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Effects of intracerebroventricular and intra-accumbens melanin-concentrating hormone agonism on food intake and energy expenditure

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Guesdon B, Paradis É, Samson P, Richard D. Effects of intracerebroventricular and intra-accumbens melanin-concentrating hormone agonism on food intake and energy expenditure. Am J Physiol Regul Integr Comp Physiol 296: R469–R475, 2009. First published January 7, 2009; doi:10.1152/ajpregu.90556.2008.—The brain melanin-concentrating hormone (MCH) system represents an anabolic system involved in energy balance regulation through influences exerted on the homeostatic and nonhomeostatic controls of food intake and energy expenditure. The present study was designed to further delineate the effect of the MCH system on energy balance regulation by assessing the actions of the MCH receptor 1 (MCHR1) agonism on both food intake and energy expenditure after intracerebroventricular (third ventricle) and intra-nucleus-accumbens-shell (intraNAcSH) injections of a MCHR1 agonist. Total energy expenditure and substrate oxidation were assessed following injections in male Wistar rats using indirect calorimetry. Food intake was also measured. Pair-fed groups were added to evaluate changes in thermogenesis that would occur regardless of the meal size and its thermogenic response. Using such experimental conditions, we were able to demonstrate that acute MCH agonism in the brain, besides its orexigenic effect, induced a noticeable change in the utilization of the main metabolic fuels. In pair-fed animals, MCH significantly reduced lipid oxidation when it was injected in the third ventricle. Such an effect was not observed following the injection of MCH in the NAcSh, where MCH nonetheless strongly stimulated appetite. The present results further delineate the influence of MCH on energy expenditure and substrate oxidation while confirming the key role of the NAcSh in the effects of the MCH system on food intake.

brain; feeding behavior; substrate oxidation; energy balance

IN THE BRAIN, SEVERAL INTERCONNECTED neurons receiving inputs from peripheral and central signals exert complex controls on food intake and energy expenditure, which are the two inescapable determinants of energy balance (6, 13, 23, 31). In recent years, a few groups of investigators have directed their attention to one of the regulatory determinants of energy balance, namely the melanin-concentrating hormone (MCH) system. MCH-containing neurons are concentrated within the lateral hypothalamus (LH) and the adjacent zona incerta from where they project to the rest of the brain (7), in a pattern that generally conforms to the distribution of the MCH receptor 1 (MCHR1, the functional MCH receptor in rats and mice) (17).

Evidence that the MCH system is involved in body weight regulation has emerged from various sources (24, 29). Hypothalamic expression of MCH mRNA is upregulated during starvation in lean mice, as well as in genetically obese ob/ob mice (30), and MCH overexpression leads to obesity and to an increased susceptibility to high-fat feeding (19). In addition, acute or chronic intracerebroventricular administration of MCH or MCHR1 agonists stimulate feeding in rats and mice (11, 15, 30, 32, 36), while ablations of MCH (35, 37), MCH neurons (2), and MCHR1 (9, 21) have all been reported to promote fat loss mainly by increasing energy expenditure.

The critical brain circuitries and mechanisms responsible for the different effects of MCH in the regulation of energy balance remain to be delineated. MCH is not only regarded as a homeostatic regulator of energy balance, but it is also seen as being involved in the hedonic control of food intake. The MCH system apparently links the LH to the brain reward system. Recently, MCH was shown to increase energy intake when injected in the nucleus accumbens shell (NAcSh) (14), which represents a key structure of the brain reward system. This area receives MCH projections (7) and abundantly expresses the MCHR1 (33). As NAcSh is involved in reward (20, 27), MCH projections toward NAcSh have been suggested to be one key component of a hypothalamic-limbic circuit that could integrate the “homeostatic” and “hedonic” regulations of energy balance (6, 34). It is however not known whether these projections are specifically involved in the control of food intake or if they could also directly or indirectly participate in the control of energy expenditure. The present study was designed to further delineate the effect of the MCH system on energy balance by assessing the effects of the MCHR1 agonism on both food intake and energy expenditure after intracerebroventricular (third ventricle) and intraNAcSH injections of a MCHR1 agonist.

MATERIALS AND METHODS

Animals. Male Wistar rats (Charles River Laboratories, St. Constant, QC, Canada), weighing ~250 g at the beginning of the experiments, were housed singly at 23 ± 1°C, under a 12:12-h light-dark cycle (lights on at 0700), first in stainless-steel cages, and then in Plexiglas calorimetric chambers (see below). Initially, they had free
access to tap water and a standard stock diet (Charles River Rodent Diet no. 5075; 18 kcal % protein and 4.5 kcal % fat; Ralston Products, Woodstock, ON, Canada). Following the surgery, the animals were trained to eat their daily ration of food (standard stock diet) between 1700 to 0800. This feeding schedule was designed to lower appetite during the early light phase (40). The animals were cared for and handled in conformance with the Canadian Guide for the Care and Use of Laboratory Animals, and the protocols used were approved by our institutional animal care and use committee.

Surgery. After a few days of habituation, two groups of eight rats were permanently implanted with stainless-steel guide cannulas (Plastics One, Roanoke, VA). One group was implanted with a 22-gauge single guide cannula aimed at the third ventricle using the following stereotoxic coordinates: 2.3 mm posterior to bregma on the midline and 8.5 mm ventral to the brain surface (26). The other group was implanted with a 26-gauge double guide cannula for bilateral injections into the NAcSh. It was placed to end 1 mm above the injection point in the NAcSh: 1.7 mm anterior to bregma, 0.75 mm each side from midline, and 5.5 mm ventral to the brain surface (26). The guide cannulas were secured with screws and cranioplastic cement (Dentsply International, York, PA). Sterile obturators were inserted into the guide cannulas to prevent them from clogging and to reduce the potential for brain infection. The obturators were checked daily, cleaned with a sterile saline solution, and replaced when needed. The cannula placement in the third ventricle was confirmed by evaluating the dipsogenic response to ANG II (50 ng). Only animals drinking more than 5 ml of water in 1 h were used in the study (40). Cannula placement was also histologically verified in every group at the end of the study.

Procedure and test conditions. The following protocol was first applied to intracerebroventricular-cannulated rats and then reproduced in NAcSh-cannulated rats. After surgery, rats were transferred into metabolic chambers (see below), where they were housed for the duration of the protocol. During the recovery period (~10 days), they were weighed each day at 0830 and then progressively familiarized with the drug injection procedure. Briefly, the obturator was removed and a 28-gauge injector (Plastics One) was inserted into the guide cannula. Once the injector was inserted, the rat was placed into an open cage and allowed to move freely during the 1-min injection, which was controlled by an infusion pump. After the injection, the injector was left in place during 30 s before being removed, and the sterile obturator was then put back. Four days before the beginning of the experiment, all the rats received an injection of 0.9% saline.

The experiment consisted of three test sessions, during which each rat was submitted to three different test conditions: control, MCH, and MCH-pair-fed (MCH-PF). The control and the MCH test conditions consisted in the injection of either the vehicle (0.9% saline) or the MCHR1 agonist [Compound A (36) kindly provided by Merck Research Laboratories, Rahway, NJ]. Compound A is a truncated analog of MCH whose biophysical properties have previously been described as being very close to the full-length MCH molecule (4, 5). It has a clear effect on food intake (36), which is comparable to that of MCH (Guesdon, B and Richard, D, unpublished data) and therefore represents a useful tool to investigate the MCH system. Injections were performed at 0900, followed by a 2-h period of ad libitum access to food while whole body metabolism measurements were recorded in metabolic chambers. The MCH-PF test condition also consisted of a MCHR1 agonist injection at 0900, but this time, the animals were pair-fed with controls.

During the whole protocol, each rat was submitted to the three different test conditions, with a 48-h washout period between each session. In line with what was reported in similar experimental designs with orexigenic or anorexigenic drugs (41, 42), a 48-h washout period was chosen between test days, as such period allowed body weight and food intake (measured before the test days) to return to stable and normal values. Furthermore, to reduce the variability between the data collected in the different conditions, the rats were assigned to the test conditions in a randomized order, according to a counterbalanced design.

Last, intracerebroventricularly and intraNAcSh-cannulated rats followed the same experimental design and were injected with 5 pg/rat of the MCHR1 agonist (14, 36). Only the injected volume changed; this volume was 4 μl for the intracerebroventricular injections and 1 μl (0.5 μl per side) in the case of bilateral intraNAcSh injections.

Feeding behavior and indirect calorimetry. The metabolic chambers (AccuScan Instruments, Columbus, OH) consisted of rectangular, air-proof cages (30 × 30 × 20 cm), which were linked to an open-circuit, flow-through calorimetric device connected to a computer-controlled system of data acquisition. In these chambers, it was possible to restrict access to food, to measure food intake, and to estimate locomotor activity (via a grid of invisible infrared light beams, allowing us to determine the animal’s position 16 times per s), while analyzing the rat metabolism through indirect calorimetry (estimation of the whole body metabolism through the measurement of respiratory exchanges).

The 2-h ad libitum period of feeding started immediately after the injection, when the rats were returned to their chambers (from 0900 to 1100). Other investigators used a similar 2-h period of measurement to determine the orexigenic effect induced by the MCH agonism (14, 36). It is noteworthy that a 30-min delay was necessary to let the indirect calorimetry system stabilize after the chambers closed and, accordingly, the first indirect calorimetry measurements reported were those obtained at 0930. Oxygen consumption (VO2) and carbon dioxide production (VCO2) were measured every 14 min over ~3 h, from the 29th to the 197th min after the injection. This 3-h duration was selected as previous results on core body temperature (43) and heart rate (22) have indicated that most of the MCH (or MCHR1 agonist) effects on body metabolism happen within the first 3 h following administration of the peptide.

The collection of VO2 and VCO2 data allowed for the estimation of energy expenditure (kcal/min), glucose oxidation (Gox, g/min), and lipid oxidation (Lox, g/min) according to classic formulas (12, 38): energy expenditure = 3.91 VO2 + 1.10 VCO2; Gox = 4.57 VCO2 – 3.23 VO2; and Lox = 1.69 VO2 – 1.69 VCO2. Energy expenditure, Gox, and Lox were converted into watts (J/s) assuming 1 kcal = 4.18 kJ, 15.65 J/g for glucose, and 39.6 J/g for lipids. Protein oxidation was not taken into account in the calculations because it was considered negligible over a short time period, under a standard high carbohydrate diet, and in the postingestive state, that is, when other fuels are usually preferred (3, 12, 38). Moreover, to limit energy expenditure related to thermoregulation, the temperature in the metabolic cage was set at 26°C ± 1.

Histology. Rats were killed with 1.5 ml of a mixture containing 20 mg/ml ketamine and 2.5 mg/ml xylazine. Subsequently, brains were removed, placed in a 10% sucrose, 10% formaldehyde PBS for 4 days, sectioned coronally, and stained with cresyl violet. Cannula tracks were determined by visual inspection under a microscope. These inspections revealed that injection sites for two animals from the intraNAcSH group lay outside the targeted structure. Data from these animals were excluded from subsequent data analyses.

Statistical analysis. Rats whose cannulas were not correctly placed were removed from data analyses. Values are reported as means ± SE. ANOVAs were used to assess differences between the various conditions and were followed by post hoc Scheffe’s tests, when appropriate. For statistical analyses, calorimetric and locomotor activity data were aggregated by 90-min intervals. However, comparisons for specific time periods were also reported on the figures. The results were considered significant with P values < 0.05. The data were analyzed by the statistical package program StatView 1992-98 (SAS Institute, Cary, NC).
RESULTS

Orexigenic effect of an acute MCHR1 agonist injection in the third ventricle and in the NAcSh. The amount of food ingested during the 2-h period following the injections is reported in Fig. 1A. Under the control condition, food intake following saline injection was similarly low in both intracerebroventricularly and intraNAcSh-cannulated rats (2.78 g ± 0.84 vs. 2.51 g ± 0.86, respectively). Comparing the MCH condition with the control condition, we observed that the injection of the MCHR1 agonist induced a noticeable threelfold increase in food intake. This robust orexigenic effect was comparable for both injection sites: 9.26 g ± 1.11 in rats injected in the ventricle and 7.99 ± 0.68 in rats injected in the NAcSh. In the pair-fed condition, the intake was restricted at 2.5 g to be comparable to that observed under the control condition. Under both control and MCH-PF conditions, food intake began immediately after the rats were returned to their chambers and was almost completed 30 min later, before the respiratory exchanges recordings began. Under the MCH condition, the hyperphagia induced by the MCHR1 agonist injection was not completed at this time, as shown in Fig. 1B.

Quantitative and qualitative metabolic changes after MCHR1 agonist injection in the third ventricle. Different variables pertaining to energy metabolism, such as energy expenditure, Gox, and Lox were calculated from the first 3 h of \( V_{\text{O}}_2 \) and \( V_{\text{CO}}_2 \) measurements in intracerebroventricularly injected rats (Fig. 2). In the control condition, we observed a slight, yet not significant, increase in energy expenditure at the beginning of the recording period (Fig. 2A). Analysis of the two main components of energy expenditure, Gox and Lox, reveals that this was mainly due to a small rise in Gox, while Lox remained generally stable (Fig. 2, B and C). In the MCH condition, we observed a progressive, yet nonsignificant increase in energy expenditure, which was sustained for a period that was longer than that of the control condition. In fact, this was clearly due to an elevated Gox, which quickly increased and remained significantly elevated until the end of the recording period (Fig. 2B, Table 1). A significant concomitant decrease in Lox was also detected when the rats were treated with MCH (Fig. 2C, Table 1). In the MCH-PF condition, no increase in energy expenditure was observed. In contrast, during the first half of the recording period (Fig. 2A), pair-fed rats treated with MCH seemed to have energy expenditure levels that were lower than that of the other groups. Further, analysis of Gox and Lox revealed that animals under MCH-PF experienced qualitative metabolic changes (Fig. 2, B and C, Table 1). During the first half of the recording period, (the early postdigestive phase), Lox levels in MCH-PF rats were significantly decreased (compared to control rats), while Gox levels tended to increase (nonsignificant difference at \( P = 0.068 \)), before returning to the control levels in the second part of the recording period. Measurements of locomotor activity, in arbitrary units, did not reveal any significant difference in the behavioral activation of rats following the different test conditions (Table 1).

Quantitative and qualitative metabolic changes after MCHR1 agonist injection in the NAcSh. Similar to intracerebroventricularly injected rats, intraNAcSh-injected rats displayed a transient increase in energy expenditure in the beginning of the recording period. However, two major differences were detected between the intraNAcSh and intracerebroventricular groups. First, although the MCH condition induced similar evolutions of substrate oxidation (increased Gox paralleled by decreased Lox), energy expenditure in the intraNAcSh-injected rats was not augmented compared with control condition (Fig. 3, A–C). Secondly, in the MCH-PF conditions, neither energy expenditure nor lipid oxidation was affected following bilateral MCH-R1 agonist injection compared with control (Fig. 3, A and C, Table 2). Measurement of locomotor activity did not reveal any significant differences between the different conditions. However, the locomotor activity of rats under the MCH condition tended to be lower than that of other conditions during the first 90 min (Table 2).

DISCUSSION

The present results confirm the orexigenic effect of the MCH agonism. When injected in either the third ventricle or the NAcSh, the MCH agonist compound A induced a noticeable increase (above control rats) in food intake. The increase was accompanied by a rise in Gox and a reduction in Lox, likely inherent to the MCH-agonism-induced hyperphagia. The hyperphagia was also accompanied by enhanced energy expenditure when the MCH agonist was intracerebroventricularly injected. The present study also emphasized the ability of the MCH agonist to reduce Lox, even when the confounding effect of MCH-agonism-induced hyperphagia on energy expenditure...
and substrate oxidation was controlled by restricting the intake of the rats treated with the MCH agonist (pair-feeding condition). In pair-fed animals, the intracerebroventricular injection of the MCH agonist tended to decrease energy expenditure, while significantly reducing fat oxidation. The reduction in Lox in pair-fed rats was not observed when the MCH agonist was injected in the NAcSh, whose function appears to extensively contribute to the orexigenic role of the MCH agonism.

Results from previous investigations have emphasized the ability of MCH (11, 30, 32) and MCH receptor agonists (36) to stimulate food intake. In line with those results, we observed a noticeable hyperphagic effect of the MCH agonism in this study. The sites of MCH orexigenic action, which possibly include the medial hypothalamic nuclei, such as the arcuate and paraventricular nuclei (1), clearly also comprise the nucleus accumbens (14). The latter could be of major importance. Indeed, the bilateral intraNAcSh injection of a MCHR1 agonist induced a noticeable threefold increase in food intake, which was comparable to that produced by the injection of the agonist in the 3rd ventricle. That the NAcSh could be a major site for the orexigenic action of MCH is in agreement with the role played by this substructure in food intake (39) and with the high density of the MCHR1 mRNA in the NAcSh (33).

In rats with free access to food, the intracerebroventricular administration of the MCH agonist led to an increase in energy expenditure (above control rats) that most probably resulted from MCH agonism-induced hyperphagia. Such an increase was not seen in pair-fed rats and was accompanied with an increase in Gox and a reduction in Lox. Food intake per se is known to increase Gox and to reduce Lox (8). The pattern of substrate oxidation seen in rats given the MCH agonist in the NAcSh was similar to that seen following the intracerebroventricular administration of the peptide. It is worthy of mention that the injection of MCH in the NAcSh did not generate a postprandial increase in energy expenditure comparable to that seen following the intracerebroventricular injection. The cause of this is not known and remains to be discovered. Given the potential role played by the nucleus accumbens in motivated behaviors (16), one can argue that the intraNAcSh MCH agonism can reduce the energy expenditure associated with the locomotion component. This result warrants further investigation since we detected a tendency for a reduced locomotion in rats injected the MCH agonist in the NAcSh.

Since calorimetry measurements were launched after the start of the feeding period, the reported metabolic rates were greatly influenced by the amount of food eaten during the meal.

| Table 1. Energy expenditure, glucose, and lipid oxidation, and locomotor activity during the 3-h recording period in intracerebroventricularly cannulated rats |
|---------------------------------|-----------------|----------------|
|                                | Control         | MCH            |
|                                | MCH-PF          |
| **EE, J/kg**                   |                 |                 |
| 0–90 min                       | 53.07 (2.47)    | 53.35 (1.52)   |
| 90–180 min                     | 52.55 (2.16)    | 56.25 (2.56)   |
| **Gox, J/kg**                  |                 |                 |
| 0–90 min                       | 14.54 (2.35)    | 27.20 (2.01)*  |
| 90–180 min                     | 15.08 (2.66)    | 32.37 (3.03)*  |
| **Lox, J/kg**                  |                 |                 |
| 0–90 min                       | 38.78 (2.88)    | 26.41 (2.47)†  |
| 90–180 min                     | 37.73 (3.46)    | 24.15 (1.03)*  |
| **Activity**                   |                 |                 |
| 0–90 min                       | 22.85 (6.7)     | 25.23 (6.37)   |
| 90–180 min                     | 15.24 (3.33)    | 16.93 (3.03)   |

Data are expressed as means ± SE. *Significantly different from the two other groups; P < 0.001. †Significantly different from control; P < 0.05. MCH, melanin-concentrating hormone; PF, pair-fed; EE, energy expenditure; Gox, glucose; Lox, lipid oxidation; Activity, locomotor activity.
The pair-feeding condition, which prevents MCH-induced hyperphagia, proved therefore to be critical to demonstrate the specific metabolic effects of the central MCH agonism. This condition was not included in a previous investigation (22). In the first 2 h after the acute injection of the MCHR1 agonist, we observed a slight yet not significant decrease in the energy expenditure of pair-fed rats. This was revealed to arise from a major drop in the part of energy expenditure coming from Lox, which was not totally compensated by a concomitant increase in Gox. That the acute MCH agonism in a MCH-PF condition can reduce Lox, while tending to reduce energy expenditure has not been observed before, but it is in line with previously reported findings emphasizing the role of MCH in the control of energy expenditure. Overexpression of MCH in mice reduces their metabolic rate (19), whereas ablation of the genes encoding either MCH (37) or MCHR1 (9, 21) markedly enhances energy expenditure.

The mechanism whereby the MCH system influences energy expenditure is not totally understood. Genetic disruption of the MCH system has been reported to increase motor activity, Lox (21) and also to stimulate expression of uncoupling protein 1 (UCP-1) in brown adipose tissue (BAT) (35), a major center for regulatory thermogenesis in rats. This last result suggests that the MCH system may affect energy metabolism by inhibiting the thermogenic activity of BAT. Such an inhibition of BAT thermogenesis could be of major importance in the effect of MCH on energy expenditure, as MCH neurons projecting toward caudal brain stem, including the raphe pallidus, connect to the sympathetic outflow to BAT (25, 43). Besides, blockade of MCH expression by antisense MCH oligonucleotide in cold-exposed rats, while having little impact on feeding, has been demonstrated to cause a loss in body weight together with an increase in BAT mass and UCP-1 expression (28). Chronic infusions of MCH increased lipogenic activity and decreased rectal temperature and UCP-1 expression in mice pair fed to control (18), while acute injection of MCH in the 4th ventricle reduced body core temperature (43). The decrease in Lox seen in MCH-PF-treated rats in this study does not contradict a potential suppressive effect of MCH agonism on meal-induced thermogenesis in BAT, which is a major fat oxidizer when stimulated.

In contrast to what it did on food intake, the bilateral injection of the MCH agonist in the NAcSH in pair-fed rats did not affect energy expenditure and Lox. This finding suggests that, beyond its role in feeding, the impact of NAcSh MCH projections on energy homeostasis might be rather weak. Without discarding the hypothesis that these projections could influence energy expenditure through spontaneous or volitional physical activity, we are inclined to suggest that they may be more dedicated to the hedonic control of food intake. Other...

Table 2. Energy expenditure, glucose, and lipid oxidation, and locomotor activity during the 3-h recording period in intra-nucleus-accumbens-shell-cannulated rats

<table>
<thead>
<tr>
<th>Activity</th>
<th>Control</th>
<th>MCH</th>
<th>MCH-PF</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–90 min</td>
<td>50.31 (1.77)</td>
<td>48.81 (1.59)</td>
<td>48.01 (1.37)</td>
</tr>
<tr>
<td>90–180 min</td>
<td>48.31 (1.29)</td>
<td>48.33 (1.69)</td>
<td>46.52 (1.28)</td>
</tr>
<tr>
<td>Gox, J/kg</td>
<td>15.42 (1.37)</td>
<td>19.97 (1.48)*</td>
<td>13.75 (1.98)</td>
</tr>
<tr>
<td>0–90 min</td>
<td>15.33 (2.75)</td>
<td>24.22 (2.01)*</td>
<td>10.56 (2.62)</td>
</tr>
<tr>
<td>90–180 min</td>
<td>35.13 (2.15)</td>
<td>29.07 (1.35)*</td>
<td>34.49 (2.37)</td>
</tr>
<tr>
<td>Lox, J/kg</td>
<td>33.22 (2.84)</td>
<td>24.35 (1.07)*</td>
<td>36.18 (3.33)</td>
</tr>
<tr>
<td>0–90 min</td>
<td>52.95 (18.13)</td>
<td>30.20 (5.42)</td>
<td>51.40 (12.85)</td>
</tr>
<tr>
<td>90–180 min</td>
<td>10.69 (3.22)</td>
<td>12.81 (4.24)</td>
<td>7.13 (0.76)</td>
</tr>
</tbody>
</table>

Data are expressed as means ± SE. *Significantly different from the two other groups; P < 0.001.
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