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

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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.

melanocortin; hypothalamus; 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 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

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(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 α-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 α-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 α-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 α-MSH would acutely activate neuronal substrates in energy regulation pathways. To identify these sites, we injected rats in the third ventricle with α-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 μl 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 α-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 μl of sterile, 0.9% preservative-free saline (Fujisawa USA, Deerfield, IL) or an equal volume of human α-MSH (Peninsula Laboratories, Belmont, CA) in saline using an injector (Plastics One) attached by polyethylene tubing to a 25-μl glass syringe (Hamilton, Reno, NV). A study comparing doses of 0, 2.5, 25, and 50 μg α-MSH (n = 5–7/group) 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 the 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 α-MSH infusion. On the basis of preliminary results showing that a single intracerebroventricular injection of 10 μg α-MSH reduces 4-h food intake in Wistar rats and that a chronic intracerebroventricular infusion of 96 μg/day is no more effective than 24 μg/day at reducing food intake and body weight after 6 days (data not shown), we selected a dose of 24 μg/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 (Azlet model 2001, Palo Alto, CA) delivering either 1 μg/μl human α-MSH in 0.9% saline vehicle at a rate of 1 μl/h (24 μg/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 α-MSH group (n = 9) was also included to determine if some responses to intracerebroventricular α-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 α-MSH-treated partner during the previous 24-h period. Animals received continuous intracerebroventricular infusions of either α-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 α-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 μl of saline (n = 5) or 20 μg α-MSH in saline (n = 10). Injections were performed between 1000 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 post-fixed in paraformaldehyde for 1 wk before assay for c-Fos-like immunoreactivity (cFLI).

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,

Brea, CA). Plasma leptin levels were determined by radioimmunoassay using a rat-specific antibody (Linco, St. Louis, MO) (21).

Analysis of body composition. Postmortem body fat and nonfat mass were measured by dual-energy X-ray absorptiometry (DEXA; QDR 1500, Hologic, Waltham, MA) using software designed for rats. The DEXA scanner was calibrated to quantify nonfat mass as lean tissue comprised of 60% water, excluding bone mass. A tissue calibration scan was performed every 4 h to correct for DEXA performance variables over the course of the analysis. Intra-assay coefficient of variation for fat mass was 6.3%. Validation of DEXA for analysis of body fat stores in rats has been documented previously (3, 24, 44).

In situ hybridization to AgRP, POMC, CRH, and MCH mRNA. Brains from *experiment 2* were immediately frozen on crushed dry ice and subsequently sectioned coronally at 14 μm 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 ^{33}P, as previously described (36). Hybridization for CRH mRNA was performed using a ^{33}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 (μCi/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 μm 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 (±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 ≤0.05 between group mean values was considered statistically significant.

RESULTS

Experiment 1: Acute Effect of Intracerebroventricular α-MSH Infusion on Food Intake

As compared with intracerebroventricular vehicle, intracerebroventricular administration of 2.5 μg of

α-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 ± 0.5 vs. 4.6 ± 0.8 g, $P < 0.05$) after 25 μg intracerebroventricular α-MSH, and no additional food intake suppression was detected at the 50-μg dose. Twenty-four hours after α-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 α-MSH Infusion

Food intake. Cumulative food intake over 6 days was reduced by 10.7% (119.3 ± 5.0 vs. 106.5 ± 3.9 g, $P < 0.05$) in rats receiving 24 μg/day α-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 ± 2.3 vs. 7.6 ± 0.9 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 α-MSH was no longer detected. By design, the intake of the saline-treated, pair-fed group was matched to that of the α-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 ± 0.8% in α-MSH-treated rats and 3.1 ± 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 α-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 α-MSH-infused groups.

Relative to ad libitum-fed controls, total body fat mass determined by DEXA analysis was 2.2 g less in
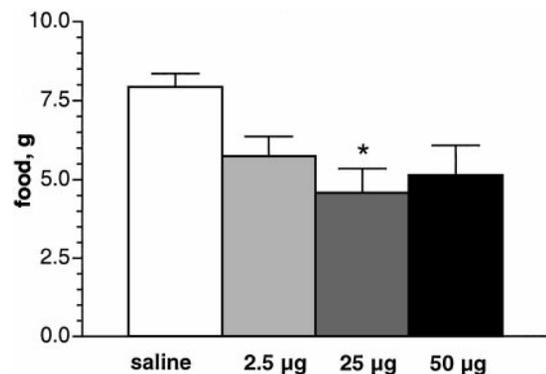
Fig. 1. Effect of acutely administered intracerebroventricular α-melanocyte-stimulating hormone (α-MSH) on 4-h food intake at 0-, 2.5-, 5-, 25-, and 50-μg doses. *$P < 0.05$ vs. saline ($n = 5$–7 animals/group).

Table 1. *Daily and cumulative food intake*

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

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. 2*A*). Plasma glucose levels were not significantly different among groups (Fig. 2*A*). 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, $P < 0.05$)(Fig. 2*B*). Plasma corticosterone levels were increased by 51.0% in the α-MSH-infused group compared with the saline-infused group (94.1 ± 33.13 vs. 62.3 ± 15.5 ng/ml) and by 7.2% in the saline pair-fed group (66.8 ± 14.22 ng/ml) (Fig. 2*B*), although neither difference reached statistical significance.

Table 2. *Body weight and body composition*

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

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.
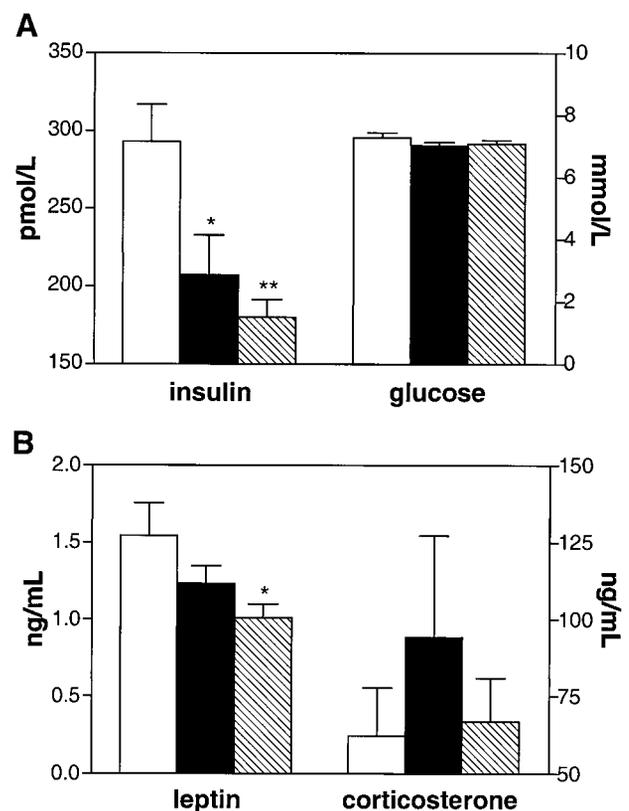
Fig. 2. Mean ± SE plasma values after 6-day infusion of α-MSH. *A*: plasma insulin levels, *$P < 0.05$ vs. saline controls, **$P < 0.01$ vs. saline controls; plasma glucose levels, P = not significant (NS). *B*: plasma leptin levels, *$P < 0.05$ vs. saline controls; plasma corticosterone levels, P = 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-
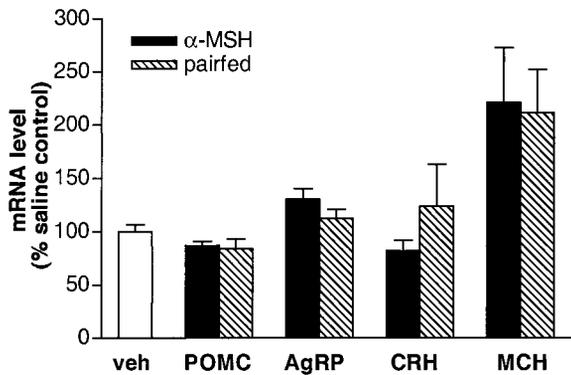
Fig. 3. Effect of chronic α-MSH infusion on hypothalamic neuropeptide expression determined by in situ hybridization. Values are means ± SE. Levels of proopiomelanocortin (POMC) mRNA in the region of the arcuate nucleus rostral to the ventromedial hypothalamic nucleus (VMN); levels of agouti-related peptide (AgRP) mRNA in the midregion of the arcuate nucleus (Arc) at the level of the VMN; levels of corticotropin-releasing hormone (CRH) mRNA in the paraventricular nucleus (PVN); levels of melanin-concentrating hormone (MCH) mRNA in the midregion of the lateral hypothalamic area (LHA), at the level of the VMN. The α-MSH group is represented by solid bars ($n = 8$), the saline group by open bars ($n = 4$ for MCH mRNA, $n = 5$ for all others), and the saline pair-fed group by hatched bars ($n = 8$).

broventricular 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 brain stem 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 lPBN, 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 Nle4DPhe7alpha (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-
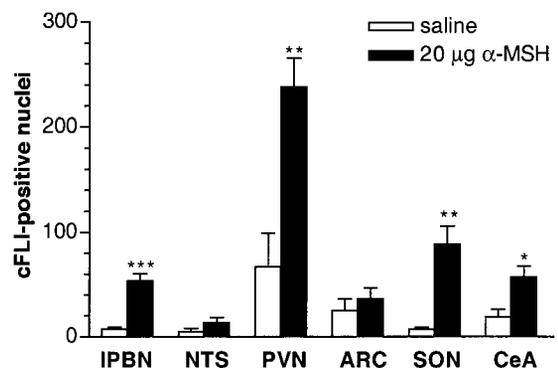
Fig. 4. Mean ± SE c-Fos-like immunoreactivity (cFLI)-positive nuclei of the brain 2 h after intracerebroventricular administration of 20 µg α-MSH ($n = 10$) or saline ($n = 5$). Parabrachial nucleus (PBN), ***$P < 0.001$ vs. saline; nucleus of the solitary tract (NTS), $P =$ NS; PVN, **$P < 0.01$ vs. saline; Arc, $P =$ NS; supraoptic nucleus (SON), **$P < 0.01$ vs. saline; central nucleus of the amygdala (CeA), *$P < 0.05$ vs. saline ($n = 4$–10 animals/group).

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. Thus 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 lPBN, as previously demonstrated with MTII (40) and NDP-MSH (6; Fig. 5). c-Fos immunostaining of the lPBN 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 lPBN 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 lPBN.

The parvocellular PVN, CeA, and lPBN 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
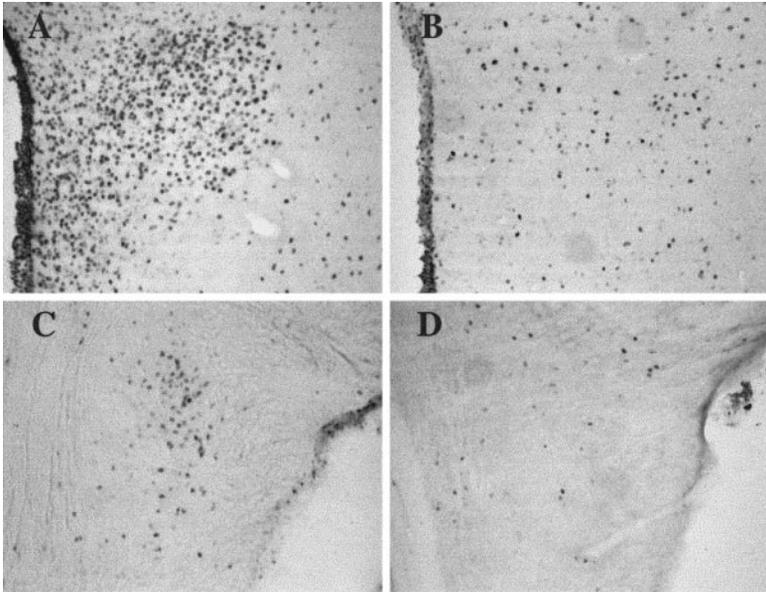
Fig. 5. α-MSH dramatically increases number of c-Fos-positive nuclei in the PVN and lateral PBN (*A*, *C*) compared with saline controls (*B*, *D*).

anorectic properties of leptin can be blocked by pretreatment with the melanocortin receptor antagonist SHU-9119 (38) and that mice deficient in MC-4 receptor 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 mediated 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 adipose-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 leptin. In addition, CNS melanocortin signaling must involve pathways not activated by leptin, because a pronounced increase of cFLI-positive nuclei was seen in the lPBN 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 hypothalamus. 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 expression 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 contribute to the response to MC-4-receptor stimulation, although we were unable to demonstrate significant effects of α-MSH on either CRH or MCH mRNA levels. Thus other neurons, such as thyrotropin-releasing hormone 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
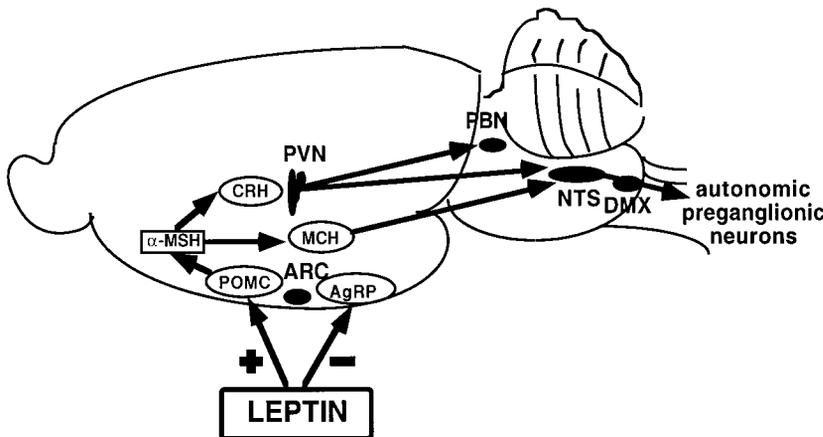
Fig. 6. Proposed model for the role of melanocortins in leptin signaling. Leptin regulates hypothalamic gene expression via leptin receptors located on POMC and AgRP neurons. Leptin indirectly regulates food intake by upregulating POMC expression and increasing α-MSH levels in the PVN, thereby activating melanocortin signaling in CRH and MCH neurons of the hypothalamus. Hypothalamic projections from the PVN and LHA transduce energy balance information to the NTS and subsequently to autonomic preganglionic neurons. DMX, dorsal motor nucleus of the vagal nerve.

melanocortin system, but also by inhibiting anabolic signaling systems such as NPY/AgRP neurons in the Arc. As weight loss induced by chronic intracerebroventricular α-MSH proceeds, therefore, the decline in adiposity signaling (in the form of circulating insulin and leptin) to the CNS is proposed to activate NPY/AgRP neurons and thereby offset the anorectic action of α-MSH. 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.

REFERENCES

1. **Baskin DG, Schwartz MW, Seeley RJ, Woods SC, Porte D Jr, Breininger JF, Jonak Z, Schaefer J, Krouse M, Burghardt C, Campfield LA, Burn P, and Kochan JP.** Leptin receptor long-form splice-variant protein expression in neuron cell bodies of the brain and co-localization with neuropeptide Y mRNA in the arcuate nucleus. *J Histochem Cytochem* 47: 353–362, 1999.

2. **Bergendahl M, Wiemann JN, Clifton DK, Huhtaniemi I, and Steiner RA.** Short-term starvation decreases POMC mRNA but does not alter GnRH mRNA in the brain of adult male rats. *Neuroendocrinology* 56: 913–920, 1992.

3. **Bertin E, Ruiz J-C, Mourot J, Peiniau P, and Portha B.** Evaluation of dual-energy x-ray absorptiometry for body-composition assessment in rats. *J Nutr* 128: 1550–1554, 1998.

4. **Boston BA, Blaydon KM, Varnerin J, and Cone RD.** Independent and additive effects of central POMC and leptin pathways on murine obesity. *Science* 278: 1641–1644, 1997.

5. **Brady LS, Smith MA, Gold PW, and Herkenham M.** Altered expression of hypothalamic neuropeptide mRNAs in food-restricted and food-deprived rats. *Neuroendocrinology* 52: 441–447, 1990.

6. **Brown KS, Gentry RM, and Rowland NE.** Central injection in rats of alpha-melanocyte stimulating hormone analog: effects on food intake and brain Fos. *Regul Pept* 78: 89–94, 1998.

7. **Cheung CC, Clifton DK, and Steiner RA.** Proopiomelanocortin neurons are direct targets for leptin in the hypothalamus. *Endocrinology* 138: 4489–4492, 1997.

7a.**Cowley MA, Pronchuk N, Fan W, Dinulescu DM, Colmers WF, and Cone RD.** Integraton of NPY, AGRP and melanocortin signals in the hypothalamic paraventricular nucleus: evidence for a cellular basis for the adipostat. *Neuron* 24: 155–163, 1999.

8. **Dunbar JC and Lu H.** Leptin-induced increase in sympathetic nervous and cardiovascular tone is mediated by proopiomelanocortin (POMC) products. *Brain Res Bull* 50: 215–221, 1999.

9. **Elias CF, Saper CB, Maratos-Flier E, Tritos NA, Lee C, Kelly J, Tatro JB, Hoffman GE, Ollmann MM, Barsh GS, Sakurai T, Yanagisawa M, and Elmquist JK.** Chemically defined projections liking the mediobasal hypothalamus and the lateral hypothalamic area. *J Comp Neurol* 402: 442–459, 1998.

10. **Elmquist JK, Bjorbaek C, Ahima RS, Flier JS, and Saper CB.** Distributions of leptin receptor mRNA isoforms in the rat brain. *J Comp Neurol* 395: 535–547, 1998.

11. **Fan W, Boston B, Kesterson R, Hruby V, and Cone R.** Role of melanocortinergic neurons in feeding and the agouti obesity syndrome. *Nature* 385: 165–168, 1997.

12. **Fekete C, Legradi G, Mihaly E, Huang Q-H, Tatro JB, Rand WM, Emerson CH, and Lechan RM.** Alpha-melanocyte-stimulating hormone is contained in nerve terminals innervating thyrotropin-releasing hormone-synthesizing neurons in the hypothalamic paraventricular nucleus and prevents fasting-induced suppression of prothyrotropin-releasing hormone gene expression. *J Neurosci* 20: 1550–1558, 2000.

13. **Green PK, Wilkinson CW, and Woods SC.** Intraventricular corticosterone increases the rate of body weight gain in underweight adrenalectomized rats. *Endocrinology* 130: 269–275, 1992.

14. **Grill HJ, Ginsberg AB, Seeley RJ, and Kaplan JM.** Brainstem application of melanocortin receptor ligands produces long-lasting effects on feeding and body weight. *J Neurosci* 18: 10128–10135, 1998.

15. **Hagan MM, Rushing PA, Schwartz MW, Yagaloff KA, Burn P, Woods SC, and Seeley RJ.** Role of the CNS melanocortin system in the response to overfeeding. *J Neurosci* 19: 2362–2367, 1999.

16. **Hahn TM, Breininger JF, Baskin DG, and Schwartz MW.** Coexpression of Agrp and NPY in fasting-activated hypothalamic neurons. *Nat Neurosci* 1: 271–272, 1998.

17. **Haskell-Luevano C, Nikiforovich G, Sharma SD, Yang Y-K, Dickinson C, Hruby VJ, and Gantz I.** Biological and conformational examination of stereochemical modifications using the template melanotropin peptide, Ac-Nle-c[Asp-His-Phe-Arg-Trp-Ala-Lys]-NH2, on human melanocortin receptors. *J Med Chem* 40: 1738–1748, 1997.

18. **Havel PJ, Hahn TM, Sindelar DK, Baskin D, Weigle DS, and Schwartz MW.** Effects of uncontrolled diabetes and insulin treatment on food intake, the hypothalamic melanocortin system, and muscle uncoupling protein-3 (UCP3) expression (Abstract). *Diabetes* 48: 288, 1999.

19. **Huszar D, Lynch C, Fairchild-Huntress V, Dunmore J, Fang Q, Berkemeier L, Gu W, Kesterson R, Boston B, Cone R, Smith F, Campfield L, Burn P, and Lee F.** Targeted disruption of the melanocortin-4 receptor results in obesity in mice. *Cell* 88: 131–141, 1997.

20. **Jackson RS, Creemers JW, Ohagi S, Raffin-Sanson ML, Sanders L, Montague CT, Hutton JC, and O'Rahilly S.** Obesity and impaired prohormone processing associated with mutations in the human prohormone convertase 1 gene. *Nat Genet* 16: 303–306, 1997.

21. **Landt M, Gingerich RL, Havel PJ, Mueller WM, Schoner B, Hale JE, and Heiman ML.** Radioimmunoassay of rat leptin: sexual dimorphism reversed from humans. *Clin Chem* 44: 565–570, 1998.

23. **Ludwig DS, Mountjoy KG, Tatro JB, Gillette JA, Frederich RC, Flier JS, and Maratos-Flier E.** Melanin-concentrating hormone: a functional melanocortin antagonist in the hypothalamus. *Am J Physiol Endocrinol Metab* 274: E627–E633, 1998.

24. **Makan S, Bayley HS, and Webber CE.** Precision and accuracy of total body bone mass and body composition measurements in the rat using x-ray based dual photon absorptiometry. *Can J Physiol Pharmacol* 75: 1257–1261, 1997.

25. **Marsh DJ, Hollopeter G, Huszar D, Laufer R, Yagaloff KA, Fisher SL, Burn P, and Palmiter RD.** Response of melanocortin-4 receptor-deficient mice to anorectic and orexigenic peptides. *Nat Genet* 21: 119–122, 1999.

26. **Mizuno TM, Kleopolulos SP, Bergen HT, Roberts JL, Priest CA, and Mobbs CV.** Hypothalamic pro-opiomelanocortin mRNA is reduced by fasting in ob/ob and db/db mice, but is stimulated by leptin. *Diabetes* 47: 294–297, 1997.

27. **Mizuno TM and Mobbs CV.** Hypothalamic agouti-related protein messenger ribonucleic acid is inhibited by leptin and stimulated by fasting. *Endocrinology* 140: 814–817, 1999.

28. **Mountjoy K, Mortrud M, Low M, Simerly R, and Cone R.** Localization of the melanocortin-4 receptor (MC4-R) in neuroendocrine and autonomic control circuits in the brain. *Mol Endocrinol* 8: 1298–1308, 1994.

29. **Murphy B, Nunes CN, Ronan JJ, Harper CM, Beall MJ, Hanaway M, Fairhurst AM, Van der Ploeg LH, MacIntyre DE, and Mellin TN.** Melanocortin mediated inhibition of feeding behavior in rats. *Neuropeptides* 32: 491–497, 1998.

30. **Naggert JK, Fricker LD, Varlamov O, Nishina PM, Rouille Y, Steiner DF, Carroll RJ, Paigen BJ, and Leiter EH.** Hyperproinsulinaemia in obese fat/fat mice associated with a carboxypeptidase E mutation which reduces enzyme activity. *Nat Genet* 10: 135–142, 1995.

31. **Poggioli R, Vergoni AV, and Bertolini A.** ACTH (1–24) and alpha-MSH antagonize feeding behavior stimulated by kappa opiate agonists. *Peptides* 7: 843–848, 1986.

32. **Qu D, Ludwig DS, Gammeltoft S, Piper M, Pelleymounter MA, Cullen MJ, Mathes WF, Przypek J, Kanarek R, and Maratos-Flier E.** A role for melanin-concentrating hormone in the central regulation of feeding behaviour. *Nature* 380: 243–247, 1996.

33. **Rossi M, Kim MS, Morgan DGA, Small CJ, Edwards CMB, Sunter D, Abusnana S, Goldstone AP, Russell SH, Stanley SA, Smith DM, Yagaloff K, Ghatei MA, and Bloom SR.** A C-terminal fragment of agouti-related protein increases feeding and antagonizes the effect of alpha-melanocyte stimulating hormone in vivo. *Endocrinology* 139: 4428–4431, 1998.

34. **Schwartz MW, Baskin DG, Bukowski TR, Kuijper JL, Foster D, Lasser G, Prunkard DE, Porte D, Woods SC, Seeley RJ, and Weigle DS.** Specificity of leptin action on elevated blood glucose levels and hypothalamic neuropeptide Y gene expression in *ob/ob* mice. *Diabetes* 45: 531–535, 1996.

35. **Schwartz MW, Seeley RJ, Campfield LA, Burn P, and Baskin DG.** Identification of targets of leptin action in rat hypothalamus. *J Clin Invest* 98: 1101–1106, 1996.

36. **Schwartz MW, Seeley RJ, Weigle DS, Campfield LA, Burn P, and Baskin DG.** Leptin increases hypothalamic proopiomelanocortin (POMC) mRNA expression in the rostral arcuate nucleus. *Diabetes* 46: 2119–2123, 1997.

37. **Schwartz MW, Sipols AJ, Marks JL, Sanacora G, White JD, Scheurinck A, Kahn SE, Baskin DG, Woods SC, Figlewicz DP, and Porte D Jr.** Inhibition of hypothalamic neuropeptide Y gene expression by insulin. *Endocrinology* 130: 3608–3616, 1992.

38. **Seeley RJ, Thiele TE, Van Dijk G, Baskin DG, Yagaloff KA, Fisher SL, Burn P, and Schwartz MW.** Melanocortin receptors in leptin effects. *Nature* 390: 349, 1997.

39. **Sipols AJ, Baskin DG, and Schwartz MW.** Effect of intracerebroventricular insulin infusion on diabetic hyperphagia and hypothalamic neuropeptide gene expression. *Diabetes* 44: 147–151, 1995.

40. **Thiele TE, Van Dijk G, Yagaloff KA, Fisher SL, Schwartz MW, Burn P, and Seeley RJ.** Central infusion of melanocortin agonist MTII in rats: assessment of c-Fos expression and taste aversion. *Am J Physiol Regulatory Integrative Comp Physiol* 274: R248–R254, 1998.

41. **Tsujii S and Bray GA.** Acetylation alters the feeding response to MSH and beta-endorphin. *Brain Res Bull* 23: 165–169, 1989.

42. **Vaisse C, Clement K, Guy-Grand B, and Froguel P.** A frameshift mutation in human MC4R is associated with a dominant form of obesity. *Nat Genet* 20: 113–114, 1998.

43. **Wilson BD, Bagnol D, Kaelin CB, Ollmann MM, Gantz I, Watson SJ, and Barsh GS.** Physiological and anatomical circuitry between agouti-related protein and leptin signaling. *Endocrinology* 140: 2387–2397, 1999.

44. **Wolden-Hanson T, Marck BT, Smith L, and Matsumoto AM.** Cross-sectional and longitudinal analysis of age-associated changes in body composition of male brown Norway rats: association of serum leptin levels with peripheral adiposity. *J Gerentol* 54: B99–107, 1999.