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Chronic MCH-1 receptor modulation alters appetite, body weight and adiposity in rats

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Abstract

Central administration of the neuropeptide melanin-concentrating hormone (MCH) stimulates feeding in rodents. We studied the effects of intracerebroventricular (i.c.v.) administration of an MCH-1 receptor agonist (Compound A) and an MCH-1 receptor antagonist (Compound B) on feeding in satiated rats. Compound B (10 μg, i.c.v.) blocked the acute orexigenic effect of Compound A (5 μg, i.c.v.). In an experiment designed to either stimulate or inhibit MCH-1 receptor signaling over an extended period, rats received continuous i.c.v. infusions of vehicle (saline), Compound A (30 μg/day), Compound B (30 or 48 μg/day) or neuropeptide Y (24 μg/day, as positive control) via implantable infusion pumps. Continuous MCH-1 receptor activation recapitulated the obese phenotype of MCH-over-expressor mice, manifest as enhanced feeding (+23%, $P<0.001$), caloric efficiency and body weight gain (+38%, $P<0.005$) over the 14-day period relative to controls. Chronic MCH-1 receptor activation also elevated plasma insulin and leptin levels significantly. Conversely, continuous MCH-1 receptor antagonism led to sustained reductions in food intake (−16%, $P<0.001$), body weight gain (−35%, $P<0.01$), and body fat gain relative to controls, without an effect on lean mass. Antagonism of the MCH-1 receptor may be an effective approach for the treatment of obesity.

Keywords: MCH (melanin-concentrating hormone); Food intake; Body weight; Neuropeptide Y; Obesity

1. Introduction

Melanin-concentrating hormone (MCH) is a nonadecapeptide with an amino acid sequence highly conserved among fish, rats and humans (Vaughan et al., 1989). MCH was first isolated from chum salmon pituitary and shown to regulate melanin pigment aggregation in melanocytes (Kawauchi et al., 1983). Neuroanatomical studies in the rat demonstrated that MCH gene expression is restricted to the lateral hypothalamic area and zona incerta with extensive fiber projections throughout the brain (Bittencourt et al., 1992). Lesions of the lateral hypothalamic area produce profound hypophagia and extreme weight loss in rodents, indicating its importance in feeding regulation (Teitelbaum et al., 1969; Bernardis and Bellinger, 1996).

Many studies suggest that MCH plays a role in feeding and energy metabolism in rodents (for review, see Griffond and Baker, 2002; Boutin et al., 2002). Central administration of MCH promotes feeding in rats and mice (Qu et al., 1996; Rossi et al., 1997) and antagonizes the actions of alpha-melanocyte-stimulating hormone (α-MSH), an anorexigenic melanocortin peptide (Sanchez et al., 1997; Grill et al., 1998; Ludwig et al., 1998). The adipocyte hormone leptin inhibits hypothalamic MCH gene expression (Shimada et al., 1998; Kokkotou et al., 2001) while fasting increases hypothalamic MCH mRNA levels in both leptin-deficient *ob/ob* and wild type mice (Qu et al., 1996; Kokkotou et al, 2001). Importantly, prepro-MCH-deficient mice are lean, hypophagic and have an increased metabolic

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rate (Shimada et al., 1998) in contrast to transgenic mice over-expressing the prepro-MCH gene which develop hyperphagia and mild obesity on a high-fat diet (Ludwig et al., 2001).

Two G-protein coupled receptors for MCH have been identified, MCH-1 and MCH-2 receptors, however, only MCH-1 receptor is found in rodents (Saito et al., 2000; An et al., 2001; Hill et al., 2001; Mori et al., 2001; Sailer et al., 2001; Wang et al., 2001; Tan et al., 2002). Both MCH receptors are selective for MCH and are not activated by other neuropeptide derivatives of the prepro-MCH gene, including neuropeptide E-I (NEI), neuropeptide G-E (NGE) and MCH-gene-overprinted-polypeptide (MGOP) (Sailer et al., 2001; Saito et al., 1999; Chambers et al., 1999). MCH-1 receptor mRNA and protein are located in rat cerebral cortex, caudate putamen, hippocampus, amygdala, hypothalamus and thalamus (Hervieu et al., 2002; Saito et al., 2001). MCH-1 receptor deficient (*Mch1r* − / −) mice are lean (like pre-pro-MCH deficient mice), but are hyperphagic when maintained on regular chow (Chen et al., 2002; Marsh et al., 2002). *Mch1r* − / − mice are less susceptible to diet-induced obesity due to their hyperactivity and increased energy expenditure (Chen et al., 2002; Marsh et al., 2002). In addition, the MCH-1 receptor is essential for the orexigenic effects of MCH in mice (Marsh et al., 2002).

To better understand the physiological function of the MCH-1 receptor in mammals, we utilized a potent MCH-1 receptor agonist peptide, Compound A, a truncated analog of MCH which efficiently binds to and activates MCH-1 and MCH-2 receptors (detailed in Bednarek et al., 2001, 2002a) and a selective MCH-1 receptor antagonist peptide, Compound B (Bednarek et al., 2002b). Here we characterize the acute effects of the novel MCH-1 receptor agonist and the selective MCH-1 receptor antagonist on food intake, body temperature and locomotor activity in rats. In addition, in lean rats we evaluate the effects of chronic intracerebroventricular infusions of the MCH 1 receptor agonist, Compound A, the MCH-1 receptor antagonist, Compound B, and neuropeptide Y on food intake, body weight, plasma hormone levels, body composition and on MCH, pro-opiomelanocortin (POMC), neuropeptide Y and melanocortin MC_4 receptor hypothalamic gene expression.

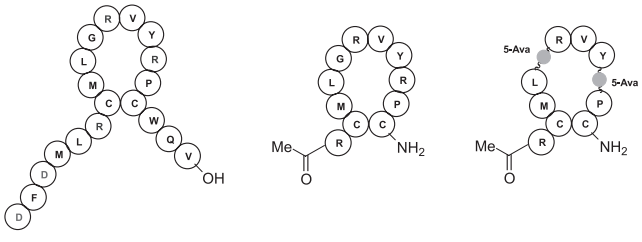
2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats with indwelling stainless steel guide cannulas placed intracerebroventricularly (i.c.v.) into the right lateral ventricle of the brain were purchased from Charles River Laboratories (Wilmington, DE). Rats were housed singly in a temperature-and humidity-controlled room with a 12:12 light–dark cycle (4:00 p.m. EST lights off). Rats were maintained with ad libitum access to water and regular pelleted rat chow (Harlan Teklad, Madison, WI, Diet 7012, 14.8% kcal from fat) prior to study. Rats were approximately 10 weeks of age when studied. Guide cannula placement was confirmed by evaluating angiotensin II-induced water intake. Only i.c.v. cannulated rats shown to drink at least 5 ml of water in 60 min in response to angiotensin II (40 ng, Peninsula Laboratories, Belmont, CA) injection were used in the experiments. All animal procedures were approved by the Merck Research Laboratories Institutional Animal Care and Use Committee (Rahway, NJ).

2.2. Peptide synthesis

Rat neuropeptide Y was purchased from Peninsula Laboratories (San Carlos, CA) and neuromedin U (rat; amino acid residues 1–23) was purchased from Phoenix Pharmaceuticals (Belmont, CA). Peptides Compound A and Compound B were synthesized at Merck (Rahway, NJ) and SynPep (Dublin, CA) (Fig. 1, for details see Bednarek et al., 2001, 2002a,b). All peptides were dissolved in 0.9% saline.



	MCH	Compound A agonist	Compound B antagonist
^{h}MCH-1R EC_{50}(nM):	35	22	N.D.
^{h}MCH-2R EC_{50}(nM):	44	8	K_b > 10,000 (nM)
^{r}MCH-1R EC_{50}(nM):	35	18	K_b = 4.4 (nM)

Fig. 1. Structures of MCH, MCH-1 receptor agonist (Compound A) and antagonist (Compound B) peptides.

2.3. Acute effects of Compound A on feeding and body weight

The dose-dependence of the orexigenic effects of Compound A was evaluated using pre-fed animals challenged at the beginning of the dark phase. Rats were housed singly in shoe-box cages with Nalgene metabolism cage feeders (Mini-Mitter, Sunriver, OR). Rats were acclimated to the feeding cages for a minimum of 2 days prior to study. Animals were exposed to a milled, purified moderate high fat diet (31.8% kcals from fat, primary source of fat from corn oil, D12266B, Research Diets, New Brunswick, NJ) for ~6 h (from 0900–1500 h) a few days before pre-treatment satiation to condition them to the diet. Rats were fed fresh powdered moderate high fat diet for 6 h during the daytime prior to compound dosing. Sixty min before dark onset, 4 μl of vehicle (sterile saline) or 1, 5, or 15 μg (0.72, 3.6, or 10.8 nmol) of Compound A were administered into the lateral ventricle as a bolus. Rats were fed milled moderate high fat diet overnight. Food intake data were measured continuously at 5 min intervals in an automated food intake monitoring system (each food cup was placed on a balance connected to a computer which recorded the weight of food remaining at pre-determined time intervals). Cumulative food intake over time (6 h) was determined for each animal. Rats were weighed before dosing and on the following morning (approx. 18 h after treatment).

2.4. Acute effects of Compound B on feeding and body weight

Rats were housed singly in shoe-box cages with Nalgene metabolism cage feeders. Rats were acclimated to the feeding cages for a minimum of 2 days prior to study. Thirty to sixty min before the dark cycle, a 4 μl bolus of vehicle (sterile saline) or peptides (5 μg (3.6 nmol) of Compound A, 5 μg of Compound A combined with 10 μg (8.6 nmol) of Compound B, or 10 μg of Compound B in a 4 μl volume) was administered into the lateral ventricle and then the rats were fed milled moderate high fat diet overnight. Food intake data were measured continuously at 5 min intervals in an automated food intake monitoring system (as described above). Cumulative food intake over time (6 h) was determined for each animal. Rats were weighed before dosing and on the following morning (approx. 18 h after treatment) to determine treatment effects on overnight body weight.

2.5. Locomotor activity, body temperature and food intake effects of Compound A and Compound B

We evaluated the effects of Compound A and Compound B administration on locomotor activity, core body temperature and appetitive behavior of rats. Rats were housed individually in metabolic cages with Nalgene cage feeders. Food intake was assessed by the frequency and duration of beam breaks recorded upon entry of the animals to the food cups (Mini-Mitter). The amount of food and water consumed was also recorded. Locomotor activity and core body temperature changes were measured using implantable transmitters (e-mitters). Cannulated rats ($n=26$) were anesthetized with ketamine/Dormitor® (medetomidine; Pfizer) (100 and 1 mg/ml, respectively; 50 μl/100 g body weight, i.m.), implanted i.p. with e-mitters (Mini-Mitter), and given the reversing agent Antisedan® (Atipamezole HCl; Pfizer) (5 mg/ml; 50 μl/100 g of body weight, i.m.). Neuromedin U peptide was used as a control for increased locomotor activity and body temperature (30, 31).

Rats were satiated on milled moderate high fat diet in the daytime (for approx. 6 h; 0900–1500 h) and then compounds [Compound A (5 μg; 3.6 nmol), Compound B (10 μg; 8.6 nmol) or neuromedin U (3 μg; 1.1 nmol)] or saline (control) were injected as a bolus i.c.v. into the lateral ventricle 30–60 min prior to lights-off. Rats were fed milled moderate high fat diet overnight. Food cups, water bottles and rats were weighed in the morning.

2.6. Effects of chronic Compound A, Compound B and neuropeptide Y administration on food intake, body weight and body composition

Lean adult male Sprague–Dawley rats (367 g average body weight) were anesthetized with ketamine/Dormitor® (100 and 1 mg/ml, respectively; 50 μl/100 g body weight, i.m.), before s.c. implantation of Alzet osmotic minipumps (model 2001, Alza, Palo Alto, CA) which were connected via polyethylene tubing to the intracerebral cannula for central infusion of saline, Compound A, Compound B or neuropeptide Y. Rats were then given the reversing agent Antisedan® (Pfizer) (5 mg/ml; 50 μl/100 g of body weight, IM). The MCH-1 receptor agonist Compound A, the selective MCH-1 receptor antagonist Compound B, and neuropeptide Y were dissolved in saline and infused into lateral ventricle for 14 days. Neuropeptide Y, an orexigenic peptide, was used as a positive control for increased feeding and body weight gain. Rats were housed individually in Nalgene metabolic cages, fed moderate high fat diet on the day of minipump implantation and for the duration of the infusion study. Treatment groups consisted of the following (7 rats per treatment group): vehicle (saline), Compound A (1.25 μg/0.5 μl/h; 30 μg/day; 21.5 nmol/day), Compound B (1.25 μg/0.5 μl/h; 30 μg/day; 25.7 nmol/day), Compound B (2 μg/0.5 μl/h; 48 μg/day; 41.1 nmol/day) and neuropeptide Y (1 μg/0.5 μl/h; 24 μg/day; 5.6 nmol/day). Food intake and body weights were recorded daily. After 14 days of infusion, rats were euthanized by CO_2 inhalation and tissues including brains, plasma, interscapular brown adipose tissue, epididymal and retroperitoneal white adipose tissue (fat pads) were excised and weighed. Blood was collected by cardiac puncture into heparinized tubes, centrifuged, and plasma was separated and stored at −80 °C for subsequent radioimmunoassays.

2.7. Dual energy X-ray absorptiometry

We analyzed whole body composition by dual energy X-ray absorptiometry (DEXA; QDR 4500A, Hologic, Waltham, MA) of rats treated centrally with saline, Compound A, Compound B or neuropeptide Y. QDR 4500A Small Animal Studies software version 9.0 was used. The body composition of the rats was determined on the day of osmotic minipump implantation and at the termination of the study (after 14 days of treatment). Rats were anesthetized with a cocktail of ketamine/Dormitor® (50 μl/100 g body weight, i.m.) prior to DEXAscan analysis.

2.8. Radioimmunoassays

Plasma concentrations of leptin and insulin were measured using commercially available rat radioimmunoassay kits RL-83K and RI-13K (Linco Research, St. Charles, MO) according to manufacturer's instructions. Glucose, cholesterol, and triglyceride levels were measured in serum using a Roche Hitachi 911 Automated Clinical Chemistry Analyzer.

2.9. Hypothalamic gene expression

Messenger RNA levels were quantified by in situ hybridization using techniques as previously described (Shearman et al., 1999). Coronal brain sections (16 μm thickness) were cut on a Leica cryostat at −20 °C and thaw-mounted onto slides coated with Vectabond (Vector Laboratories, Burlingame, CA). Riboprobes complementary to melanocortin MC_4 receptor, MCH, neuropeptide Y and POMC were generated from cDNA fragments. The templates for neuropeptide Y and POMC probe generation were PCR-generated cDNA fragments subcloned into the TA vector (PCR2.1, Invitrogen). Probes included rat neuropeptide Y (nt 75-426 of the rat sequence assigned to GenBank accession number NM_012614) and POMC (nt 220-697, exon 3, of GenBank number J00579.1. MCH cDNA (mouse sequence, nt 25–548, Breton et al., 1993) was subcloned into pGEMT-easy vector (Promega, Madison, WI). Full-length rat melanocortin MC_4 receptor (nt 148-1146, GenBank number U67863) cDNA was subcloned into pCI-neo vector.

Antisense and sense (control) cRNA probes were produced from linearized plasmid DNA by in vitro transcription in the presence of [^{35}S]α-thio-UTP (1100–1300 Ci/mmol, New England Nuclear, Natick, MA). Probes were purified by extraction and ethanol precipitation prior to use. Probe quality and size were confirmed by determining ^{35}S incorporation into trichloroacetic acid-precipitable material, by gel electrophoresis and autoradiography of the gel.

Pre-hybridization, hybridization and wash conditions were as previously described (Shearman et al., 1999). Probes (50–70 μl at 10^7 c.p.m./ml) were applied to each slide. Coverslipped slides were incubated overnight in humidified chambers at 55 °C. Following wash steps, slides were air dried and apposed to Kodak SB-5 film for 10–12 days. Densitometric analysis of hybridization intensity on the film was performed using an IBM computer-based imaging system and Scion-Image software. Data are expressed as absolute optical density (OD) values as determined by calibration with a Kodak photographic step tablet. Radioactive standards (^{14}C, 20 μm thickness; American Radiolabeled Chemicals, St. Louis, MO) were included on each film to confirm that OD values were within linear range of the film. Statistical assessment was accomplished by analysis of variance of OD values. Data were also analyzed by Dunnett's *t*-test, with $P<0.05$ as the significance level.

2.10. Statistical analysis

All results are presented as the mean ± S.E.M. Statistical significance was assessed by unpaired two-tailed Student's *t*-test, and analysis of variance (ANOVA) with Dunnett's post-hoc test using StatView software (Abacus, CA) and Microsoft Excel. Differences were considered significant at the two-tailed $P<0.05$ value.

3. Results

3.1. Acute food intake effects of Compound A

In pre-fed rats, i.c.v. administration of the MCH-1 receptor agonist Compound A increased food consumption in a dose-and time-dependent manner over the 18 h duration of the study. In comparison to vehicle-treatment, the acute orexigenic effects of Compound A were most evident within the first 6 h following peptide administration (Fig. 2). The extents of the increases in food intake evoked by Compound A at 1, 5 and 15 μg were +315% ($P<0.01$), +409% ($P<0.05$) and +611% ($P<0.01$), respectively, at 3 h; +68% ($P<0.05$), +76% (NS) and +122% ($P<0.05$), respectively, at 6 h; and 0% (NS), +3% (NS) and +47% ($P<0.05$), respectively, at 18 h. No significant differences in overnight body weight gain were observed between the treatment groups, although the 15 μg treated group gained the most weight overnight (+10 g).

3.2. Acute food intake effects of Compound B

The food intake of rats given Compound B (10 μg, i.c.v.) alone did not differ from that of vehicle-treated animals (Fig. 3A). The MCH-1 receptor antagonist Compound B attenuated the orexigenic effects of Compound A from 2 to 4 h post-dosing (−31% vs. Compound A at 3 h, $P<0.05$) (Fig. 3B). In pre-fed rats, 5 μg of the MCH-1 receptor agonist, Compound A, increased food intake markedly for the first 6 h compared to vehicle treatment (+127%, $P=0.057$ at 1 h; +57%, $P<0.05$ at 3 h, +35%, $P<0.05$ at 6 h, Fig. 2A). The major effect of Compound A was

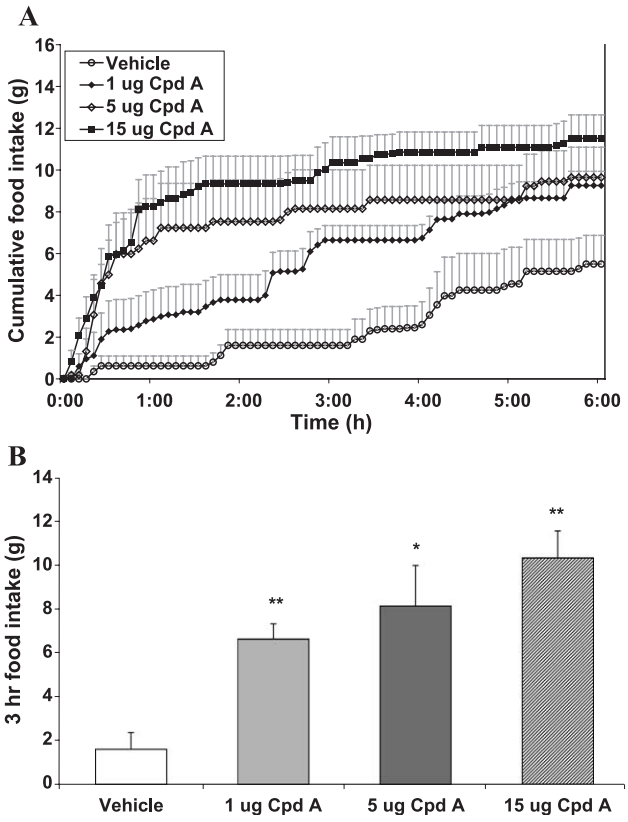


Fig. 2. (A) Cumulative ad libitum food intake following i.c.v. administration of vehicle (saline; 5 μg) or Compound A (1, 5, 15 μg) in pre-fed rats. Plotted values are mean ± S.E.M. of four to eight rats per treatment group. (B) Food intake of lean rats in first 3 h following i.c.v. treatment with saline or Compound A (1, 5 and 15 μg). *$P<0.05$ significantly different from saline; **$P<0.01$ significantly different from saline.

observed in the first 2 h after dosing. There were no significant effects of Compound A or Compound B alone or in combination on overnight body weight gain (body weight changes ranged from − 3 to +6 g).

3.3. Locomotor activity, body temperature and feeding effects of Compound A and Compound B

The overall pattern of responses during the 6 h period indicated an increased feeding duration with Compound A treatment and decreased feeding duration with the control neuromedin U. The MCH-1 receptor agonist Compound A increased food intake as measured by feeding duration during the first 2 h post-dosing compared to vehicle (+110%, $P<0.05$; Fig. 4A). Neuromedin U decreased feeding duration significantly from 2 to 4 h post-dosing (−97%, $P<0.05$ vs. vehicle at 2 h, Fig. 4A). No significant differences were observed with Compound B; there was a trend, however, for decreased feeding duration. Feeding frequency correlated well with feeding duration (data not shown). There were no significant effects of the MCH related peptides on water intake, indicating a specific effect on food intake.

Compound A increased cumulative gross motor activity significantly from 0 to 2 h post-dosing (+223% at 2 h, $P<0.05$, Fig. 4B) and no effect was detected from 2–4 or 4–6 h. Similarly, neuromedin U increased activity from 0 to 2 h post-dosing (+329% at 2 hr, $P<0.05$, Fig. 4B). Compound B treatment did not alter locomotor activity significantly at any time point examined. Neuromedin U had a biphasic effect on core body temperature; it increased body temperature at 1 and 6 h post-dosing (Fig. 4C). Neither Compound A nor Compound B had any appreciable effect on core body temperature.

3.4. Food intake and body weight gain from 14-day infusion study

The effects of continuous i.c.v. infusion with Compound A, Compound B and neuropeptide Y on cumulative food intake are depicted in Fig. 5A. The MCH-1 receptor agonist Compound A increased food intake significantly as early as day 7 of infusion and by day 14 cumulative food intake in Compound A-treated animals exceeded that of the vehicle

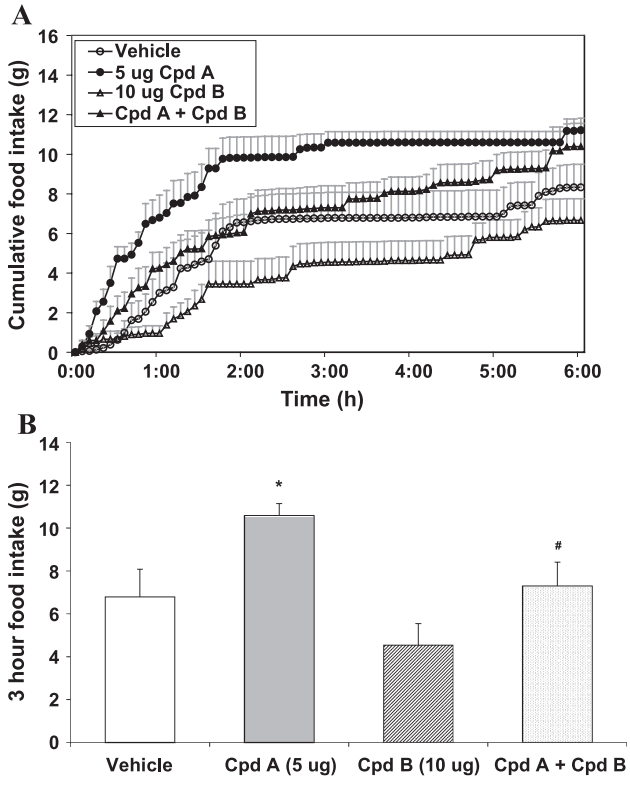


Fig. 3. (A) Cumulative ad libitum food intake following i.c.v. administration of vehicle (saline; 5 μg), Compound A (5 μg), Compound B (10 μg) or a combination of Compound A (5 μg) and Compound B (10 μg) in pre-fed rats. Plotted values are mean ± S.E.M. of six to seven rats per treatment group. (B) Food intake of lean rats during the first three hours following ICV treatment with saline, Compound A (5 μg), Compound B (10 μg) or Compound A (5 μg) and Compound B (10 μg). *$P<0.05$ significantly different from saline, #$P<0.05$ significantly different from Compound A alone.

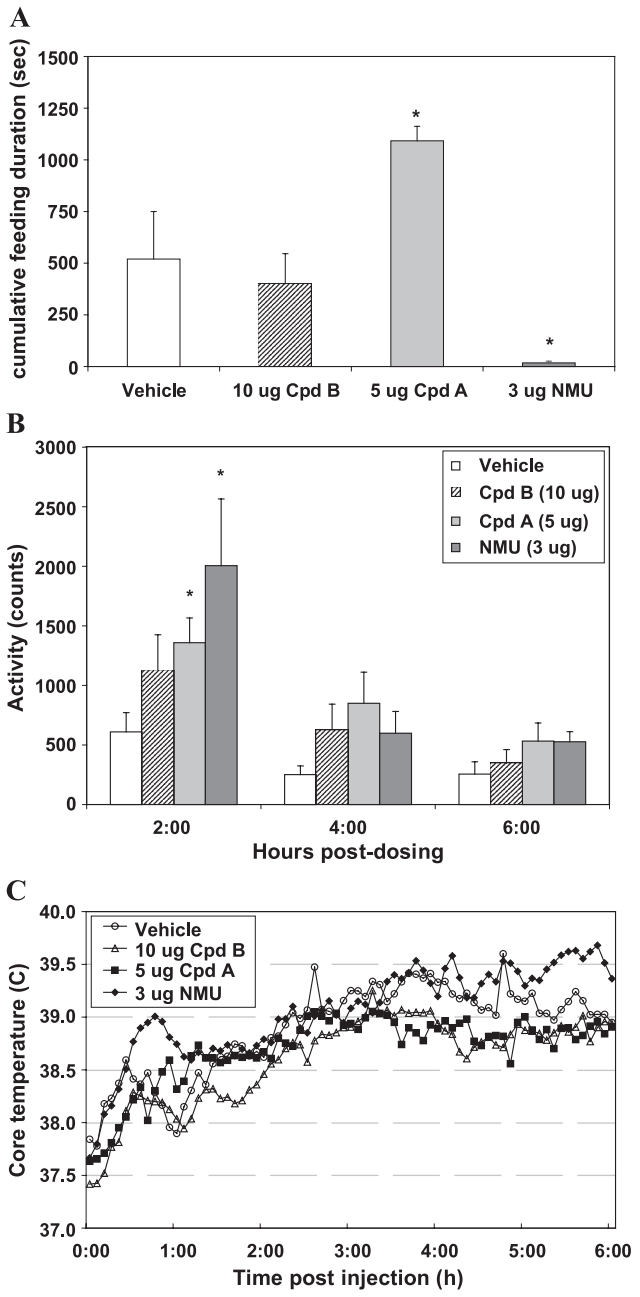


Fig. 4. (A) Feeding duration in first 2 h post-dosing, (B) locomotor activity and (C) core body temperature during the dark period of pre-fed rats given i.c.v. injections of vehicle (saline; 5 μg; $n=6$), Compound A (5 μg; $n=7$), Compound B (10 μg; $n=7$) or neuromedin U (3 μg; $n=6$). *$P<0.05$ denotes statistical difference with the vehicle-treated group.

group by 23% ($P<0.0005$). Infusion of the MCH-1 receptor antagonist Compound B at 30 or 48 μg/day suppressed food intake as early as day 3 and by day 14 cumulative food intake was decreased by 16% ($P<0.001$ vs. vehicle) and 13% ($P<0.05$ vs. vehicle) at 30 and 48 μg, respectively. Neuropeptide Y elicited a more rapid stimulation of feeding that was evident by day 3 of infusion. Neuropeptide Y infusion caused a robust hyperphagia, yielding a 91% increase in cumulative food consumption compared to vehicle treatment by day 14 ($P<0.001$). In terms of mean daily food intake, Compound A-infused rats consumed more on a daily basis than controls from days 3 to 14, inclusive (Fig. 5B). The amount of food consumed by rats treated with Compound B at either 30 or 48 μg/day was slightly lower than controls on most treatment days. The daily hyperphagia observed in neuropeptide Y infused rats appeared to diminish by day 13.

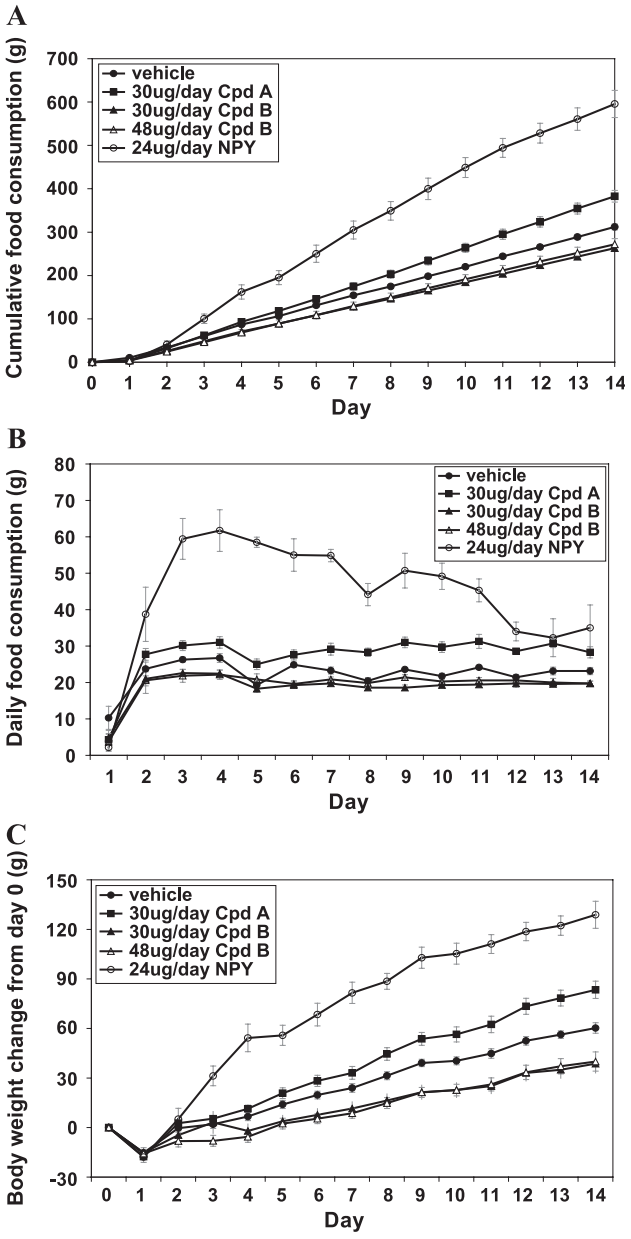


Fig. 5. Effect of lateral i.c.v. treatment for 14 days with vehicle (saline; $n=7$), Compound A (30 μg/day; $n=7$), Compound B (30 μg/day; $n=7$), Compound B (48 μg/day; $n=7$), or neuropeptide Y (24 μg/day; $n=7$) on cumulative food intake (A), daily food intake (B) and body weight change (C). Both food intake and body weight were significantly decreased in Compound B treated animals compared with the vehicle-treated group. Neuropeptide Y and Compound A infusion significantly increased food intake and body weight compared with the vehicle-treated group.

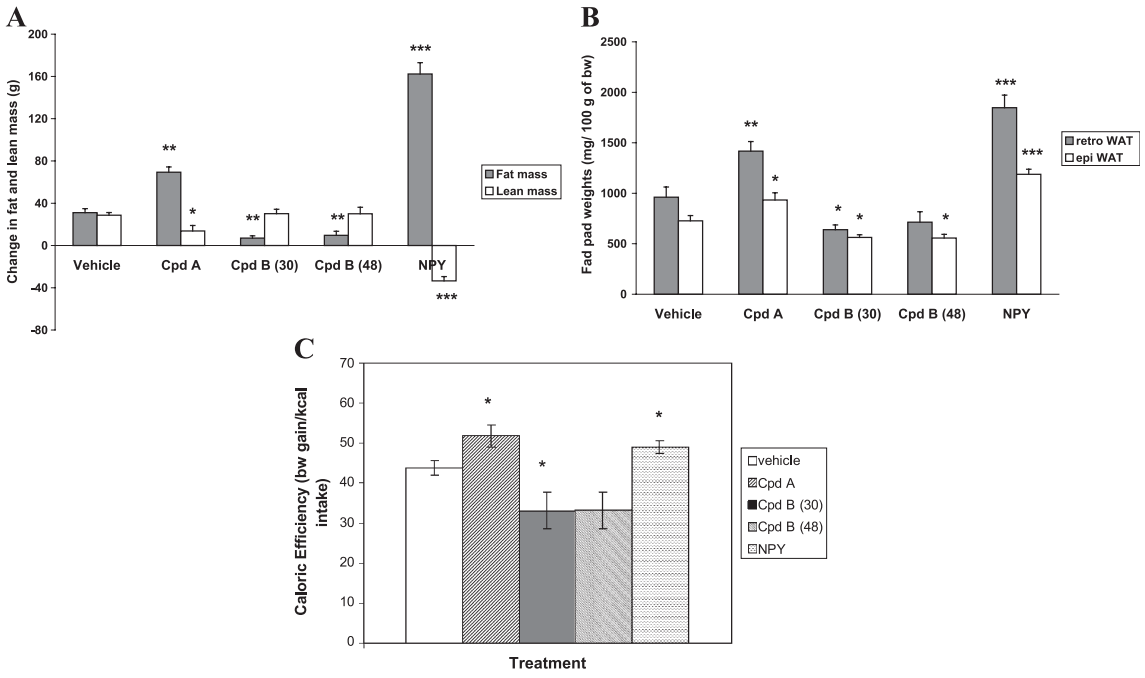


Fig. 6. Body composition including (A) fat and lean mass gain from pre-treatment period and (B) fat pad weights of rats infused centrally for 14 days with vehicle (saline; $n=7$), Compound A (30 μg/day; $n=7$), Compound B (30 μg/day; $n=7$), Compound B (48 μg/day; $n=7$), or neuropeptide Y (24 μg/day; $n=7$). (C) Caloric efficiency (mg body weight gain/kcal intake). *$P<0.05$, **$P<0.01$, ***$P<0.001$ denote statistical difference with the vehicle-treated group.

Compound A increased body weight gain above that of vehicle-treated rats from days 8 to 14 (+38%, $P<0.005$ on day 14; Fig. 5C). Compound B at 48 μg/day reduced body weight gain from days 3 to 14 (−33%, $P<0.01$ on day 14). The 30 and 48 μg/day doses of Compound B were equivalent in their suppressive effects on food intake and body weight gain. Neuropeptide Y infusion increased body weight gain dramatically from day 3 of infusion and this was maintained throughout the study (+115%, $P<0.001$ on day 14). There was no evidence of reduced efficacy of neuropeptide Y, Compound A or Compound B on body weight gain over time.

3.5. Body composition and feed efficiency

Body composition analysis on day 14 revealed significantly increased fat mass, % body fat, and fat pad weights of rats chronically infused with the orexigenic peptides Compound A or neuropeptide Y (Fig. 6A). Relative to vehicle on day 14, Compound A increased fat mass by 222% (69 ± 5 g vs. 31 ± 4 g, $P<0.0001$) and neuropeptide Y infusion increased fat mass by 522% (+139 ± 11 g, $P<0.0001$ vs. vehicle). Compound A did not have an appreciable effect on lean mass gain relative to baseline (day 0). However, when compared to the vehicle group, the lean mass gain of Compound A treated rats was significantly reduced (gain of 14 ± 5 g vs. 29 ± 3 g for vehicle, $P<0.05$). Associated with dramatic increases in adiposity, neuropeptide Y infusion resulted in a marked reduction in lean mass by 34 g (10%) relative to day 0 (Fig. 6A). Compound B, at 30 and 48 μg/day, decreased fat mass and % body fat significantly (fat mass was reduced by 22% and 35%, respectively, on day 14 compared to day 1). Compound B treatment decreased epididymal and retroperitoneal fat pad weights significantly (Fig. 6B). Chronic infusion of Compound A or Compound B did not affect bone mineral content. In contrast, continuous neuropeptide Y infusion for 14 days increased bone mineral content (2.7 ± 0.2 g vs. 1.4 ± 0.2 g, $P<0.001$ vs. vehicle).

Feed efficiency was calculated to address possible effects of the MCH 1 receptor peptides on energy expenditure. The feed efficiency on day 14 (mg body weight gain/kcal food intake) revealed that the orexigenic peptides, Compound A and neuropeptide Y, increased caloric efficiency significant-

Table 1
Effects of central administration of MCH-1R peptides and NPY on serum parameters

	Glucose (mg/dL)	Insulin (ng/mL)	Leptin (mg/mL)	Cholesterol (mg/dL)	Triglycerides (mg/dL)
Vehicle	268 ± 23	0.6 ± 0.2	4.3 ± 1.2	80 ± 3	188 ± 40
Cpd A	280 ± 8	1.5 ± 0.2[a]	18.8 ± 2.4[c]	94 ± 6	188 ± 17
Cpd B (30 μg)	260 ± 9	0.5 ± 0.2	2.4 ± 0.5	69 ± 8	162 ± 24
Cpd B (48 μg)	242 ± 5	0.9 ± 0.2	3.9 ± 0.7	71 ± 5	183 ± 60
NPY	263 ± 10	3.2 ± 0.6[b]	76.2 ± 11.2[c]	234 ± 26[c]	95 ± 12[a]

Data are means ± S.E.M. $N=7$ rats per treatment group.
[a] $P<0.05$ vs. vehicle.
[b] $P<0.01$ vs. vehicle.
[c] $P<0.001$ vs. vehicle.

ly ($P<0.05$ vs. vehicle) (Fig. 6C). In contrast, the MCH-1 receptor antagonist peptide Compound B evoked decreases in caloric efficiency relative to vehicle treatment ($P=0.05$ for 30 μg; $P=0.06$ for 48 μg), suggesting that Compound B infusion increased energy expenditure (Fig. 6C).

3.6. Endocrine parameters

After 14 days of infusion, the orexigenic peptides Compound A and neuropeptide Y increased plasma leptin and insulin levels significantly (Table 1). The mean plasma leptin and insulin levels of vehicle-treated rats were 4.32 and 0.61 ng/ml, respectively. The MCH-1 receptor agonist Compound A increased leptin levels to 18.8 ng/ml ($P<0.001$ vs. vehicle) and insulin to 1.5 ng/ml ($P<0.05$ vs. vehicle). Neuropeptide Y infusion caused robust increases in plasma leptin to 76.2 ng/ml ($P<0.001$ vs. vehicle) and insulin to 3.2 ng/ml ($P<0.01$ vs. vehicle). Compound B (MCH 1 receptor antagonist) infusion, at either dose, did not alter plasma leptin or insulin levels in comparison to vehicle-treated rats. Neuropeptide Y treated rats showed a significant elevation in plasma cholesterol (234 mg/dL vs. 80 mg/dL, $P<0.001$) and reduction in triglyceride levels (95 mg/dL vs. 188 mg/dL, $P<0.05$), without an effect on glucose levels (263 mg/dL vs. 268 mg/dL, NS). Cholesterol, triglyceride and glucose levels were not altered by Compound A or Compound B treatment.

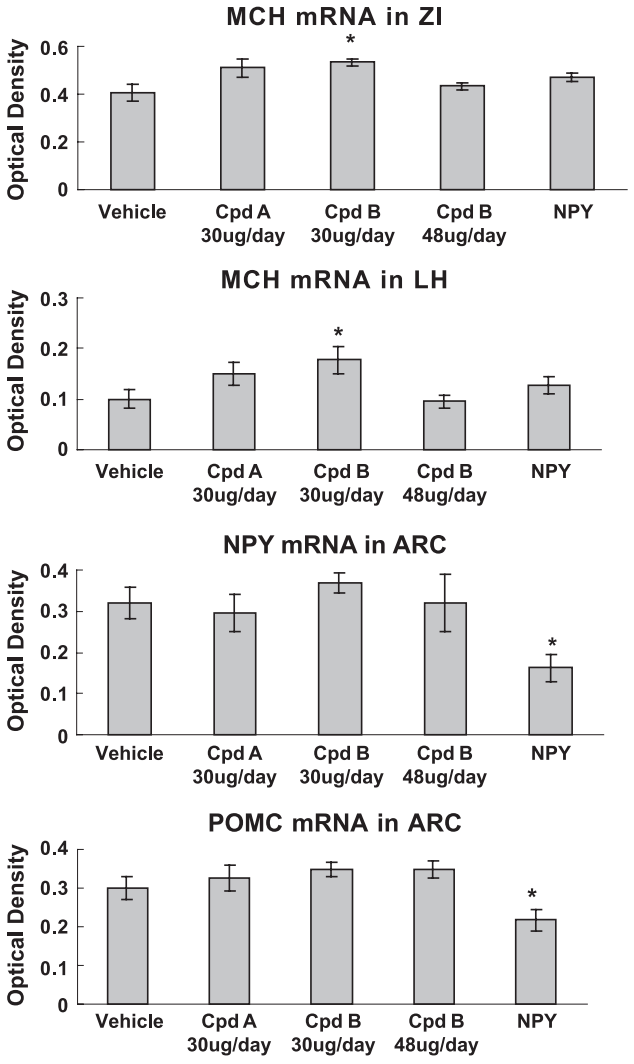


Fig. 7. Expression of mRNA for MCH in lateral hypothalamus and zona incerta, and neuropeptide Y and POMC in the arcuate hypothalamic nucleus of rats infused centrally for 14 days with vehicle (saline; $n=7$), Compound A (30 μg/day; $n=7$), Compound B (30 μg/day; $n=7$), Compound B (48 μg/day; $n=7$), or neuropeptide Y (24 μg/day; $n=7$). *$P<0.05$ denotes statistical difference with the vehicle-treated group. Levels of mRNA are expressed as optical density values (in arbitrary units).

3.7. Hypothalamic gene expression

Infusion with the MCH-1 receptor antagonist Compound B for 14 days elevated MCH mRNA expression in the lateral hypothalamus and zona incerta ($P<0.01$ for LH and $P<0.05$ for ZI vs. vehicle treatment, Fig. 7). Central neuropeptide Y infusion for 14 days reduced POMC and neuropeptide Y mRNA expression in the arcuate nucleus ($P<0.05$ vs. vehicle treatment for both) with no change in hypothalamic MCH mRNA levels. Compound B infusion non-significantly reduced neuropeptide Y mRNA expression in the arcuate nucleus. Compound A infusion did not alter expression of the neuropeptides examined. None of the peptide treatments altered melanocortin MC_4 receptor gene expression in the paraventricular nucleus of the hypothalamus (data not shown).

4. Discussion

Here we show that central administration of the MCH-1 receptor agonist Compound A into the lateral ventricle of satiated rats prior to dark onset increases food intake and that this hyperphagia can be blocked pharmacologically by the MCH-1 receptor antagonist Compound B. We used a moderate high fat (MHF) diet in our studies because we determined empirically that it provided a substantially bigger window for detecting effects of the MCH-1 receptor peptides on food intake over regular, low fat chow. It was more difficult to discern the orexigenic effects of Compound A when standard low fat chow was used. Others have shown that MCH-1 receptor stimulation can increase food intake acutely in rats (Suply et al., 2001). The MCH-1 receptor agonist Compound A increased both feeding frequency and duration of feeding bouts for several hours after dosing. Importantly, acute treatment with the MCH-1 receptor antagonist, Compound B, did not alter locomotor activity, core body temperature or feeding behavior under our experimental conditions. In contrast, our control, neuromedin U, decreased feeding duration significantly from 2 to 4 h post-dosing, elevated core body temperature at 1 and 6 h post-dosing and increased locomotor activity from 2 to 6 h post-dosing, similarly to previous reports (Howard et al., 2002; Nakazato et al., 2000).

We demonstrated that continual modulation of the MCH-1 receptor can elicit lasting changes in appetite, body weight gain, and body composition. Continuous infusion of the MCH-1 receptor agonist Compound A for 14 days led to substantial increases in food intake, body weight gain, and plasma leptin and insulin levels (mimicking hormonal changes of obesity), without a concomitant increase in plasma cholesterol levels. This effect on body weight gain is in contrast to a study by Rossi et al. (1997) which reported that repeated i.c.v. (third ventricle) injections of MCH to lean rats over 8 days did not affect body weight. Possible explanations for the observed differences in efficacy between the studies include the strain of rat, diets, and mode of administration (intermittent injection vs. continuous infusion). We found that central infusion of the MCH-1 receptor agonist Compound A, like the potent orexigenic peptide neuropeptide Y, selectively increased fat mass and decreased lean mass. However, the magnitude of the obesity phenotype after Compound A infusion was not as pronounced as with NPY infusion. Central infusion of MCH-1 receptor agonist Compound A recapitulated the obese phenotype of the MCH-over-expressor mice (Ludwig et al., 2001). Others have recently shown similar effects of MCH infusion on feeding and body weight gain in rats (Della-Zuana et al., 2003). Chronic MCH infusion was reported to increase hypothalamic levels of NPY mRNA, but this effect was strain-dependent (Della-Zuana et al., 2003). We did not observe any changes in hypothalamic expression of MCH, melanocortin MC_4 receptor, neuropeptide Y or POMC mRNA levels following MCH-1 receptor agonist (Compound A) infusion.

The results after treatment with the MCH-1 receptor antagonist Compound B for 14 days were consistent with the lean phenotype of prepro-MCH deficient mice (Shimada et al., 1998). Our results are also in agreement with the sustained feeding and body weight effects of an MCH-1 receptor antagonist given intraperitoneally to diet-induced obese rats (Borowsky et al., 2002). Compound B, at 30 and 48 μg/day, significantly decreased fat mass, as well as epididymal and retroperitoneal fat pad weights without effect on lean mass. The decreases in body weight and fat mass may not be explained solely by a reduction in food intake, suggesting that additional factors such as enhanced thermogenesis may play a role. To address this issue, caloric efficiency was calculated; this ratio indicates how efficiently the food energy was used for accretion of body mass. The feeding efficiency of rats infused with the MCH-1 receptor antagonist, Compound B, was lower than that of vehicle, Compound A, or neuropeptide Y treated animals, suggesting that MCH-1 receptor antagonist treatment increases energy expenditure. In contrast, treatment with orexigenic compounds Compound A and neuropeptide Y significantly increased caloric efficiency, indicative of decreases in metabolic rate/energy expenditure. The effects of Compound A on caloric efficiency were as pronounced as that of neuropeptide Y. Central neuropeptide Y treatment has been shown previously to depress metabolic rate (Balasko et al., 1999). We did not measure energy expenditure using a calorimeter, but the decrease in feed conversion efficiency with chronic MCH-1 receptor antagonist treatment provides indirect evidence for an increase in energy expenditure. Indeed, *Mch1r* −/− mice have increased metabolic rate relative to wild-types during the dark cycle and exhibit a lower respiratory quotient, an indicator of metabolic fuel preference, during the light cycle (Marsh et al., 2002). The increased metabolic rate seen in *Mch1r* −/− mice was temporally correlated with increased locomotor activity, indicating that this increase could be secondary to the hyperactivity (Marsh et al., 2002). The food intake effects of 14-day MCH-1 receptor antagonist infusion differ from the observed hyperphagia of the *Mch1r* −/− mice (Chen et al., 2002; Marsh et al., 2002). However, *Mch1r* −/− mice are hyperactive and we did not detect an effect of acute MCH-1 receptor antagonist treatment on locomotor activity. These differences may be attributable to the lack of the MCH-1 receptor during development in *Mch1r* −/− mice, to potential compensatory mechanisms, or to species differences.

We found that infusion of the MCH-1 receptor antagonist Compound B for 14 days elevated MCH mRNA expression in the lateral hypothalamus and zona incerta of rats. This finding is consistent with evidence that fasting up-regulates hypothalamic MCH gene expression (Qu et al., 1996). Neuropeptide Y and POMC mRNA levels in the arcuate were not affected by chronic MCH-1 receptor antagonism. The lack of effect of chronic MCH-1 receptor blockade on neuropeptide Y and POMC mRNA levels is in agreement with the reported normal neuropeptide expression in *Mch1r* deficient mice (Chen et al., 2002; Marsh et al., 2002).

Our findings with central neuropeptide Y infusion and moderate high fat feeding are in agreement with other reports of chronic neuropeptide Y administration to normal rats producing hyperphagia, hyperinsulinemia, hypercorticosteronemia, increased body weight and fat storage (Zarjevski et al., 1993; McMinn et al., 1998; Raposinho et al., 2000). We also observed decreased triglyceride levels, increased cholesterol levels and greater bone mineral content in rats chronically infused with neuropeptide Y. By in situ hybridization, we found that 14-day infusion of neuropeptide Y (24 μg/day) into the lateral ventricle decreased arcuate POMC and neuropeptide Y mRNA levels substantially without effects on hypothalamic MCH or melanocortin MC_4 receptor mRNA levels. Others have reported that an infusion of neuropeptide Y (12 μg/day) into the third ventricle of male Long-Evans rats for a shorter duration (4.5 days) had no effect on arcuate POMC levels (McMinn et al., 1998). However, the authors observed elevated plasma leptin and insulin and decreased neuropeptide Y mRNA levels in the arcuate following 4.5 day i.c.v. infusion of neuropeptide Y, suggesting that elevated leptin and insulin signaling feed back to inhibit neuropeptide Y gene expression. It is possible that the down-regulation of POMC gene expression we observed with 14-day neuropeptide Y

infusion was mediated via changes in insulin and leptin. In another study, 7-day neuropeptide Y infusion decreased hypothalamic POMC and neuropeptide Y gene expression as measured by Northern blot analysis (Raposinho et al., 2000). Interestingly, neuropeptide Y over-expressing mice become obese when fed a high sucrose diet, indicating that diet palatability can modulate effects of neuropeptide Y on obesity (Kaga et al., 2001). In our study, rats were fed a moderate high fat diet containing high amounts of sucrose; this diet exposure might have impacted the magnitude of the orexigenic response we observed with central neuropeptide Y infusion.

These results suggest that MCH-1 receptor agonism and neuropeptide Y infusion in rats may increase adiposity by divergent pathways because the hormonal effects of these treatments differed (triglyceride and cholesterol changes were observed with neuropeptide Y infusion but not with Compound A infusion). In addition, neuropeptide Y and POMC RNA levels were down-regulated with neuropeptide Y infusion but not following Compound A (MCH-1 receptor agonist) infusion, although both treatments elevated plasma insulin and leptin levels. Alternatively, the greater adiposity and weight gain induced by neuropeptide Y infusion may have resulted in the observed differences.

Here we demonstrate that chronic MCH-1 receptor blockade is capable of producing long-term reductions in appetite, caloric efficiency and body weight gain. These findings support the importance of MCH in the regulation of energy homeostasis and suggest that an MCH-1 receptor antagonist may be effective for the treatment of obesity.

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