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ORIGINAL ARTICLE

Chronic MCH infusion causes a decrease in energy expenditure and body temperature, and an increase in serum IGF-1 levels in mice

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Abstract Melanin concentrating hormone (MCH) is an orexigenic peptide secreted from the lateral hypothalamus. Various observations suggest a role for MCH in energy expenditure in transgenic mice; however, the influence of MCH on energy expenditure and body temperature in WT mice was inadequately studied. Therefore, our first goal was to characterize the influence of chronic intracerebroventrical MCH infusion on energy homeostasis in mice. Our second goal was to explore the effect of MCH on the GH–insulin like growth factor 1 (IGF-1) axis in vivo. We have recently published that MCH directly increased GH-secretion from pituitary cells in vitro, suggesting that MCH may exert part of its effects on energy balance via direct pituitary hormone regulation. Mice were centrally infused with MCH for 14 days, resulting in a significant increase in food intake, body weight, fat mass and plasma IGF-1 levels, while decreasing body temperature and energy expenditure. Our data emphasize the role of MCH as a key regulator of energy homeostasis by means of appetite regulation, regulation of energy expenditure, and an integrator of energy balance with the neuroendocrine system regulating pituitary hormone secretion. They also support the notion that MCH may have a physiologic role in GH regulation that may, in turn, contribute to its effect on body weight.

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Introduction

Melanin concentrating hormone (MCH) is an orexigenic (appetite stimulating) peptide secreted from the lateral hypothalamus. It plays an important role in the central control of appetite and body weight, and its MCHR1 receptor (which is functional in humans as well as in rodents) is considered to be a major potential target for the pharmacological treatment of obesity ([1], reviewed by Pissios et al. [2]). MCH levels increase during a fast, are high in *ob*/*ob* mice [3] and in obese Zucker rats [4], and acute intracerebroventrical (ICV) MCH injections increase feeding in rats and mice [3, 5–7]. Moreover, rats treated with an MCHR1 antagonist are hypophagic and lose weight [8]. Chronic ICV infusion of MCH to rats stimulated feeding and resulted in obesity in one experiment [6], but others reported a transient [5] or no effect [9] on feeding and body weight. In mice, chronic ICV infusion of MCH increased food intake and body weight especially on a moderately high fat diet [10, 11]. A similar response to MCH was reported in sheep, which increased food intake in response to acute MCH injections, but not in response to a chronic infusion [12].

Genetic studies revealed that MCH has an effect on energy balance beyond feeding: mice lacking MCH or its receptor were found to be lean, had an increased metabolic rate, and enhanced thermogenesis [13–15], *ob*/*ob* mice lacking MCH, as compared to "regular" *ob*/*ob* mice, weighed less despite no change in their food intake, and this was attributed to increased energy expenditure in the

double KO animals [16]. Moreover, ICV MCH-infused mice pair fed to control animals gain weight compared to the control animals, indicating a lower energy expenditure in the treated animals [10]. Nevertheless, in rats, chronic ICV MCH-infusion had no effect on energy expenditure (measured by oxygen consumption) or activity level, but did produce a mild suppressive effect on mean arterial blood pressure and heart rate [9]. In spite of all these observations suggesting a role for MCH in energy expenditure in transgenic mice, the influence of MCH on energy expenditure and body temperature (one of the main routes for energy expenditure) in WT mice was inadequately studied. One study [10] measured rectal temperature at a single time point (early afternoon) and found no significant difference between ICV MCH and vehicle-infused mice. Therefore, our first goal was to better characterize the influence of chronic ICV MCH infusion on energy homeostasis in mice, and specifically to study its effect on body temperature and oxygen consumption.

Although MCH mediates energy homeostasis via regulation of food intake and energy expenditure, a few lines of evidence have suggested that MCH may also influence the hypothalamic–pituitary axis. MCH-R1 was reported to be expressed in the pituitary gland of both rodents and humans [17–19]. Moreover, MCH was detected around the hypophysial portal vessels [20] and projections from the lateral hypothalamus to the median eminence were described [20, 21], suggesting a role for MCH in the regulation of pituitary hormone secretion. A few investigators have shown that upon ligand binding, MCHR1 is capable of stimulating pituitary hormone secretion. The secretion of TSH, ACTH, and gonadotropins was directly affected by MCH [22, 23]. The gut-derived peptide ghrelin which normally increases pituitary GH expression and secretion had no effect on GH mRNA expression levels in the pituitary gland of MCH-receptor knockout mice, suggesting that MCHR is involved in the acute effect of ghrelin on pituitary GH expression [24]. Finally, we have recently published that MCH directly increased GH secretion from human pituitary cells in vitro, providing support for the notion that MCH may exert part of its effects on energy balance via direct pituitary hormone regulation. However, since the physiological relevance of this finding remained unclear, our second goal in this study was to explore the effect of MCH on the GH–insulin like growth factor 1 (IGF-1) axis in vivo.

Results

The effect of chronic MCH treatment on food intake

Both groups consumed similar amounts of food at baseline (non significant). While the control mice, treated with the aCSF infusion lacking MCH, did not change their average daily food intake during the treatment period (3.37 ± 0.14 g/day and 3.54 ± 0.16 g/day respectively, non significant), MCH-treated mice significantly increased their average daily food intake by 34% during this time (from 3.21 ± 0.15 to 4.31 ± 0.15 g/day) and consumed significantly more food then the control group (t-test, $t = -2.12$, df $= 11$, $P < 0.05$, Fig. 1a). Analyzing the effect of MCH infusion on daily food intake, we found

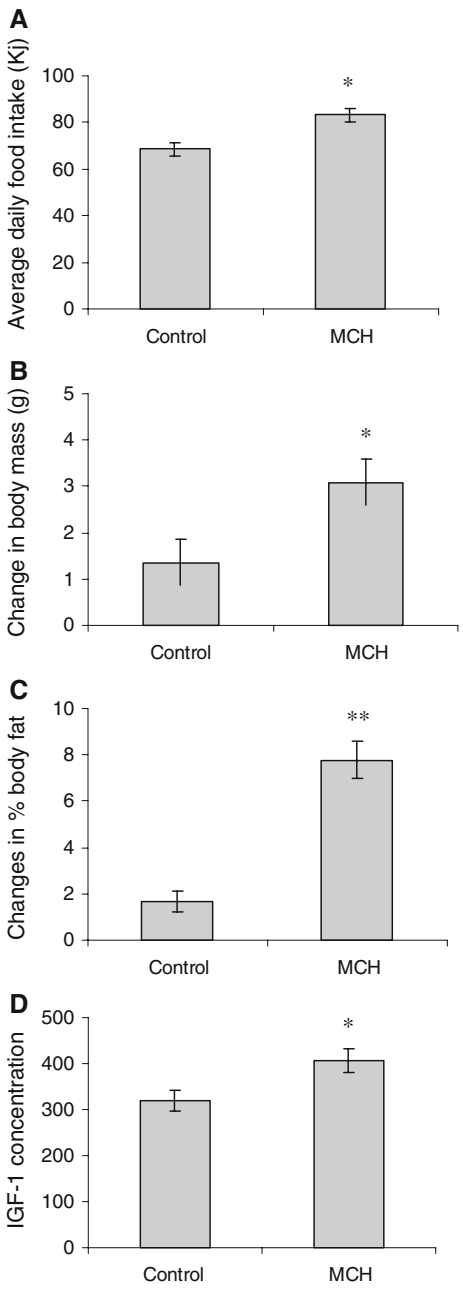

Fig. 1 Average daily food intake (g), weight gain (g), and fat gain (%) during 14 days ICV MCH infusion, and IGF-1 plasma concentration (ng/ml) at the end of the infusion. Values represent average ± SE, $n = 6$, * $P < 0.05$, ** $P < 0.01$

that although the MCH-treated mice consumed more food on average every day, the difference was marginal on the day of implantation and became statistically significant only on the fourth day after the beginning of the infusion (repeated measures ANOVA, treatment effect: $F = 6.97$, df $= 1$, $P < 0.0001$, Fisher LSD post-hoc df $= 108$, $P < 0.05$ on day 4, $P = 0.07$ on day 0, Fig. 2a).

The effect of chronic MCH treatment on body weight

On the day after the osmotic minipumps were implanted, the body mass of MCH-treated mice increased significantly and was significantly higher than that of the control mice (repeated measure ANOVA, $F = 27.52$, df $= 11$, $P < 0.05$, Fisher LSD post-hoc, Fig. 2b). The difference in body mass between the groups disappeared the next day, and was significant again from 5 days post-implantation to the end of the experiment. In total, body weight of the MCH-treated mice increased by 3.08 ± 0.3 g (15%) during the ICV MCH infusion, whereas body weight of the control group increased only by 1.3 ± 0.3 g (repeated measure ANOVA, $F = 27.52$, df $= 11$, $P < 0.05$, Fisher LSD post-hoc, Fig. 1b).

The effect of chronic MCH treatment on body composition

Both groups increased body fat during the experiment. However, body fat of the ICV MCH-infused mice increased 4.6-fold compared to the control mice (7.78 ± 0.78 vs. $1.68 \pm 0.45\%$, respectively, t-test, $t = -6.9$, df $= 11$, $P < 0.001$, Fig. 1c).

The effect of chronic MCH treatment on body temperature

Body temperature was averaged to 3 h intervals and then analyzed. ICV MCH infusion had a significant effect on body temperature (repeated measure ANOVA, $F = 39$, df $= 1$, $P < 0.001$), which was lower in ICV MCH-infused mice toward the end of the dark-phase almost all days of the treatment (Fisher LSD post-hoc, df $= 1008$, $P < 0.05$, Fig. 3a). Before treatment, the average body temperature of

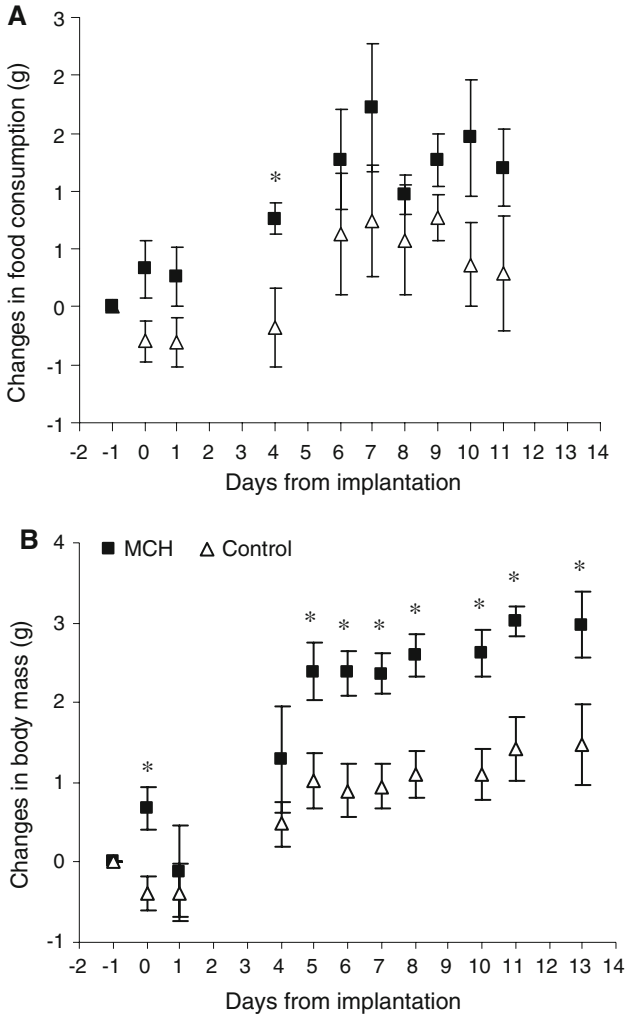

Fig. 2 Changes in food intake (**a**, average ± SE) and body mass (**b**, average ± SE) of ICV MCH infused (*black squares*) and control (*white triangles*) mice. Day 0 is the day the infusion started. $N = 6$, * $P < 0.05$

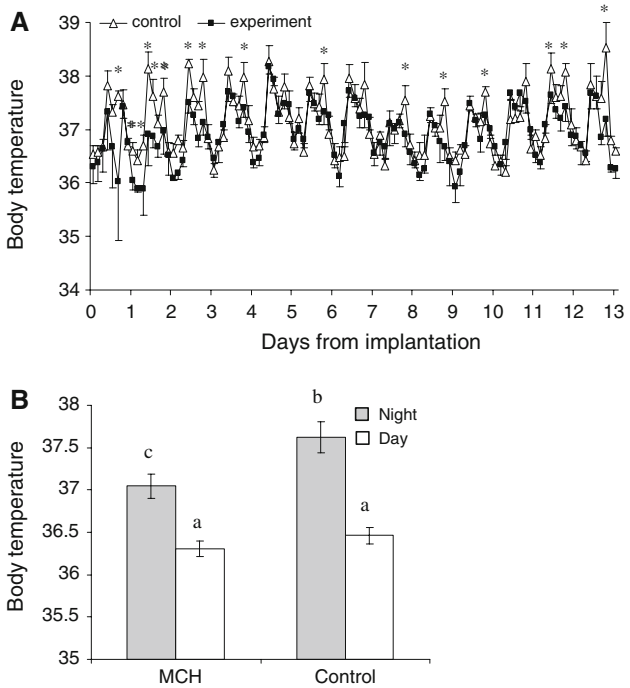

Fig. 3 Daily rhythm (**a**) and average body temperature (°C, average ± SE) during the night and day (**b**) of ICV MCH infused (*black squares*) and control (*white triangles*) mice during 13 days of infusion. Columns with different letters are significantly different from each other ($P < 0.001$), $n = 6$, * $P < 0.05$

both groups was 37°C. MCH treatment decreased the average body temperature of the mice to 36.67 ± 0.06°C, a measure that was significantly lower than that of the control mice (37.04 ± 0.08°C). This difference was secondary to a decrease in the core body temperature of the MCH-treated mice during the dark-phase (repeated measure ANOVA on average body temperature during day and night, $F = 257$, df = 1, $P < 0.001$, Fisher LSD post-hoc, Fig. 3b).

The effect of chronic MCH treatment on energy expenditure

ICV MCH infusion had a significant effect on oxygen consumption (repeated measure ANOVA, $F = 12.6$, df = 1, $P < 0.001$), which was lower in ICV MCH-infused mice toward the end of the dark-phase (Fisher LSD post-hoc, df = 288, $P < 0.05$, Fig. 3). MCH-treated mice had an average oxygen consumption of 1.04 ± 0.028 ml/g/h, which was 15% lower than that of the control group (1.22 ± 0.03 ml/g/h, *t*-test, $t = -3.95$, df = 48, $P < 0.001$). Since there was a significant difference in body mass between the groups at the end of the experiment, we also calculated oxygen consumption per animal, which again was found to be significantly lower in the MCH-treated mice, despite the fact that these animals weighed more than the control group. This difference is contributed, again, to the lower oxygen consumption of the MCH-infused mice during the dark-phase (repeated measure ANOVA on average oxygen consumption during day and night, $F = 13.48$, df = 1, $P < 0.001$, Fisher LSD post-hoc, Fig. 4).

The effect of chronic MCH treatment on IGF-1 secretion

IGF-1 plasma levels of MCH-treated mice were 26% higher compared to the control mice (320.59 ± 22.84 vs. 406.23 ± 24.5 ng/ml, respectively, *t*-test, $t = -2.5$, df = 11, $P < 0.05$, Fig. 1).

Discussion

The study presented here focused on further defining the in vivo role of the hypothalamic peptide MCH in energy balance in mice. Mice were centrally treated with MCH for 14 days. This treatment resulted in a significant increase in food intake, body weight, fat mass, and plasma IGF-1 levels, while decreasing their body temperature and energy expenditure.

One day after the implantation, both groups lost weight, probably due to the effect of the surgery; however, the MCH-treated mice showed an early, transient increase in body weight during the day of the implantation, which was not evident in the control group. This increase could have resulted theoretically from the acute effect of MCH on feeding, which was reported to increase by 2–3-fold during the first few hours after an injection of MCH or an MCH agonist [3, 5–7]. However, food intake did not differ significantly between the groups during that day (although it was close to significance, with $P = 0.07$). On the following day (day 1), body weight of both groups was similar. From that day on, body mass of the MCH-infused mice was significantly higher than that of the control mice. The additional weight gain observed in the MCH-treated mice is accounted for by the increase fat mass, which was significantly higher in the MCH-treated mice.

We then proceeded to evaluate whether the difference in body weight between the two groups was secondary to variations in food intake, energy expenditure, or both. In accord with previous studies in mice [10, 11], we found that chronic MCH infusion had a continued orexigenic effect. In rats, a continuous ICV MCH infusion (12 days) resulted in an increase in food intake and body mass [6],

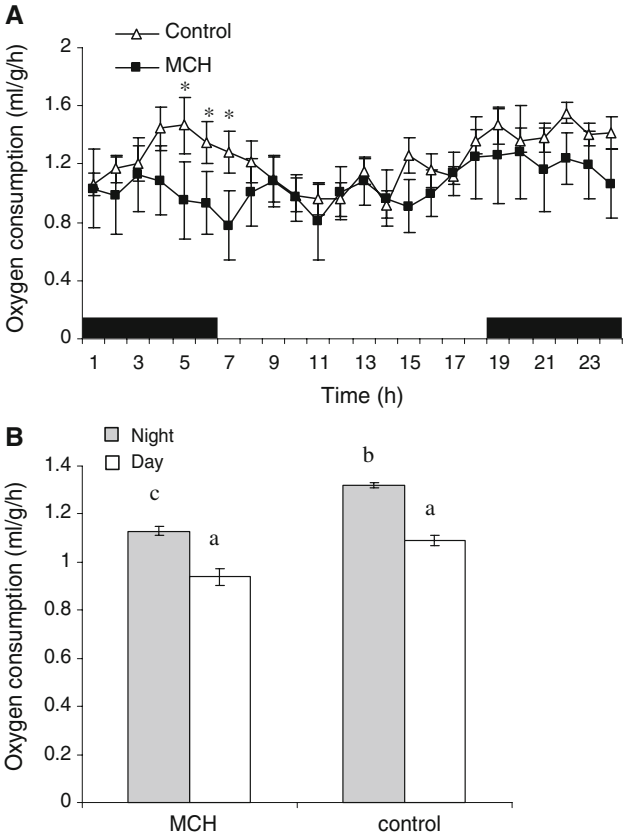

Fig. 4 Daily rhythm (**a**) of oxygen consumption (ml/g/h, average ± SE) and average oxygen consumption (ml/g/h, average ± SE) during the night and day (**b**) of ICV MCH infused (*black squares*) and control (*white triangles*) mice on the last 24 h of infusion (day 14). Columns with different letters are significantly different from each other ($P < 0.001$), $n = 6$, * $P < 0.05$

whereas repeated ICV injections of MCH had no effect on body weight, and a transient orexigenic effect lasting only for 5 days [5].

We also found that a chronic, continuous MCH infusion caused a significant decrease in metabolic rate. This is in contrary to the findings in rats, where chronic ICV MCH infusion did not affect energy expenditure [9], but in accord with genetic manipulations of the MCH system in mice showing that mice over-expressing MCH decrease their metabolic rate [25], mice lacking MCH [13, 26], or MCHR1 [14, 15] have increased resting metabolic rate, and mice lacking both leptin and MCH have a higher energy expenditure than mice lacking leptin alone [16]. These results support the finding that in mice, MCH is involved in the control of energy balance by affecting both food intake and energy expenditure.

The decrease in metabolic rate resulted at least in part from a decrease in the energy invested in thermoregulation, resulting in significantly lower body temperature mainly toward the end of the dark phase. Several observations support the involvement of MCH in the control of thermogenesis: MCH neurons in the lateral hypothalamus have sympathetic projections to brown adipose tissue (BAT) [27]. Cold exposed rats have high MCH mRNA levels in the hypothalamus, and blocking its protein synthesis results in increased uncoupling protein-1 (UCP-1) expression in BAT as well as BAT weight [28]. The involvement of MCH in thermoregulation was also demonstrated in leptin-deficient *ob*/*ob* mice. These mice are hypothermic and MCH ablation "corrects" their hypothermia [16], probably, at least in part, via activating UCP-1 in BAT, hence stimulating non-shivering thermogenesis. Finally, chronic ICV MCH infusion to mice was shown to cause a decrease in UCP-1 mRNA levels in BAT [10], although no differences between rectal temperature of MCH-treated and control mice were detected, presumably resulting from measuring only one time point and the methods of measurement. Based on all the above, it is possible that in this study the chronic MCH infusion resulted in inhibition of UCP-1, which resulted in lower non-shivering thermogenesis capacity and therefore lower body temperature.

It is interesting to note that the reduction in body temperature occurred in spite of the expected increase in diet-induced thermogenesis due to higher food consumption in the MCH-treated mice. BAT was shown to be involved in diet-induced thermogenesis [29, 30], and it is possible that diet-induced thermogenesis was also decreased due to the changes in UCP-1 expression in BAT and in BAT mass. Another possible explanation for the decreased energy expenditure in the MCH-treated animals is a decrease in their physical activity. Indeed, MCHR-1-deficient mice are hyperactive [14] and MCH-deficient mice show profound hyperactivity in response to fasting [31]. However, it was previously found that chronic ICV MCH infusion to mice did not change their spontaneous motor activity during either the light or the dark periods [11].

GH secretion from the pituitary gland is known to be regulated by both Growth Hormone Releasing Hormone (GHRH) and Ghrelin which both increase its secretion and by somatostatin which decreases it. Based on our previous results showing a direct stimulation of GH-secretion from pituitary cell cultures by MCH [17], and on works of others showing an effect of MCH on other pituitary hormone secretions [22, 23], we suggest that MCH is a novel direct regulator of GH-secretion, although an indirect effect such as activation of the hypothalamic GHRH by MCH may have theoretically contributed to this effect. IGF-1, a peptide secreted mainly from the liver in response to GH, was increased in mice treated with MCH as compared to control animals. In accord, MCHR KO mice have significantly lower serum IGF-1 levels compared to WT mice [24], further supporting this hypothesis. Since obesity is associated with decreased GH secretion [32], the fact that IGF-1 was increased parallel to the weight gain of the mice in this study is counter-intuitive and further supports a direct effect of MCH on the pituitary gland. IGF-1 is known to have an anabolic effect on bone formation. Interestingly, MCHR KO mice have osteoporosis [33] that may be explained by their relative leanness. Our findings raise the question whether it is possible that the osteoporosis seen in these animals may be also contributed by IGF-1 deficiency. The fact that the MCHR-1 is expressed in the human and rodent pituitary gland [17–19], the ability of MCH to induce GH-secretion from human and mice pituitary tissue in vitro [17] and the ability of chronic continuous MCH infusion to increase plasma IGF-1 levels (this article), together support the notion that MCH may have a physiologic role in GH regulation that may, in turn, contribute to its effect on body weight.

Taken together, our data emphasize the role of MCH as a key regulator of energy homeostasis by means of appetite regulation, regulation of energy expenditure, and an integrator of energy balance with the neuroendocrine system regulating pituitary hormone secretion.

Materials and methods

All procedures were conducted in accordance with and approved by the Institutional Animal Ethics Committee (Permit No. L-05-003).

Animals

Twelve 12-week-old C57bl/6 mice (Harlan) were individually caged, under controlled temperature and light

conditions (25°C, 12 h light dark cycle) and fed rodent chow (Koffolk Serial No. 19510) ad libitum.

Body weight and food intake

Body weight was measured daily using electronic scales (Sartorius, ±0.1 g). To measure individual ad libitum food and energy intake, the mice were given weighed (Sekel, ±0.01 g) commercial rodents pellet (Koffolk Serial No. 19510, 21% protein, 4.5% fat, 19.3 kJ/g). Leftovers were collected after 48 or 72 h, dried to a constant mass at 60°C, and weighed. Energy content of the pellets (19.3 kJ/g) was measured using a bomb calorimeter (Gallenkamp) calibrated by ascending mass of benzoic acid (Analar, 26.45 kJ/g).

Body temperature

Body temperature was measured using temperature sensitive single stage implanted transmitter (Sirtrack, New Zealand, accuracy: ±0.5°C, resolution: 0.0625°C) and an RX-900 scanner-receiver (Televit, Sweden). Data were collected every 6 min throughout the experiment.

Transmitter implantation

Mice were anesthetized with isoflurane in medical grade oxygen using an anesthetic machine (Ohmeda, 1.5 vol.%, 1 l/min) and implanted with the single stage implanted transmitters in the abdominal cavity. Both the abdominal wall and the skin were sutured with absorbable surgical suture, with cutting needle (5-0 Dexon) and the incision was treated with topical antibiotic (silver sulfadiazine 1%; Silverol Cream). Prophylactic antibiotics (Baytril 5% 24 mg/kg) and artificial tear ointment (to prevent desiccation) were administered preoperatively. At least 2 weeks of recovery from surgery were allowed before initiation of data collection.

Implantation of Alzet osmotic minipumps

Alzet osmotic minipumps (Alzet osmotic minipump Model No. 2002, Alzet Corp., Palo Alto, CA, USA), which deliver their contents at a rate of 0.5 µl/h for 2 weeks, were each connected by means of a polyethylene catheter to a stainless steel cannula (Brain Infusion kit, Alzet) and loaded either with 10 nM of MCH (Bachem AG Switzerland) in artificial cerebrospinal fluid (10 µg/day) or with a similar solution without MCH. This dose was chosen based on other published results, using similar doses in order to evaluate the effect of ICV–MCH on pituitary hormone secretions in vivo [5, 6, 9–11]. Mice were anesthetized by isoflurane as described above, their skulls were carefully exposed, and a small hole was drilled with a 25-gauge needle above the lateral ventricle (1 mm posterior and 1.5 mm lateral to the bregma). The tip of the brain infusion cannula was inserted into the hole, and the cannula was glued to the skull (Luctite 454). To complete the procedure, the pump was inserted s.c. on the mouse's back, and the cut skin over the skull was sutured. An antibiotic (1% oxytetracycline) was added to the drinking water for 1 week. The correct placement of the ICV infusion was verified after termination of experiment by ICV infusion of Evans Blue dye.

Body composition

Fat mass was measured before implantation of the transmitters (at the beginning of the experiment) and at the end of the experiment (after the transmitters were removed) using Double X-ray Absorption (DEXA scanner; Lunar Piximus II). During the measurements, mice were anesthetized with isoflurane (Rhodic, Abbott Laboratories, Maidenhead, UK) using an anesthetic machine (Ohmeda) with medical-grade oxygen (2 vol.%, 1 l/min).

Energy expenditure

Oxygen consumption rates $\dot{V}_{O_2}$, ml O_2/g h_{STPD} as an indirect indicator for energy expenditure were measured in our animals in an open system following [34] on the last 48 h of the infusion. $\dot{V}_{O_2}$ was calculated using Eq. 4 of [35]: $\dot{V}_{O_2} = [V_E \times (FI_{O_2} - -F_{EO_2})]/(1 - -FI_{O_2})$, where V_E is the rate of airflow out of the cage (ml/min_{STPD}), FI_{O_2} is the fractional concentration of O_2 entering the cage, and F_{EO_2} is the fractional concentration of O_2 of out flowing air. STPD is standard temperature (0°C), pressure (760 mmHg), and dry air. Oxygen consumption rate was measured in the individual cage by covering the cage with a transparent Perspex plate with two holes. Airflow through the cages was 200 ml/min and was continuously monitored using a McMillan flow meter (±1 ml/min).

Sampled air was desiccated (Silica gel blue; Fluka, Buchs, Switzerland), CO_2 was absorbed (Soda lime; Merck, Darmstadt, Germany), and F_{O_2} was measured using a paramagnetic O_2 analyzer (Taylor Servomax, ±0.001%). Flow meters were calibrated before every measurement using dry air (without CO_2) at different flow rates through a calibration cylinder (Model No. 1053; Brooks, Chelmsford, MA, USA). The O_2 analyzer was calibrated before every measurement using nitrogen versus dry air. V_E and F_{O_2} were sampled continuously, averaged, and saved every 20 s. For the analysis, we used only the last 24 h of measurements.

Hormone measurements

Since the secretion of GH is highly pulsatile, we tested blood levels of IGF-1 rather than GH. Liver IGF-1 production is directly stimulated by GH, accurately reflects the levels of GH, and can be easily measured in the blood. Moreover, IGF-1 levels do not fluctuate greatly throughout the day, and are routinely used by physicians for evaluating GH alternations. IGF-1 levels were measured using IGF-1 RIA kit (Diagnostic Systems Laboratories (DSL), Texas, USA).

Statistical analysis

Data were analyzed using Student's *t*-test; or repeated measures ANOVA. Analysis was performed with Statistica 7.0 program (Statsoft Inc., Tulsa, OK, USA). Statistical significance was determined at $P < 0.05$.

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