \)-MSH were secondary to reduced food intake. The amount of chow provided to each pair-fed animal on each treatment day was equal to the measured amount of chow consumed by its \( \alpha \)-MSH-treated partner during the previous 24-h period. Animals received continuous intracerebroventricular infusions of either \( \alpha \)-MSH or saline for 6 days and were lightly anesthetized by brief exposure to carbon dioxide before decapitation between 1100 and 1300 on the 6th day of infusion. Trunk blood, carcasses, and brains were collected upon decapitation. Blood was centrifuged, and plasma was stored at −20°C and brains were stored at −80°C. Carcasses were stored at −20°C.

**Experiment 3: acute effect of \( \alpha \)-MSH on c-Fos-like immunoreactivity in the brain.** Three weeks after cannula placement in the third ventricle, male Long-Evans rats were assigned to one of two weight-matched groups and injected in the third ventricle with either 5 \( \mu \)l of saline (\( n = 5 \)) or 20 \( \mu \)g \( \alpha \)-MSH in saline (\( n = 10 \)). Injections were performed between 1600 and 2000, after which the animals were immediately returned to their home cages and their food hoppers were removed. After 110 min, rats were anesthetized with pentobarbital sodium (60 mg ip) and transcardially perfused with isotonic saline of neutral pH followed by a 4% paraformaldehyde solution. Brains were removed immediately and postfixed in paraformaldehyde for 1 wk before assay for c-Fos-like immunoreactivity (cFILI).

Assays and Data Analyses

**Plasma assays.** Radioimmunoassays were used to measure plasma levels of corticosterone and immunoreactive insulin as previously described (13, 37). Plasma glucose was determined by the glucose oxidase method (Beckman Instruments,
Brains from mRNA. previously (3, 24, 44). Analysis of body fat stores in rats has been documented of variation for fat mass was 6.3%. Validation of DEXA for variables over the course of the analysis. Intra-assay coefficient performed every 4th to correct for DEXA performance vari-
ables.

to quantify nonfat mass as lean tissue comprised of 60%
software designed for rats. The DEXA scanner was calibrated for DEXA performance vari-
ables.

Experiment 1: Acute Effect of Intracerebroventricular administration of 2.5 \( \mu \text{g} \) on crushed dry ice and subsequently sectioned coronally at 14 \( \mu \text{m} \) in a cryostat and mounted on RNase-free slides. Riboprobes complementary to rat POMC (a generous gift of Dr. Robert Steiner), MCH, and AgRP mRNAs (constructs provided by Dr. Tina M. Hahn) were used for hybridization after labeling with \( ^{32} \text{P} \), as previously described (36). Hybridization for CRH mRNA was performed using a \( ^{32} \text{P} \)-labeled antisense oligonucleotide probe based on cDNA sequences of rat CRH genes, as described elsewhere (39). Slides for in situ hybridization (ISH) to POMC mRNA were selected from the region of the Arc rostral to the ventromedial hypothalamic nucleus (VMN), and sections for MCH and AgRP were taken from the midregion of the Arc, at the level of the VMN. Slides for CRH mRNA were selected from the PVN. All slides were selected by an investigator blinded to the treatment group. Labeled slides were washed under high-stringency conditions and opposed to X-ray film to generate autoradiographs, which were analyzed by computer densitometry. With the use of a standard curve, autoradiographic optical density and hybridization area were determined on six to eight sections per rat using the MCID computer densitometry system (Imaging Research, St. Catherine’s, Ontario, Canada). The product of hybridization area (pixels) and density (\( \mu \text{Ci/pixel} \)) was used as an index of overall neuropeptide mRNA levels, which are expressed as percentage of mean control values (34–36, 39).

cFLI. Each postfixed brain was rinsed two to three times in PBS and sectioned at 50 \( \mu \text{m} \) on a Vibratome in a PBS bath. Coronal sections taken from the forebrain and horizontal slices from the hindbrain were processed for cFLI as described in detail elsewhere (40). Sections were mounted on slides, and the number of cFLI-positive cell nuclei was quantified in specific brain areas using the MCID computer grain counting system (Imaging Research).

### Statistical Analyses

All statistical analyses were carried out using Prism 2.01 (GraphPad Software, San Diego, CA) statistical software. Data are presented as group mean values (\( \pm \text{SE} \)). For experiments with greater than two study groups, comparisons were performed with one-way ANOVA and Newman-Keuls post hoc test. A Student’s \( t \)-test was used for two-group comparisons. A \( P \) value \( \leq 0.05 \) between group mean values was considered statistically significant.

### RESULTS

**Experiment 1: Acute Effect of Intracerebroventricular \( \alpha \)-MSH Infusion on Food Intake**

As compared with intracerebroventricular vehicle, intracerebroventricular administration of 2.5 \( \mu \text{g} \) of \( \alpha \)-MSH 2 h before the dark cycle caused a nonsignificant 27.5% decrease of cumulative 4-h food intake in nonfasted rats (Fig. 1). Food intake was significantly suppressed by 42.1% (7.9 \( \pm \ 0.5 \) vs. 4.6 \( \pm \ 0.8 \) \( \text{g} \), \( P < 0.05 \)) after 25 \( \mu \text{g} \) intracerebroventricular \( \alpha \)-MSH, and no additional food intake suppression was detected at the 50-\( \mu \text{g} \) dose. Twenty-four hours after \( \alpha \)-MSH infusion, cumulative food intake and body weight across all dose groups did not differ significantly from vehicle-treated control values.

**Experiment 2: Chronic Intracerebroventricular \( \alpha \)-MSH Infusion**

**Food intake.** Cumulative food intake over 6 days was reduced by 10.7% (119.3 \( \pm \ 5.0 \) vs. 106.5 \( \pm \ 3.9 \) \( \text{g} \), \( P < 0.05 \)) in rats receiving 24 \( \mu \text{g/day} \) \( \alpha \)-MSH as a continuous intracerebroventricular infusion compared with saline-treated controls fed ad libitum (Table 1). Analysis of the time course of this effect demonstrated a 39.9% inhibition of food intake that occurred during the first 24 h (12.7 \( \pm \ 2.3 \) vs. 7.6 \( \pm \ 0.9 \) \( \text{g} \), \( P < 0.05 \)), with food intake returning to near normal levels on subsequent days. After an initial suppression of energy intake, therefore, the anorectic effect of \( \alpha \)-MSH was no longer detected. By design, the intake of the saline-treated, pair-fed group was matched to that of the \( \alpha \)-MSH group and was also significantly below that of controls fed ad libitum.

**Body weight and composition.** As summarized in Table 2, mean body weight at baseline was similar among the three groups of rats. When expressed as a percentage of initial body weight, weight at the time of death was decreased by 3.9 \( \pm \ 0.8 \)% in \( \alpha \)-MSH-treated rats and 3.1 \( \pm \ 0.9 \)% in the saline-infused pair-fed animals (\( P < 0.01 \) for both comparisons) and remained unchanged in the saline-infused control group. When expressed as a percentage of final body weight of the saline control group, weight was decreased by 4.3% in the \( \alpha \)-MSH group and 3.6% in the pair-fed group. Final body weight was not significantly different between the saline-infused pair-fed and the \( \alpha \)-MSH-infused groups.

Relative to ad libitum-fed controls, total body fat mass determined by DEXA analysis was 2.2 \( \text{g} \) less in
**Table 1. Daily and cumulative food intake**

<table>
<thead>
<tr>
<th></th>
<th>Saline</th>
<th>α-MSH</th>
<th>Pair-fed</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Food Intake, g</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Day 1</strong></td>
<td>12.6 ± 2.3</td>
<td>7.6 ± 0.9*</td>
<td>5.1 ± 1.1†</td>
</tr>
<tr>
<td><strong>Day 2</strong></td>
<td>19.9 ± 0.9</td>
<td>17.6 ± 1.1</td>
<td>16.6 ± 1.1</td>
</tr>
<tr>
<td><strong>Day 3</strong></td>
<td>18.7 ± 0.7</td>
<td>18.0 ± 1.2</td>
<td>17.5 ± 1.1</td>
</tr>
<tr>
<td><strong>Day 4</strong></td>
<td>22.8 ± 0.9</td>
<td>20.7 ± 0.9</td>
<td>20.7 ± 0.9</td>
</tr>
<tr>
<td><strong>Day 5</strong></td>
<td>23.1 ± 1.1</td>
<td>22.0 ± 1.0</td>
<td>21.6 ± 0.8</td>
</tr>
<tr>
<td><strong>Day 6</strong></td>
<td>22.0 ± 1.1</td>
<td>20.6 ± 0.7</td>
<td>20.6 ± 0.7</td>
</tr>
<tr>
<td><strong>Cumulative</strong></td>
<td>119.3 ± 5.0</td>
<td>106.5 ± 3.9*</td>
<td>102.0 ± 3.7*</td>
</tr>
</tbody>
</table>

Values are means ± SE. Effect of chronic α-melanocyte-stimulating hormone (α-MSH) infusion on daily and cumulative food intake, saline vs. α-MSH and saline pair-fed, *P < 0.05 vs. saline controls, †P < 0.01 vs. saline controls by 1-way ANOVA.

α-MSH-infused animals and 0.9 g less in the pair-fed group [P = not significant (NS) between all groups], whereas lean mass was reduced by 15.7 and 13.7 g in the α-MSH and pair-fed groups, respectively (P = NS between all groups, Table 2). Total weight loss was, therefore, comprised of decreases in both fat and lean mass, and percent body fat was not significantly different in either the α-MSH-infused or pair-fed groups compared with the ad libitum-fed controls (−0.3% in the α-MSH-infused group and +0.3% in the pair-fed group).

**Plasma values.** Nonfasting plasma insulin levels were reduced by 29.3% in the α-MSH group vs. saline-treated rats fed ad libitum (207.3 ± 25.7 vs. 293.1 ± 23.9 pmol/l, P < 0.05) and by 38.5% in the pair-fed group vs. saline (180.3 ± 11.1 pmol/l, P < 0.01 vs. saline-infused rats fed ad libitum), but the α-MSH and pair-fed groups were not significantly different (Fig. 2A). Plasma glucose levels were not significantly different among groups (Fig. 2A). Plasma leptin was decreased nonsignificantly by 20.1% in the α-MSH group compared with the saline group (1.2 ± 0.1 vs. 1.5 ± 0.2 ng/ml) and was decreased by 34.4% in the saline pair-fed group compared with controls fed ad libitum (1.0 ± 0.1 ng/ml, P < 0.05 vs. saline-infused rats fed ad libitum), but the α-MSH and pair-fed groups were not significantly different (Fig. 2B). Plasma corticosterone levels were increased by 51.0% in the α-MSH-infused group compared with the saline-infused group (9.4 ± 3.9 vs. 62.3 ± 15.5 ng/ml) and by 7.2% in the saline pair-fed group (66.8 ± 14.22 ng/ml) (Fig. 2B), although neither difference reached statistical significance.

**Table 2. Body weight and body composition**

<table>
<thead>
<tr>
<th></th>
<th>Saline</th>
<th>α-MSH</th>
<th>Pair-Fed</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Initial body wt, g</strong></td>
<td>365.9 ± 5.8</td>
<td>362.1 ± 7.6</td>
<td>361.9 ± 7.8</td>
</tr>
<tr>
<td><strong>Body wt at death, g</strong></td>
<td>367.4 ± 7.2</td>
<td>347.8 ± 7.9*</td>
<td>350.5 ± 9.4*</td>
</tr>
<tr>
<td><strong>Fat at death, g</strong></td>
<td>25.3 ± 1.4</td>
<td>23.1 ± 1.0</td>
<td>24.4 ± 1.5</td>
</tr>
<tr>
<td><strong>Lean body mass at death, g</strong></td>
<td>313.5 ± 8.1</td>
<td>297.8 ± 7.0</td>
<td>299.8 ± 8.1</td>
</tr>
<tr>
<td><strong>%Body fat at death</strong></td>
<td>6.9 ± 0.5</td>
<td>6.6 ± 0.2</td>
<td>7.2 ± 0.3</td>
</tr>
</tbody>
</table>

Values are means ± SE. Baseline and body weight at death and body composition at death after chronic infusion as determined by dual-energy X-ray absorptiometry analysis, saline vs. α-MSH and saline pair-fed. *P < 0.05 vs. saline.

**Fig. 2. Mean ± SE plasma values after 6-day infusion of α-MSH. A: plasma insulin levels, *P < 0.05 vs. saline controls; plasma glucose levels, P = not significant (NS). B: plasma leptin levels, *P < 0.05 vs. saline controls; plasma corticosterone levels, P = NS. The saline group is represented by open bars (n = 7), the α-MSH group by solid bars (n = 9), and the saline pair-fed group by hatched bars (n = 9).**

**ISH.** As expected, hybridization to both POMC and AgRP mRNA in the hypothalamus was detected only in the Arc. POMC mRNA levels in the rostral Arc were nonsignificantly suppressed by 13.1 ± 4.0 and 16.1 ± 9.7% in the α-MSH and pair-fed groups relative to saline controls fed ad libitum (Fig. 3). By comparison, AgRP mRNA levels in the Arc were elevated by 30.4 ± 10.1 and 12.6 ± 8.6% in the α-MSH and pair-fed groups, respectively, compared with ad libitum-fed controls, although neither difference reached statistical significance. CRH mRNA levels in the PVN were decreased by 18.2 ± 10.4% and increased by 24.2 ± 38.9% in the α-MSH and pair-fed groups, respectively, compared with saline controls (P = NS for both comparisons). MCH mRNA levels in the LHA were increased by 121.1 ± 51.3 and 111.3 ± 41.0% in the α-MSH and pair-fed groups compared with saline controls (P = NS for both comparisons).

**Experiment 3: Acute Effect of α-MSH on cFLI in the Brain**

In the brain stem, the number of nuclei that stained positively for cFLI in the lateral parabrachial nucleus (LPBN) was increased by 627% of intracerebroventricularly saline-injected control levels 2 h after intracere-
The melanocortin system is proposed to be an important hypothalamic pathway involved in energy homeostasis (25, 38). To compare the response to acute and chronic central melanocortin receptor stimulation and to investigate the effect of increased central nervous system (CNS) melanocortin signaling on hypothalamic and brainstem areas associated with energy homeostasis, we infused α-MSH into the third cerebral ventricle of adult male rats. We found that a single intracerebroventricular injection of α-MSH at a dose of 20 μg (7.4 ± 1.9 vs. 53.8 ± 7.0 cFLI-positive nuclei, P < 0.001) and was nonsignificantly increased by 185% in the NTS. In the forebrain, α-MSH also increased fos staining in the supraoptic nucleus (SON), the PVN, the central nucleus of the amygdala (CeA), and in the Arc. The number of nuclei with cFLI in the SON was increased with intracerebroventricular α-MSH by 1,142% (7.2 ± 1.9 vs. 89.4 ± 17.0 cFLI-positive nuclei, P < 0.01), in the PVN by 254% (67.3 ± 32.2 vs. 238.0 ± 27.9 cFLI-positive nuclei, P < 0.01), and in the CeA by 204% (19.0 ± 7.6 vs. 57.7 ± 10.4 cFLI-positive nuclei, P < 0.05). The number of cFLI-positive nuclei in the Arc was nonsignificantly increased by 46% (Fig. 4).

**DISCUSSION**

**Summary of Findings**

The melanocortin system is proposed to be an important hypothalamic pathway involved in energy homeostasis (25, 38). To compare the response to acute and chronic central melanocortin receptor stimulation and to investigate the effect of increased central nervous system (CNS) melanocortin signaling on hypothalamic and brainstem areas associated with energy homeostasis, we infused α-MSH into the third cerebral ventricle of adult male rats. We found that a single intracerebroventricular injection of α-MSH, at a dose that reduces 4-h intake, activated neurons (as indicated by increased cFLI in cell nuclei) in several forebrain areas, as well as in the IPBN, an important metabolic relay area of the brain stem. A reduction in body weight, however, was not observed after a single intracerebroventricular α-MSH injection. In contrast, continuous intracerebroventricular administration of α-MSH for 6 days did result in sustained decreases of both food intake and body weight, although the anorectic response to chronic infusion of α-MSH was greatest during the first 24 h of infusion and was not significant thereafter. Moreover, a chronic infusion of α-MSH did not selectively reduce body fat and failed to alter expression of specific hypothalamic neuropeptide mRNAs. Therefore, although α-MSH acts in the CNS to induce a state of negative energy balance, its efficacy wanes with chronic administration, suggesting that a competing mechanism attenuates its effectiveness.

**Acute Injection of α-MSH**

To investigate the acute effects of intracerebroventricular α-MSH on food intake, we measured food consumption in response to different doses of α-MSH administered to conscious, nonfasted rats 2 h before the beginning of the nocturnal cycle. We found that, although a 25-μg injection of α-MSH reduced food intake by 42.1% over 4 h compared with saline-injected controls (P < 0.05, experiment 1), a compensatory increase of food intake occurred subsequently, such that neither 24-h food intake nor body weight was significantly affected. Higher doses were no more effective in their ability to suppress food intake or to induce weight loss.

This finding differs from the effects of the synthetic melanocortin receptor agonist MTII, which, at a dose of 1 nmol icv, elicits a more sustained anorexia and a significant weight loss after 24 h (40) despite an initial response (at 4 h) comparable to that of 25 μg (15 nmol) of α-MSH. A 10-μg icv (6 nmol) dose of Nle14DPhe7α (NDP-MSH), an α-MSH analog, also maintains a significant 34% suppression of food intake over a 24-h period compared with vehicle control (6). Because MTII and NDP-MSH are engineered to bind the MC-4 receptor with higher affinity and, as synthetic compounds, may be cleared less readily than α-MSH (17), the prolonged effects of MTII and NDP-MSH on food intake could reflect a sustained increase of neuronal melanocortin signaling. It is therefore possible that the short duration of α-MSH-induced an-
orexia compared with MTII and NDP-MSH is due simply to a relatively short-lived stimulation of central melanocortin receptors, in which case, a chronic intracerebroventricular infusion of α-MSH should elicit a more durable anorexic response. To investigate this hypothesis, we measured the effect of continuous intracerebroventricular administration of α-MSH for 6 days.

**Chronic Infusion of α-MSH**

Although both vehicle- and α-MSH-treated animals experienced reduced food intake and body weight on the first day of infusion (presumably related to minipump implantation), animals infused with α-MSH consumed 39.9% less food than saline-infused, ad libitum-fed controls. Thus, whereas an acute intracerebroventricular injection of 25 μg α-MSH suppressed food intake at 4 h, but not at 24 h, continuous infusion of a comparable dose (24 μg) induced a cumulative reduction of food intake that was sustained for at least 24 h with no compensatory increase of food intake over the subsequent 6 days of α-MSH infusion. This demonstration that the duration of α-MSH-induced anorexia is increased by chronic intracerebroventricular infusion supports the hypothesis that sustained melanocortin signaling is necessary to achieve the more durable anorexia induced by MTII or NDP-MSH. By day 2, however, food intake differed by only 11.6% between these two groups and was only 5.9% lower for the remainder of the study. This attenuation of α-MSH-induced anorexia during continuous infusion is consistent with the development of either melanocortin receptor downregulation, compensatory responses involving other neuronal pathways, or both.

By day 6, the α-MSH-infused group weighed 4.3% less than the saline-infused controls, whereas saline-infused animals pair-fed to the α-MSH group exhibited a 3.6% weight loss that was not significantly different from that seen in the α-MSH-infused group. Although these findings suggest that decreased food intake induced by chronic infusion of α-MSH is sufficient to explain the measured weight reduction, they do not exclude an effect of α-MSH on energy expenditure. Postmortem body composition analysis indicated that the percentages of lean and fat mass were similar between the α-MSH and the two control groups and that lost weight was comprised of both lean and fat tissue. This weight loss induced by chronic central administration of α-MSH is modest and is not comprised preferentially of adipose tissue, as has been reported for leptin.

As expected, reduced body weight in both the α-MSH and pair-fed groups was associated with comparable reductions of plasma insulin and leptin levels relative to ad libitum-fed controls. This finding suggests that suppression of basal levels of these plasma hormones was the consequence of reduced food intake and weight loss rather than an independent effect of α-MSH on hormone secretion. However, our study did not investigate insulin secretory responses or systemic insulin action. Thus further experiments are necessary to clarify this issue. Measurements of plasma corticosterone and glucose levels also failed to demonstrate independent effects of α-MSH on these measures. Taken together, these findings suggest that in normal rats, chronic intracerebroventricular infusion of α-MSH does not substantially influence basal plasma levels of hormones involved in energy homeostasis or glucose homeostasis independent of its effect to reduce food intake and body weight.

Significant changes in hypothalamic neuropeptide mRNA levels were not observed after chronic α-MSH infusion. Although they did not reach statistical significance, the direction and magnitude of changes of POMC, CRH, MCH, and AgRP mRNA we detected are consistent with the response predicted for the modest decrease of body weight and leptin and insulin levels experienced by the α-MSH and pair-fed groups.

**Brain cFLI After α-MSH Injection**

Although not all CNS neurons express c-Fos on activation, detection of those that do provides a useful means of identifying CNS targets of specific pharmacological stimuli. We found that 2 h after 20 μg of α-MSH was administered into the third cerebral ventricle, marked increases in cFLI-positive nuclei were seen in the PVN, CeA, and the IPBN, as previously demonstrated with MTII (40) and NDP-MSH (6; Fig. 5). c-Fos immunostaining of the IPBN by α-MSH may have arisen from activation of melanocortin-sensitive hypothalamic projections to the brain stem or by direct activation of melanocortin receptors located in the IPBN by α-MSH carried from the third to the fourth ventricle. Because MTII delivered into either the lateral or fourth ventricle is equally potent in suppressing food intake and body weight (14), it is possible that α-MSH exerts direct effects in brain stem feeding areas that may explain c-Fos induction in the IPBN.

The parvocellular PVN, CeA, and IPBN are components of a pathway that integrates centers regulating food intake and body weight with satiety centers in the brain stem. The activation of all three areas by α-MSH suggests that it may enhance in the perception of satiety and thereby facilitate the consumption of smaller meals, which, over time, results in a modest decrease in body weight. However, meal size was not measured in our study, and intracerebroventricularly administered α-MSH also increases mean arterial blood pressure via an action that may involve the same brain nuclei (8). Further studies are therefore warranted to differentiate activation by α-MSH of cardiovascular versus energy balance circuits along this pathway.

**Perspectives**

Previous studies have shown that leptin stimulates POMC expression in the Arc (7, 36), suggesting that melanocortin signaling may be downstream of leptin in a pathway leading to anorexia. Support for this hypothesis was provided by the observation that the
anorectic properties of leptin can be blocked by pre-
treatment with the melanocortin receptor antagonist
SHU-9119 (38) and that mice deficient in MC-4 recep-
tor signaling have an attenuated response to leptin
(25). Because the leptin receptor is expressed by Arc
POMC neurons (7), these findings suggest that at least
some of leptin’s effects on energy homeostasis are me-
diated by increased α-MSH signaling via the MC-4
receptor.

Our current findings, however, suggest that CNS
leptin signaling must involve pathways additional to
melanocortin signaling, in agreement with a previous
study (4). For example, leptin’s ability to induce adi-
pose-specific weight loss was not induced by chronic
intracerebroventricular α-MSH, and the magnitude of
α-MSH-induced anorexia and weight loss with chronic
central administration is less than that seen with lep-
tin. In addition, CNS melanocortin signaling must in-
volve pathways not activated by leptin, because a
pronounced increase of cFLI-positive nuclei was seen
in the IPBN in response to intracerebroventricular
α-MSH, whereas intracerebroventricular leptin does
not have this effect (40).

These data support a model (Fig. 6) in which α-MSH
induces reductions in food intake via activation of
MC-4 receptors in both the brain stem and hypothala-
mus. On the basis of previous studies, an increase of
leptin delivery to the brain increases forebrain MC-4-
receptor signaling via both an increase in POMC ex-
pression and a decrease in AgRP expression in Arc
neurons. Increased MC-4 signaling in second-order
neurons involved in food intake regulation may then
promote anorexia. For example, activation of CRH- or
suppression of MCH-containing neurons may contrib-
ute to the response to MC-4-receptor stimulation, al-
though we were unable to demonstrate significant ef-
fects of α-MSH on either CRH or MCH mRNA levels.
Thus other neurons, such as thyrotropin-releasing hor-
mone neurons of the PVN, may also contribute to the
anorectic effects of α-MSH (12).

Leptin is hypothesized to affect food intake not only
by stimulating catabolic effector pathways such as the

Fig. 5. α-MSH dramatically increases number of c-Fos-
positive nuclei in the PVN and lateral PBN (A, C) com-
pared with saline controls (B, D).

Fig. 6. Proposed model for the role of melanocortins
in leptin signaling. Leptin regulates hypothalamic
gene expression via leptin receptors located on
POMC and AgRP neurons. Leptin indirectly regu-
lates food intake by upregulating POMC expression
and increasing α-MSH levels in the PVN, thereby
activating melanocortin signaling in CRH and MCH
neurons of the hypothalamus. Hypothalamic projec-
tions from the PVN and LHA transduce energy bal-
ance information to the NTS and subsequently to
autonomic preganglionic neurons. DMX, dorsal mo-
tor nucleus of the vagal nerve.
This scenario is consistent with current literature and provides a viable explanation for our results. If correct, the clinical efficacy of melanocortin agonists in the treatment of obesity may be limited by the effect of weight loss to activate compensatory responses that antagonize the effect of melanocortins. This possibility suggests that the efficacy of such compounds will be increased if used in combination with drugs that block such compensatory responses, and further studies are warranted to test this hypothesis.

Many thanks to Dr. Todd Thiele for expert guidance and advice on the c-Fos assay and to Dr. George Merriam and Lynna C. Smith for the use of the DEXA scanner system located at the Veterans Affairs Puget Sound Health Care System, American Lake Division in Tacoma, WA. Technical assistance for in situ hybridization was provided by Vicki Hoagland and Will Anderson. The leptin assay was performed by Kimber Stanhope, the corticosterone assay by Elizbeth Celusardo and Shannon Reed, the insulin assay by Hong Nguyen, and the glucose assay by Ruth Hollingworth. The riboprobe for POMC mRNA was a gift of Dr. Robert Steiner, and constructs for the MCH and AgRP riboprophes were provided by Dr. Tina M. Hahn.

This work was supported by grants from the National Institutes of Health (DK-12829, DK-52798, NS-32273, DK-50129, DK-35747, DK-17844, and DK-54080), the Diabetes Endocrinology Research Center, the Clinical Nutrition Research Unit and the Royalty Research Fund at the University of Washington, the American Diabetes Association, and the Veterans Affairs Merit Review Program.

REFERENCES


