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Chronic intracerebroventricular infusion of MCH causes obesity in mice

AKIRA GOMORI, AKANE ISHIHARA, MASAHIKO ITO, SATOSHI MASHIKO, HIROKO MATSUSHITA, MARIKO YUMOTO, MAKOTO ITO, TAKESHI TANAKA, SHIGERU TOKITA, MINORU MORIYA, HISASHI IWAASA, AND AKIO KANATANI
Tsukuba Research Institute, Banyu Pharmaceutical Co., Ltd., Okubo 3, Tsukuba 300–2611, Japan

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Gomori, Akira, Akane Ishihara, Masahiko Ito, Satoshi Mashiko, Hiroko Matsushita, Mariko Yumoto, Makoto Ito, Takeshi Tanaka, Shigeru Tokita, Minoru Moriya, Hisashi Iwaasa, and Akio Kanatani. Chronic intracerebroventricular infusion of MCH causes obesity in mice. *Am J Physiol Endocrinol Metab* 284: E583–E588, 2003. First published November 26, 2002; 10.1152/ajpendo.00350.2002.—Melanin-concentrating hormone (MCH) is a cyclic amino acid neuropeptide localized in the lateral hypothalamus. Although MCH is thought to be an important regulator of feeding behavior, the involvement of this peptide in body weight control has been unclear. To examine the role of MCH in the development of obesity, we assessed the effect of chronic intracerebroventricular infusion of MCH in C57BL/6J mice that were fed with regular or moderately high-fat (MHF) diets. Intracerebroventricular infusion of MCH (10 μg/day for 14 days) caused a slight but significant increase in body weight in mice maintained on the regular diet. In the MHF diet-fed mice, MCH more clearly increased the body weight accompanied by a sustained hyperphagia and significant increase in fat and liver weights. Plasma glucose, insulin, and leptin levels were also increased in the MCH-treated mice fed the MHF diet. These results suggest that chronic stimulation of the brain MCH system causes obesity in mice and imply that MCH may have a major role in energy homeostasis.

melanin-concentrating hormone; food intake; body weight; fat weight

MELANIN-CONCENTRATING HORMONE (MCH) is a cyclic amino acid peptide that was first isolated from salmon pituitaries (7). MCH is expressed predominantly in the lateral hypothalamus (18), which is well known to play an important role in the control of feeding. Hypothalamic expression of prepro-MCH (*Pmch*) mRNA is upregulated during starvation in lean mice as well as in genetically obese *ob*/*ob* mice (21). Bolus intracerebroventricular injection of MCH stimulates food intake in rats and mice (21, 23, 25). These observations are good evidence to support the role of MCH in the central regulation of feeding behavior. In addition, *Pmch*-deficient mice are lean, with accompanying hypophagia and an increased metabolic rate (28), and *Pmch* overexpression slightly increases food intake and body weight in mice (12), suggesting that MCH is also a major regulator of energy homeostasis. However, *Pmch* also encodes neuropeptide EI (NEI) and neuropeptide GE (NGE), although the physiological roles of NEI and NGE are not yet fully understood. Thus it is difficult to conclude that the phenotypes observed in the models that represent genetic manipulations of *Pmch* are attributable to the effects of MCH itself. Furthermore, repetitive intracerebroventricular injection of MCH is reported to cause tachyphylaxis in feeding stimulation and to have no effect on body weight (24), which casts doubt on the role of MCH in energy homeostasis.

In the present study, we assessed the effect of chronic intracerebroventricular infusion of MCH in mice fed a regular or a moderately high-fat (MHF) diet to clarify the role of MCH itself in the development of obesity.

MATERIALS AND METHODS

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

Animals. Male C57BL/6J mice (15 wk old, CLEA Japan, Tokyo, Japan) were used. Mice were housed individually in plastic cages under controlled temperature and humidity (23 ± 2°C, 55 ± 15%) and a 12:12-h light-dark cycle (7 PM lights off) with ad libitum access to regular diet (CE-2; CLEA Japan) and tap water for an acclimation period. All experimental procedures followed the Japanese Pharmacological Society Guideline for Animal Use.

Surgical procedure and experimental designs. After 2–3 wk of acclimation, mice were anesthetized with pentobarbital sodium (80 mg/k ip; Dainabot, Osaka, Japan). A sterile brain infusion cannula (28 gauge; Alzet, Palo Alto, CA) was stereotaxically implanted into the right lateral ventricle. The stereotaxic coordinates, using a flat skull position, 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 cannula was fixed to the skull with dental cement. The cannula was connected to an osmotic minipump (model no. 2001, Alzet) filled with 30% propylene glycol (PG) with polyvinyl chloride tubing. The pump was implanted under the skin of the back, and antibiotic (Cefamedine α, 50 mg/kg; Fujisawa Pharmaceutical, Tokyo, Japan) was injected subcutaneously. After a 7- to 14-day recovery period, mice were divided into four

Address for reprint requests and other correspondence: A. Ishihara, Tsukuba Research Institute, Banyu Pharmaceutical Co., Ltd., Okubo 3, Tsukuba 300–2611, Japan (E-mail: isihraan@banyu.co.jp).

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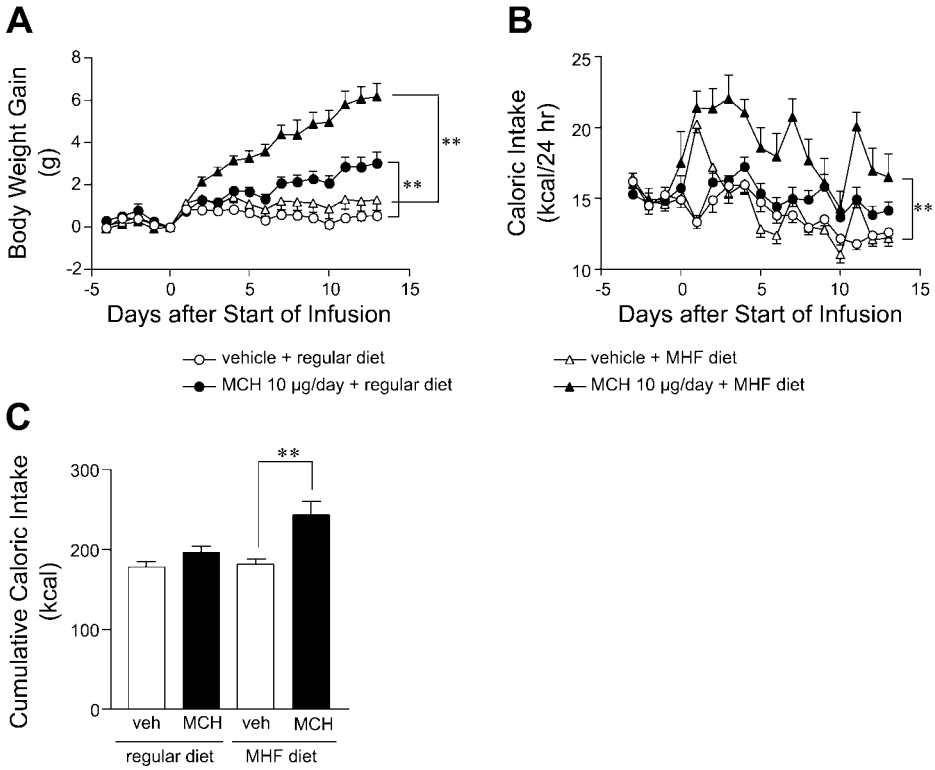

Fig. 1. Body weight change (*A*) and daily (*B*) and cumulative (*C*) caloric intake in mice receiving icv infusion of melanin-concentrating hormone (MCH) (10 μg/day) or vehicle [veh; 30% propylene glycol (PG)] under regular diet- or moderately high-fat (MHF) diet-fed conditions. Data are represented as means ± SE; $n = 10$. $^{**}P < 0.01$ vs. vehicle-treated groups.

groups to match average body weight and food intake ($n = 10$). The infusion pump was replaced with a new pump (model no. 2001, Alzet) filled with MCH (10 μg/day for 14 days) or its vehicle (30% PG) under ether anesthesia. A pair of MCH- and vehicle-treated groups was fed the regular diet throughout the experiment. In the other two groups of mice, the diet was changed from the regular diet to an MHF diet [slightly-modified diet reported by Lauterio et al. (10); Oriental BioService, Tokyo, Japan] at the start of the MCH or vehicle infusion. The MHF diet provided 52.4% energy as carbohydrate, 15.0% as protein, and 32.6% as fat (4.41 kcal/g). The regular diet provided 59.3% energy as carbohydrate, 29.2% as protein, and 11.5% as fat (3.42 kcal/g). Food intake and body weight were measured daily. After the 14-day intracerebroventricular infusion, mice were fasted for 2 h, and blood samples were collected from the infraorbital vein for measurement of plasma glucose, insulin, and leptin levels. Then the mice were killed by collecting whole blood from the heart under ether anesthesia. Epididymal, retroperitoneal, and mesenteric adipose tissues and liver were excised and weighed.

Measurement of plasma biochemical parameters. Plasma leptin and insulin levels were measured with ELISA kits (Morinaga, Kanagawa, Japan). Plasma glucose, triglyceride (TG), total, HDL-, and non-HDL-cholesterol and free fatty acid levels were measured using commercial kits [Determiner GL-E, L TGII, L TCII, L HDL-C, and L LDL-C, Kyowa Medex, Tokyo, Japan; NEFA-HA Testwako (II), Wako Pure Chemical Industries, Osaka, Japan].

Motor activity. Another set of MCH- or vehicle-infused mice was prepared for measurement of spontaneous motor activity. MCH (10 μg/day) or the vehicle was infused for 14 days under the regular diet-fed condition. Motor activity was measured during the last 3 days of the 14-day infusion by an activity monitoring system (NS-AS01, Neuroscience, Tokyo, Japan) in home cages. In brief, the activity monitor was composed of an infrared ray sensor placed over a home cage (21 × 32 × 12.5 cm), a signal amplification circuit, and a control unit. The sensor detected the movement of animals on the basis of the released infrared ray associated with their body temperature (16, 19). The data of motor activity were collected at 10-min intervals and analyzed with a computer-associated analyzing system (AB System-24A, Neuroscience).

Statistics. Data are expressed as means ± SE. Significant differences in body weight changes and daily caloric intake were analyzed by repeated-measures one-way ANOVA and the Bonferroni test. For cumulative caloric intake, blood parameters, and tissue weights, analysis by one-way ANOVA and the Bonferroni test was performed. Two-way ANOVA was performed for the interaction between factors of diet and MCH infusion. *P* values <0.05 were considered to be significant.

RESULTS

In the regular diet-fed mice, chronic intracerebroventricular infusion of MCH (10 μg/day) slightly but significantly increased body weight. Body weight gains for 14 days were 3.03 ± 0.52 g in the MCH-infused

Table 1. *Body weight before and after chronic icv infusion of MCH*

	Regular Diet		MHF Diet	
	Vehicle	MCH	Vehicle	MCH
Initial body weight	28.6 ± 0.5	28.4 ± 0.4	28.6 ± 0.6	28.7 ± 0.8
Final body weight	29.1 ± 0.5	31.4 ± 0.8†	29.9 ± 1.0	34.9 ± 0.9†

Data represent means ± SE in grams; $n = 10$. MCH, melanin-concentrating hormone; MHF, moderately high fat; icv, intracerebroventricular. †$P < 0.01$ vs. initial body weight.

Table 2. *Effect of icv infusion of MCH on tissue weights*

	Regular Diet		MHF Diet	
	Vehicle	MCH	Vehicle	MCH
Epididymal fat	0.31 ± 0.01	0.48 ± 0.07	0.65 ± 0.11*	1.16 ± 0.08‡
Mesenteric fat	0.25 ± 0.03	0.35 ± 0.06*	0.39 ± 0.08	0.79 ± 0.09‡
Retroperitoneal fat	0.10 ± 0.01	0.21 ± 0.03*	0.28 ± 0.05†	0.53 ± 0.06‡
Liver	1.51 ± 0.05	1.67 ± 0.07	1.31 ± 0.06*	1.67 ± 0.07‡

Data represent means ± SE in grams; $n = 10$. *$P < 0.05$, †$P < 0.01$ vs. vehicle-treated regular diet-fed group; ‡$P < 0.01$ vs. vehicle-treated MHF diet-fed group.

group vs. 0.55 ± 0.19 g in the vehicle-infused group (Fig. 1*A* and Table 1). The MHF diet alone did not affect body weight in the vehicle-infused mice during this experiment. The MCH infusion in the presence of the MHF diet caused a remarkable increase in body weight (gains of 6.17 ± 0.64 and 1.29 ± 0.50 g in the MCH- and in the vehicle-infused group, respectively; Fig. 1*A* and Table 1). There was a significant interaction between diet and the MCH infusion in the final body weight gain [$F(1,36) = 5.94$, $P = 0.0199$], indicating that the MCH-infused mice gained more weight when they were fed the MHF diet.

In the regular diet-fed mice, the MCH infusion showed a tendency to increase cumulative caloric intake, whereas the difference was not statistically significant (Fig. 1*C*). The vehicle-infused mice on the MHF diet showed a transient increase in food intake on *day 1*. The food intake levels returned to the basal level on *day 2*. The MCH-infused mice on the MHF diet showed a sustained hyperphagia during the experiment, and total caloric intake was significantly increased compared with that of the vehicle-infused mice (Fig. 1, *B* and *C*). There was a significant interaction between diet and the MCH infusion in cumulative caloric intake [$F(1,36) = 5.23$, $P = 0.0282$]. Thus MCH stimulated caloric intake more in the MHF diet-fed mice than in the regular diet-fed mice. Water intake was slightly increased by the MCH infusion in the MHF diet-fed mice but not in the regular diet-fed mice (data not shown).

Fourteen-day infusion of MCH increased the adipose tissue weights in both the regular diet-fed and the MHF diet-fed mice (Table 2). A significant interaction between diet and the MCH infusion was observed in epididymal [$F(1,36) = 5.07$, $P = 0.0306$] and mesenteric fat weights [$F(1,36) = 5.00$, $P = 0.0316$], indicating that MCH stimulated fat accumulation more in the MHF diet-fed than in the regular diet-fed mice. Exposure to the MHF diet reduced liver weight compared with that of the regular diet-fed mice. The MCH infusion significantly increased the liver weight in the MHF diet-fed group (Table 2).

In the regular diet-fed mice, the MCH infusion did not affect plasma glucose and insulin levels, whereas MCH increased the plasma leptin level fourfold (Fig. 2). Exposure to the MHF diet resulted in significant increases in these parameters compared with those of the regular diet-fed mice. The MCH infusion with the MHF diet significantly stimulated the increase in the plasma levels of glucose 1.2-fold, insulin 2-fold, and leptin 3-fold (Fig. 2). Significant interactions between diet and the MCH infusion were observed in the insulin and leptin levels [$F(1,35) = 4.37$, $P = 0.0439$ and $F(1,36) = 10.04$, $P = 0.0031$, respectively] but not in the glucose level.

The MCH infusion did not affect total, HDL-, or non-HDL-cholesterol levels in the regular diet-fed group (Table 3). Although these parameters were increased by the MHF diet in the vehicle-infused group, further changes were not observed by the MCH treat-

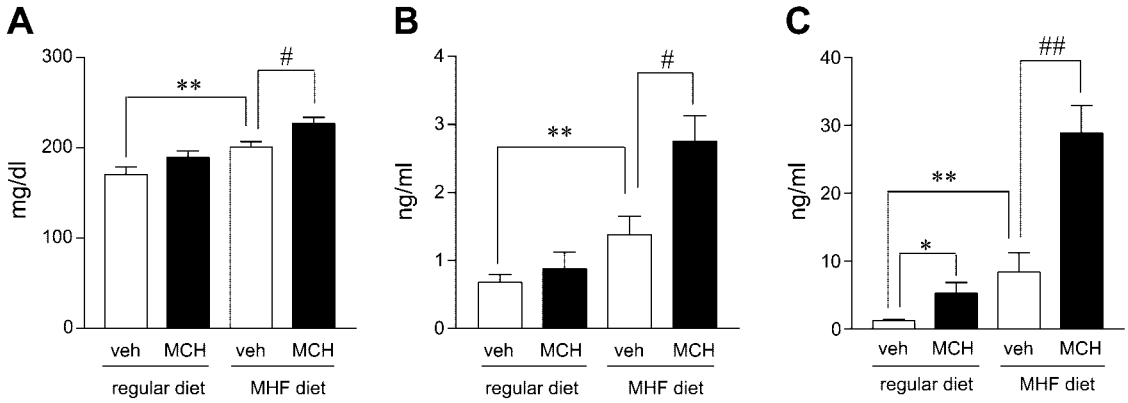

Fig. 2. Effect of icv infusion of MCH on plasma glucose (*A*), insulin (*B*), and leptin (*C*) levels. Data are represented as means ± SE; $n = 10$. *$P < 0.05$, **$P < 0.01$ vs. vehicle-treated groups; #$P < 0.05$, ##$P < 0.01$ vs. vehicle-treated MHF diet-fed group.

Table 3. *Effect of icv infusion of MCH on plasma biochemical parameters*

	Regular Diet		MHF Diet	
	Vehicle	MCH	Vehicle	MCH
Total cholesterol, mg/dl	60 ± 3	68 ± 3	114 ± 5†	120 ± 3
HDL-C, mg/dl	46 ± 3	50 ± 3	94 ± 3†	90 ± 3
Non-HDL-C, mg/dl	9.1 ± 0.7	9.1 ± 1.0	14.9 ± 1.4†	16.2 ± 1.0
Triglyceride, mg/dl	57 ± 5	86 ± 5†	37 ± 4†	45 ± 3
Free fatty acid, μeq/l	673 ± 93	566 ± 22	668 ± 29	692 ± 101

Data represent means ± SE; $n = 10$. HDL-C, HDL-cholesterol. †$P < 0.01$ vs. vehicle-treated regular diet-fed group.

ment. The plasma TG level was elevated by the MCH infusion under the regular diet-fed condition. The MHF diet decreased the plasma TG level in the vehicle-infused mice. MCH tended to increase the TG level in the MHF diet-fed mice, but the effect was not statistically significant. There was a significant interaction between diet and the MCH infusion in the TG level [$F(1,36) = 5.57, P = 0.0238$]. The plasma FFA level was not changed in any of the four groups (Table 3).

Recently, it was reported that MCH 1 receptor-deficient mice showed increased motor activity (3a, 13). To elucidate effects of chronic activation of the MCH system, we measured spontaneous motor activity. To correctly compare the results with the MCH 1 receptor-deficient mice, the experiments were conducted with mice on the regular diet. The MCH infusion did not change spontaneous motor activity during either the light or the dark cycle (Fig. 3). In addition, no notable changes were observed during the MCH infusion in the gross behavior tests (data not shown).

DISCUSSION

In this study, we showed that chronic intracerebroventricular infusion of MCH for 14 days caused body weight gain in mice fed a regular diet. MCH also caused slight but significant increases in adipose tissue weight and leptin levels. The current observation is coincident with previous reports that transgenic mice overexpressing *Pmch* developed a slight increase in body weight gain compared with that in wild-type mice under the regular diet-fed condition (12). Therefore, the phenotypes observed in the transgenic mice overexpressing *Pmch* are caused mainly by activation of the brain MCH system. However, our results are inconsistent with the observation of Rossi et al. (24), showing that repetitive MCH administration did not cause obesity. The discrepancy between that observation and ours might be due to methodological differences. Bolus injection of MCH at high doses may easily induce tachyphylaxis or compensatory hypophagia. Alternatively, continuous activation of MCH systems might be necessary to produce obesity. The effect of stress that is induced by repetitive intracerebroventricular injection should also be considered.

Although the effect of MCH on body weight regulation was relatively mild under normal energy conditions, MCH caused sustained hyperphagia and a more greatly increased body weight accompanied by hyperglycemia, hyperinsulinemia, and hyperleptinemia when the MHF diet was given. The present data are in agreement with the report that the *Pmch*-transgenic mice are highly sensitive to diet-induced obesity (12). From these results, it is suggested that MCH could produce a crucial influence on the development of obesity, especially when combined with additional environmental factor(s) such as a high-calorie diet. However, this finding may also raise the possibility that MCH influences the food preference of mice. MCH neurons project to the parabrachial nucleus, which is thought to be involved in gustatory sensation processes (31). Further investigations will be needed to address this possibility.

Recently, it was reported that MCH 1 receptor-deficient mice showed increased motor activity (3a, 13). These findings may evoke the possibility that the MCH-induced obesity in the present study may be caused, in part, by sedentary or reduced activity. However, intracerebroventricularly infused MCH did not affect spontaneous motor activity during either the light or the dark cycle. Consequently, it is not likely that the MCH-induced obesity is due to sedentary changes in behavior.

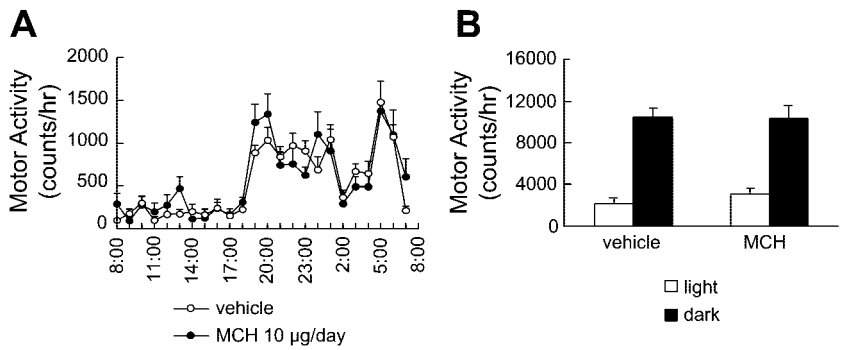

Fig. 3. Spontaneous motor activity during icv infusion of MCH (10 μg/day) or vehicle (30% PG). *A*: time course change; *B*: cumulative motor activity during light (0700–1900) and dark (1900–0700) cycles. Data are represented as means ± SE; $n = 7–8$.

Intracerebroventricular infusion of MCH stimulated adiposity. Because MCH caused hyperphagia, the MCH-induced fat accumulation may be partly due to feeding stimulation. It is reported that MCH neurons project to the brown adipose tissue, which has an important role in energy expenditure (20). In support, O_2 consumption in the MCH-deficient mice was slightly higher than in control mice (28). Thus the MCH-induced fat accumulation might also be caused by the reduction of energy expenditure. To consider the mechanism of the MCH-induced fat accumulation, it is noteworthy that MCH suppresses the hypothalamo-pituitary-thyroid axis (8). Several hormones, such as thyroid hormone and corticosterone, might also be involved in the effect of MCH. Thus MCH might produce obesity with typical metabolic and endocrine changes. Further examination of the effect of MCH on sympathetic tone and hormonal balance is of importance.

MCH did not significantly change most of the lipid parameters studied under either the regular diet- or MHF diet-fed conditions. However, the plasma TG level was significantly increased in the regular diet-fed mice, suggesting that MCH might stimulate fat synthesis in the liver. This change was not clearly observed under the MHF diet condition. Presumably, dietary fatty acid on the MHF diet inhibited an intrinsic lipogenic activity (6, 33); hence, the effect of MCH on TG level might be diminished.

Two subtypes of MCH receptors have been cloned so far. SLC-1, an orphan G protein-coupled receptor, was identified as an MCH 1 receptor (2, 3, 11, 27, 29). Another receptor subtype, named MCH 2 receptor, was recently identified in human brain (1, 5, 15, 22, 26, 32). These receptors are similarly distributed in the hypothalamic regions. However, the MCH 2 receptor is reported not to exist in rodents (30). Furthermore, MCH failed to produce body weight gain in MCH 1 receptor-deficient mice (13). Consequently, the typical obese phenotypes evoked by MCH in the present experiment are mediated by the MCH 1 receptor.

In summary, chronic intracerebroventricular infusion of MCH stimulated fat accumulation, especially when combined with the MHF diet. These results imply that MCH may have a major role in energy homeostasis. Taken together with the observation that the MCH expression level was increased in several obesity models (4, 9, 14, 17, 28), MCH may be involved in the pathogenesis of obesity syndrome. Consequently, MCH antagonists may be useful drugs for the treatment of obesity.

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