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Characterization of MCH-mediated obesity in mice

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Ito, Masahiko, Akira Gomori, Akane Ishihara, Zenjun Oda, Satoshi Mashiko, Hiroko Matsushita, Mariko Yumoto, Makoto Ito, Hideki Sano, Shigeru Tokita, Minoru Moriya, Hisashi Iwaasa, and Akio Kanatani. Characterization of MCH-mediated obesity in mice. *Am J Physiol Endocrinol Metab* 284: E940–E945, 2003. First published January 28, 2003; 10.1152/ajpendo.00529.2002.—Melanin-concentrating hormone (MCH) is a cyclic orexigenic peptide expressed in the lateral hypothalamus. Recently, we demonstrated that chronic intracerebroventricular infusion of MCH induced obesity accompanied by sustained hyperphagia in mice. Here, we analyzed the mechanism of MCH-induced obesity by comparing animals fed ad libitum with pair-fed and control animals. Chronic infusion of MCH significantly increased food intake, body weight, white adipose tissue (WAT) mass, and liver mass in ad libitum-fed mice on a moderately high-fat diet. In addition, a significant increase in lipogenic activity was observed in the WAT of the ad libitum-fed group. Although body weight gain was marginal in the pair-fed group, MCH infusion clearly enhanced the lipogenic activity in liver and WAT. Plasma leptin levels were also increased in the pair-fed group. Furthermore, MCH infusion significantly reduced rectal temperatures in the pair-fed group. In support of these findings, mRNA expression of uncoupling protein-1, acyl-CoA oxidase, and carnitine palmitoyltransferase I, which are key molecules involved in thermogenesis and fatty acid oxidation, were reduced in the brown adipose tissue (BAT) of the pair-fed group, suggesting that MCH infusion might reduce BAT functions. We conclude that the activation of MCH neuronal pathways stimulated adiposity, in part resulting from increased lipogenesis in liver and WAT and reduced energy expenditure in BAT. These findings confirm that modulation of energy homeostasis by MCH may play a critical role in the development of obesity.

melanin-concentrating hormone; body weight; liver weight; brown adipose tissue

OBESITY IS CAUSED BY AN IMBALANCE between caloric intake and energy expenditure. The hypothalamus plays an important role in the integrated regulation of this homeostatic balance. In the past several years, a number of hypothalamic neuropeptides involved in feeding and energy homeostasis were discovered. Melanin-concentrating hormone (MCH), a cyclic neuropeptide originally isolated from salmon pituitaries (8), was recently identified as a mediator of energy homeostasis. In rodents, MCH is predominantly expressed in the lateral hypothalamic area, pivotal center of energy homeostasis (1, 20). Qu et al. (15) found that MCH stimulated food intake after intracerebroventricular (ICV) administration. Furthermore, mRNA levels of MCH increased in genetic obesity models such as *ob*/*ob*, *db*/*db*, and A^Y/a mice and Zucker fatty rats (5, 14, 15, 21). These results suggest that MCH might play an important role in the regulation of energy homeostasis. In support of the potential role of MCH, prepro-MCH-deficient mice are lean and hypophagic and show a slight increase in their metabolic rate (18). Moreover, prepro-MCH overexpression in transgenic mice is marked by obesity and insulin resistance (11). However, these models do not rule out influence of the neuropeptides (N)GE and NEI, which are derived from prepro-MCH.

In a previous report (4), we demonstrated that the chronic ICV infusion of MCH rapidly causes hyperphagia and obesity in mice maintained on a moderately high-fat (MHF) diet. To address the mechanism of MCH-mediated obesity more precisely, we aim to elucidate specific mechanisms resulting from activation of the central MCH neuronal system. Because increased food intake caused by ICV-injected MCH makes it difficult to correctly interpret MCH-induced metabolic changes in peripheral tissues, we also performed a pair-feeding study in an MCH-induced obesity model.

MATERIALS AND METHODS

Materials. MCH was purchased from Peptide Institute, Osaka, Japan. All other chemicals were of analytical grade.

Animals. Male C57BL/6J mice (13 wk old, Clea Japan, Tokyo, Japan) were used. Mice were housed individually in plastic cages that were kept at 23 ± 2°C with 55 ± 15% relative humidity and on a 12:12-h light-dark cycle (7 PM lights off). Mice had ad libitum access to food (CE-2, Clea Japan) and tap water during the acclimation period. After the acclimation period, mice were given an MHF diet (Oriental Bioservice, Kyoto, Japan). The MHF diet provides 52.4% energy as carbohydrate, 15.0% as protein, and 32.6% as fat (4.41 kcal/g). All experimental procedures were in accordance with the Japanese Pharmacological Society Guideline for Animal Use.

Surgical procedure and experimental designs. Mice were anesthetized with pentobarbital sodium (80 mg/kg ip; Dain-

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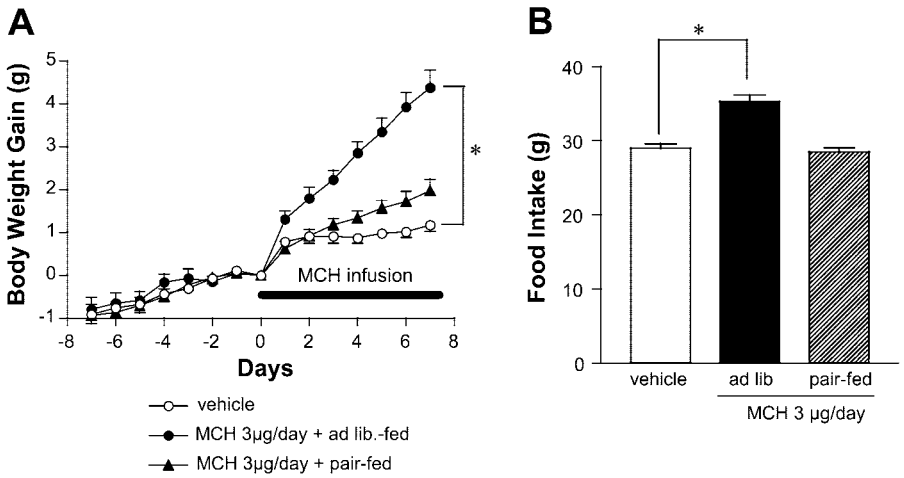

Fig. 1. Changes in body weight (*A*) and cumulative food intake (*B*) during melanin-concentrating hormone (MCH) infusion. Data are means ± SE. *$P < 0.05$ vs. vehicle group.

abot, Tokyo, Japan). A sterile brain infusion cannula (28-gauge; Alzet, Palo Alto, CA) was stereotaxically implanted into the right lateral ventricle. When a flat skull position was used, the stereotaxic coordinates were 0.4 mm posterior to the bregma, 0.8 mm lateral to the midline, and 2.0 mm from the surface of the skull. The cannulas were fixed to the skull with dental cement. The infusion cannula was connected to an osmotic minipump (Alzet, model no. 2002) that was filled with 30% propylene glycol (PG) and had polyvinyl chloride tubing. A pump was implanted under the skin of each mouse's back, and an antibiotic (Cefamedine α, 50 mg/kg; Fujisawa Pharmaceutical, Osaka, Japan) was injected subcutaneously. During the recovery period, body weight and daily food intake were measured. The mice were divided into three groups matched for average body weight: vehicle infused (vehicle group), MCH infused (ad libitum-fed group), and MCH infused and pair fed (pair-fed group). Subsequently, the pumps were replaced with MCH (3 μg/day)- or its vehicle (30% PG)-containing pumps with the mice under light ether anesthesia. Daily food intake and body weight were measured for 7 days (n = 8–9). The pair-fed group was provided the same amount of food as the vehicle group.

Measurement of rectal temperature. Five days before and seven days after the start of MCH infusion, rectal temperature of mice was measured during early afternoon with a digital thermometer (BAT-12, Physitemp Instruments, Clifton, NJ) equipped with a rectal probe (IT-14, Physitemp Instruments) inserted to a depth of 1.5 cm. The mice were gently wrapped with paper towel and lightly held during insertion of the probe. To reduce methodological stress, mice were sufficiently acclimated to the procedure before the test period. All measurements were recorded when rectal temperature reading reached a plateau.

Measurement of plasma hormone levels. After a 7-day ICV-infusion of MCH, the mice were fasted for 2 h, and blood samples were collected from the orbital vein for measurements of plasma glucose, insulin, and leptin levels. Subsequently, the mice were killed by collecting whole blood from the hearts under isoflurane anesthesia. Epididymal, retroperitoneal, and mesenteric adipose tissue and liver were excised and weighed. Glucose levels were measured by the pyranose peroxidase method (Determiner GL-E kit; Kyowa Medex, Tokyo, Japan). Triglyceride (TG) levels were measured by the glycerol kinase-glycerol-3-phosphate oxidase-peroxidase method with free glycerol elimination (Determiner L TGII, Kyowa Medex). Free fatty acid levels were measured by the acyl-CoA synthase-acyl-CoA oxidase method [NEFA-HA Test Wako (II); Wako Pure Chemical Industries, Tokyo, Japan]. Insulin and leptin levels were measured by ELISA (Morinaga, Yokohama, Japan).

Lipogenesis. A small fragment of epididymal adipose tissue (~50 mg wet wt) and a slice of liver (~25 mg wet wt) were incubated in 1 ml of Dulbecco's modified Eagle's medium (Invitrogen, Carlsbad, CA) containing 0.5 mM sodium acetate and 0.4 μCi sodium [1-^{14}C]acetate (Amersham Biosciences, Piscataway, NJ). Glass tubes were gassed for 15 s with 95% O_2-5% CO_2, capped, and incubated for 2 h in a shaking water bath at 37°C. After the incubation period, 1 ml of saline was added to the tube, and tissue was homogenized. The neutral lipids in the homogenate were extracted with chloroform-methanol (2:1, vol/vol). A portion of the lipid extract was dispersed in scintillation fluid, and its radioactivity was determined by a liquid scintillation counter (TRI-CARB2500, PerkinElmer Life Sciences, Boston, MA). The incorporation of [^{14}C]acetate into neutral lipids was expressed as acetate incorporated per gram of wet tissue in 2 h.

Fatty acid oxidation. Small fragments of intrascapular brown adipose tissue (BAT) (5–10 mg wet wt) and epididymal adipose tissue (~50 mg wet wt) were incubated in 1.5 ml of Krebs-Ringer bicarbonate, pH 7.4, containing 4% fatty acid-free bovine serum albumin and 2 μCi [1-^{14}C]palmitic acid (PerkinElmer Life Sciences) in vials. The vials were gassed for 15 s with 95% O_2-5% CO_2, capped, and incubated for 40 min in a shaking water bath at 30°C. At the end of the incubation period, the reaction was terminated by the addition of 0.5 ml of 9 M H_2SO_4, and the vials were incubated further for 1 h at 37°C. Gaseous $^{14}CO_2$ produced during incubation was trapped in filter paper soaked in 25% phenethylamine. This filter paper was contained in a trapping vial, which was connected to the incubation vial. The radioactivity of the filter paper was determined using a liquid scintillation counter. The conversion of [^{14}C]palmitic acid to

Table 1. *WAT and liver weights*

	Vehicle	Ad Lib	Pair Fed
Epididymal fat	0.51 ± 0.04	0.78 ± 0.05*	0.61 ± 0.06
Mesenteric fat	0.35 ± 0.04	0.71 ± 0.05*	0.47 ± 0.05
Retroperitoneal fat	0.18 ± 0.22	0.29 ± 0.02*	0.24 ± 0.02
Liver	1.24 ± 0.04	1.60 ± 0.04*	1.54 ± 0.04*

Data are means ± SE in grams. WAT, white adipose tissue; Ad Lib, ad libitum-fed group. *$P < 0.05$ vs. vehicle group.

Table 2. *Plasma glucose, hormone, and lipid levels and liver triglyceride levels*

	Vehicle	Ad Lib	Pair Fed
Glucose, mg/dl	217 ± 6	244 ± 10	204 ± 11
Leptin, ng/ml	5.9 ± 1.4	18.3 ± 1.8*	12.6 ± 1.5*
Insulin, ng/ml	1.2 ± 0.2	1.7 ± 0.1	1.5 ± 0.2
Triglyceride, mg/dl	40 ± 2	40 ± 3	60 ± 7*
Free fatty acid, μeq/l	461 ± 43	428 ± 22	475 ± 34
Liver triglyceride, mg/g	13.6 ± 4.6	29.9 ± 5.3*	16.3 ± 4.4
Liver glycogen, mg/g	19.1 ± 6.8	25.7 ± 7.5	40.3 ± 5.3†

Data are means ± SE. *$P < 0.05$; †$P < 0.01$ vs. vehicle group.

$^{14}CO_2$ was expressed as palmitate converted per gram of wet tissue in 40 min.

Real-time RT-PCR. TaqMan analysis was used to determine mRNA levels of uncoupling protein-1 (UCP1), acyl-CoA oxidase (ACO), and liver (L) and muscle (M) type carnitine palmitoyltransferase I (CPT I) in BAT, as well as leptin levels in white adipose tissue (WAT) and sterol-regulatory element-binding protein-1c (SREBP1c) and fatty acid synthase (FAS) in the liver. Total RNA was purified using Isogen (Nippon Gene, Tokyo, Japan). Quantity and purity were determined by absorbance at 260 and 280 nm. cDNA was synthesized from 1 μg of total RNA using TaqMan RT reagents (Applied Biosystems, Foster City, CA).

In a typical reaction, 5 ng of cDNA were mixed with 12.5 μl of TaqMan Universal PCR Master Mix (Applied Biosystems); 900 nM of forward and reverse primers; 250 nM of probe complementary to the gene of interest, which was labeled with FAM dye; 50 nM of each primer; and 200 nM of probe complementary to 18S rRNA labeled with VIC dye (Applied Biosystems) in a 25-μl total volume. The reaction was performed in a 384-well optical reaction plate using PRISM 7900HT Sequence Detection System (Applied Biosystems). The expression data were normalized to 18S rRNA expression level.

The following primers and probes were used in the real-time PCR. For UCP1 the primers were forward GCAGATATCATCACCTTCCCG, reverse CCTGGCCTTCACCTTGGAT, and probe TGGACACTGCCAAAGTCCGCCTTC. For ACO the primers were forward GCCTTTGTTGTCCCTATCCGT, reverse CGATATCCCCAACAGTGATGC, and probe AGATTGGGACCCACAAGCCTCTGCC. For CPT I-L the primers were forward CCTGCAACTTTGTGCTGGC, reverse TGAACAGCTTGAGCCTCTGCT, and probe ATGATGGACCCCACAACAACGGCA. For CPT I-M the primers were forward AATATGTCTACCTCCGAAGCAGGA, reverse CGTGAACGGCATTGCCTAG, and probe CAACTATTATGCCATGGATTTTGTGCTTATTAAGA. For leptin the primers were forward GACATTTCACACACGCAGTCG, reverse AGCCCAGGAATGAAGTCCAA, and probe TATCCGCCAAGCAGAGGGTCACTGG. For SREBP1c the primers were forward GTAGCGTCTGCACGCCCTA, reverse CTTGGTTGTTGATGAGCTGGAG, and probe ACGGAGCCATGGATTGCACATTTGAAG. For FAS the primers were forward GGCTCAGCATGGTCGCTT, reverse CTCCCGCCAGCTGTCATT, and probe AACCACCCTCTGGGCATGGCTATCTTCT.

Statistical analysis. The mean values ± SE were analyzed by a one-way analysis of variance (ANOVA), followed by a Bonferroni test for multiple comparisons. Differences were considered significant when the P value was <0.05.

RESULTS

Change in body weight and food intake. MCH strongly increased food intake and body weight gain in the ad libitum-fed group (Fig. 1). In the pair-fed group, a slight increase in body weight was observed compared with the vehicle group, but the difference was not statistically significant (1.17 ± 0.14 g in the vehicle group and 4.38 ± 0.41 g in the ad libitum-fed group, $P < 0.05$; 1.98 ± 0.26 g in the MCH-pair-fed group).

Tissue weight. As shown in Table 1, all fat pad weights significantly increased in the ad libitum-fed group, and a tendency toward increased fat pad weights was also observed in the pair-fed group; the same was true for body weight. Interestingly, not only the ad libitum-fed group but also the pair-fed group showed significant increases in liver weight (Table 1).

Plasma and liver parameters. Plasma leptin levels significantly increased in both the ad libitum-fed and the pair-fed groups (5.9 ± 1.4 ng/ml in the vehicle group, 18.3 ± 1.8 ng/ml in the ad libitum-fed group, $P < 0.05$; 12.6 ± 1.5 ng/ml in the pair-fed group, $P < 0.05$; Table 2). Similar results were obtained for leptin mRNA levels in WAT (data not shown). Plasma glucose, insulin, and free fatty acid levels were similar between groups (Table 2). In addition, plasma TG levels increased in the pair-fed group to a greater extent than in the vehicle and the ad libitum-fed groups (40 ± 2 mg/dl in the vehicle group, 40 ± 3 mg/dl in the

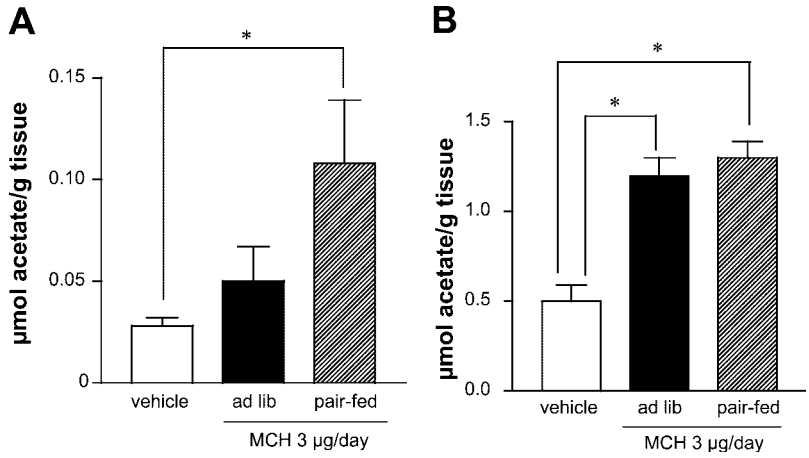

Fig. 2. Ex vivo lipogenic activity in liver (*A*) and white adipose tissue (WAT; *B*). Lipogenic activities were measured using isolated liver or WAT after infusion of vehicle or MCH, as described in MATERIALS AND METHODS. Data are means ± SE. *$P < 0.05$ by ANOVA followed by a Bonferroni test for multiple comparisons.

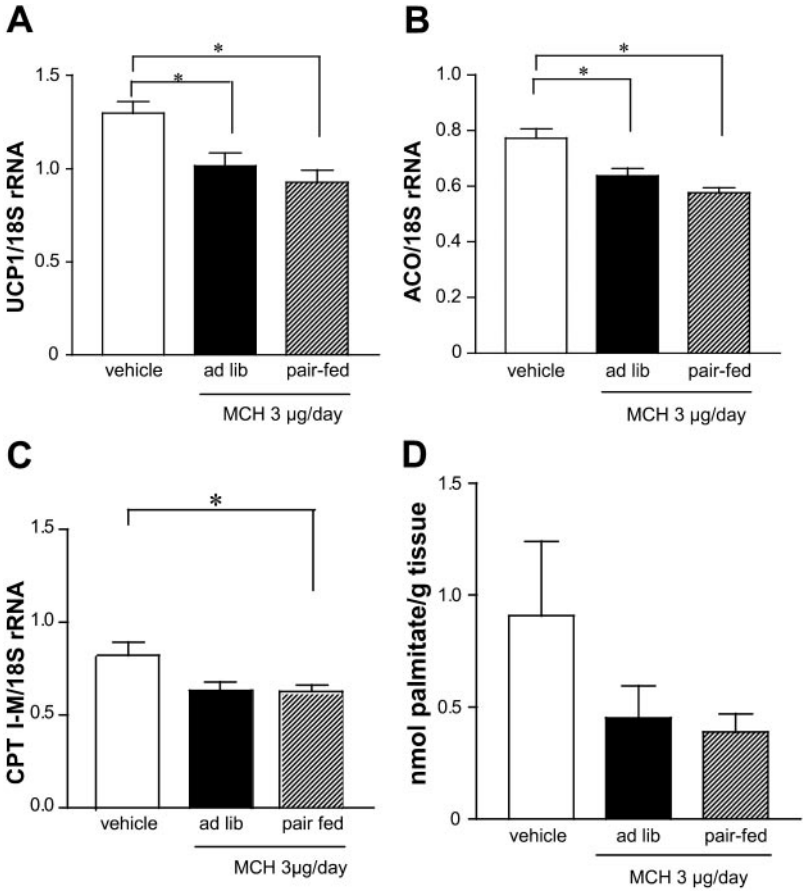

Fig. 3. Brown adipose tissue (BAT) functions. mRNA levels of uncoupling protein-1 (UCP1; *A*), acyl-CoA oxidase (ACO; *B*), and muscle type (M) carnitine palmitoyltransferase I (CPT I-M; *C*) and activities of ex vivo fatty acid oxidation (*D*) in BAT. mRNA levels were measured by the TaqMan system, as described in MATERIALS AND METHODS. Fatty acid oxidation was measured using isolated BAT after the infusion of vehicle or MCH, as described in MATERIALS AND METHODS. Data are means ± SE. *$P < 0.05$ by ANOVA followed by a Bonferroni test for multiple comparison.

MCH-ad libitum-fed group, and 60 ± 7 mg/dl in the pair-fed group; $P < 0.05$; Table 2).

TG contents in liver increased twofold in the ad libitum-fed group but not in the pair-fed group (13.6 ± 4.6 mg/g in the vehicle group, 29.9 ± 5.3 mg/g in the ad libitum-fed group, $P < 0.05$; 16.3 ± 4.4 mg/g in the pair-fed group; Table 2). By contrast, glycogen contents in liver increased twofold in the pair-fed group compared with the vehicle group (19.1 ± 6.8 mg/g in vehicle group, 25.7 ± 7.5 mg/g in the ad libitum-fed group, 40.3 ± 5.3 mg/g in the pair-fed group; $P < 0.01$; Table 2).

Lipogenesis in liver and WAT. Ex vivo lipogenic activity in liver (Fig. 2*A*) and epididymal WAT (Fig. 2*B*) was assessed. Lipogenic activity in liver increased significantly in the pair-fed group but not in the ad libitum-fed group compared with the vehicle group (0.028 ± 0.004 μmol acetate/g tissue in the vehicle group, 0.050 ± 0.017 μmol acetate/g tissue in the ad libitum-fed group, 0.108 ± 0.031 μmol acetate/g tissue in the pair-fed group, $P < 0.05$). The lipogenic activity in WAT was significantly enhanced in both the ad libitum-fed and pair-fed groups [0.498 ± 0.09 μmol acetate/g tissue (vehicle group), 1.194 ± 0.104 μmol acetate/g tissue; $P < 0.05$ (ad libitum-fed group), and 1.298 ± 0.091 μmol acetate/g tissue, $P < 0.05$ (pair-fed group)].

Molecular profiling and fat oxidation in BAT. We measured expression levels of genes related to energy expenditure and metabolism in BAT. As shown in Fig. 3*A*, UCP1 mRNA decreased significantly in both the ad libitum-fed and the pair-fed groups. Furthermore, expression levels of ACO mRNA, which is the key enzyme of β-oxidation in peroxisomes, were reduced by MCH infusion (Fig. 3*B*). Moreover, mRNA of CPT I-M, which has a crucial role in β-oxidation pathways in the mitochondria, was also reduced in the pair-fed group (Fig. 3*C*). Results for CPT I-L mRNA were similar to those for CPT I-M (data not shown).

In addition, we investigated ex vivo fatty acid oxidation activity in isolated BAT. Although this difference was not statistically significant, fatty acid oxidation activity tended to decrease in the MCH-treated groups compared with the vehicle group (0.908 ± 0.331 nmol palmitate/g tissue in the vehicle group, 0.452 ± 0.142 nmol palmitate/g tissue in the ad libitum-fed group, and 0.388 ± 0.083 nmol palmitate/g tissue in the pair-fed group; Fig. 3*D*).

Rectal temperature. Rectal temperature was measured before and after ICV infusion of MCH (Table 3).

Table 3. *Rectal temperature*

	Vehicle	Ad Lib	Pair Fed
Pre	36.8 ± 0.14	36.8 ± 0.14	36.6 ± 0.14
Post	37.2 ± 0.14	36.9 ± 0.17	35.7 ± 0.07*

Data are means ± SE in °C. Pre and Post, before and after intracerebroventricular infusion. *$P < 0.05$ vs. vehicle group.

Although rectal temperature in the vehicle and the ad libitum-fed groups did not change, ICV infusion of MCH significantly decreased this parameter in the pair-fed group.

DISCUSSION

In the ad libitum-fed group, MCH infusion caused a significant hyperphagia and an increase in body and WAT weights. These findings were consistent with previous results (4). In contrast, changes of body and WAT weights were not statistically significant in the pair-fed group. However, plasma leptin levels were significantly increased in the pair-fed group and in the ad libitum-fed group. It is well known that plasma leptin levels are correlated with adiposity (12). Additionally, significantly increased lipogenic activity in liver and WAT was observed in the pair-fed group. Furthermore, reduced rectal temperature reduction of UCP1 mRNA expression levels in BAT was observed in the pair-fed group. Taken together, these results suggest that MCH-induced obesity was caused not only by hyperphagia but also by regulation of metabolism, although direct measurement of metabolic changes such as oxygen consumption remains to be addressed. Recent literature shows that overexpression of prepro-MCH in mice reduces their metabolic rate (11). Our results support the findings that reduced metabolic rate in mice overexpressing prepro-MCH might be caused by MCH.

Liver weight was increased in the ad libitum-fed group due to the increases in TG content. Hepatic TG content was increased in the ad libitum-fed group but not in the pair-fed group. By contrast, the plasma TG level was increased in the pair-fed group but not in the ad libitum-fed group. Moreover, ex vivo lipogenic activity in liver was enhanced in the pair-fed group but not in the ad libitum-fed group. On the basis of these results, we speculate that MCH itself might have stimulated lipogenesis in liver and TG output into plasma in the pair-fed group. On the other hand, in the ad libitum-fed group, TG derived from increased food intake might be responsible for the accumulation of TG and might inhibit de novo lipogenesis in liver. It has been reported that the influx of dietary fatty acid inhibits de novo lipogenesis in the liver (7). Therefore, these data indicate that MCH may cause hepatic hypertrophy via increases in TG not only by hyperphagia but also by stimulation of fatty acid synthesis.

To confirm this hypothesis, we measured mRNA levels of liver SREBP1c, which was proposed to regulate gene expression related to lipid biosynthesis (6). However, significant changes in SREBP1c mRNA could not be seen in the three groups. Furthermore, FAS mRNA did not change (data not shown). These observations indicate that the changes in liver function by MCH may not be regulated on the transcriptional level under the control of the SREBP-1c pathway. Therefore, the mechanism by which MCH enhances lipogenic activity in the liver is still unclear. However, MCH may attenuate the protein level or activity of some lipogenic enzymes (19). Direct measurement of enzymatic activities of key molecules remains to be addressed.

Hepatic glycogen was increased in the pair-fed group but not in the ad libitum-fed group. Although the mechanisms are still unclear, MCH may increase hepatic glycogen via insulin or other hormone signals.

In the pair-fed group, rectal temperature was decreased by MCH infusion. MCH also decreased mRNA levels of UCP1, which is abundantly expressed in BAT and has an important role in thermogenesis in rodents (9). Furthermore, MCH inhibited ex vivo fatty acid oxidation and reduced mRNA levels of ACO and CPT I, which are involved in fatty acid oxidation (10, 16). The results indicate that chronic infusion of MCH may suppress BAT function. The reductions of BAT function were observed in both the ad libitum-fed and the pair-fed groups; however, rectal temperatures were similar between the vehicle and ad libitum-fed groups. It was previously reported that increases in caloric intake enhance metabolic rate (17), suggesting that the reduction in rectal temperature by MCH infusion might be masked by diet-induced thermogenesis. Thus the suppressed BAT function might, at least in part, contribute to MCH-induced body weight gain.

Herein we showed that MCH had important roles not only in appetite but also in energy metabolism. The stimulation of fat synthesis in liver and WAT and the reduction of BAT function might contribute to MCH-mediated obesity. As the next step to further define the mechanism of action of MCH-mediated obesity, evaluation of potent and selective MCH antagonists will be of interest. Recently, the antiobesity effects of the potent and selective MCH-1 receptor antagonist SNAP-7941 was reported (2). The validation of specificity of such a small molecule will be critical for our understanding of these pathways. The approach of the MCH-induced obesity model described here, in combination with MCH receptor antagonists and MCH-1 receptor-deficient mice, will help elucidate the role of MCH in energy homeostasis (3, 13).

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