Decay of food intake by MC4-R agonist MTII in Siberian hamsters in long and short photoperiods

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Schuhler, Sandrine, Tracey L. Horan, Michael H. Hastings, Julian G. Mercer, Peter J. Morgan, and Francis J. P. Ebling. Decrease of food intake by MC4-R agonist MTII in Siberian hamsters in long and short photoperiods. Am J Physiol Regul Integr Comp Physiol 284: R227–R232, 2003. First published September 27, 2002; 10.1152/ajpregu.00331.2002.—We investigated the role of the hypothalamic melanocortin system in the regulation of food intake in the Siberian hamster, which shows a profound seasonal decrease in food intake and body weight in short photoperiod (SP). In male hamsters maintained in long photoperiod (LP), intracerebroventricular injection of melanotan II (MTII) just before lights off significantly decreased food intake relative to vehicle treatment over the 6-h observation period. Similar effects were observed in age-matched hamsters after exposure to a short daylength for 9 wk, when body weight had significantly decreased. There was no clear difference in either the magnitude of response or the dose required for half-maximal inhibition of food intake in hamsters in SP compared with those in LP. MTII significantly increased grooming in both LP and SP. Our results indicate that the melanocortin system is a potent short-term regulator of food intake. However, the lack of differential response or sensitivity to MTII treatment in the obese (LP) vs. lean (SP) states does not support the hypothesis that changes in this melanocortin pathway underlie the long-term decrease in food intake that occurs in this seasonal model.

THE HYPOTHALAMUS plays a key role in the regulation of energy homeostasis via its neural and endocrine outputs. While both anabolic and catabolic components of this system have been characterized, most studies have focused on their involvement in the defense against acute energy deficits (for review see Ref. 30). Studies on a number of rodent species, including seasonal animals, provide compelling evidence that body weight is defended, at least in some species, within certain limits. Weight loss during dieting may engage these powerful compensatory mechanisms that increase hunger and reduce metabolic rate, so most dieters return to their original weight in the long term (20). Maintenance of weight loss in the long term may only be possible if therapeutic strategies are developed to counteract these compensatory mechanisms.

Studies from several animal models provide evidence that the level at which compensatory mechanisms operate can be altered. This plasticity is particularly clear in mammals that have evolved under seasonal conditions and consequently display profound annual cycles of weight gain and loss (25). For example, under increasing or long photoperiods the Siberian hamster (Phodopus sungorus) defends its maximum body weight, whereas under decreasing or short photoperiods it enters a catabolic state and gradually loses body weight (10, 22, 31), mainly through a reduction in intraperitoneal fat stores (4). The reduction in body weight takes approximately 12–15 wk to reach the maximum weight loss of up to 40% of initial weight (24, 29, 31).

The central mechanisms underlying this transition to a catabolic state are not understood. The aim of the current study was to investigate the role of the melanocortin system in this transition, since this has been shown to be an important component of the appetite-regulating system in experimental rodents and humans. Neurons expressing pro-opiomelanocortin (POMC) are localized in the ventrolateral region of the arcuate nucleus, and a major cleavage product of POMC, α-melanocyte-stimulating hormone (α-MSH), has been shown to inhibit feeding via an action on the melanocortin-4 receptor (MC4-R). MC4-R-deficient mice are hyperphagic and obese (18), and mutations in MC4-R or its associated signaling pathways have been observed in association with extreme obesity in humans (33). Intracerebroventricular injections of α-MSH and melanotan II (MTII), a specific synthetic MC3/4-R agonist, potently inhibit food intake in mice and rats (8, 11, 17, 19, 32). MC4-R mRNA is detected in

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hypothalamic sites that play important roles in the control of feeding behavior, including the ventromedial, lateral, dorsomedial, and paraventricular nuclei.

We adopted a functional approach to investigate whether changes in melanocortin mechanisms might contribute to the weight loss and reduced food intake that occurs in hamsters in short photoperiod (SP). Previous studies in Siberian hamsters have shown that the effectiveness of some putative satiety peptides (bombesin and cholecystokinin) is dependent on a change in the photoperiod, whereas sensitivity to certain orexigenic peptides (neuropeptide Y) does not change. We tested whether intracerebroventricular administration of the MC3/4-R agonist MTII could influence food intake, grooming, resting, and other activities in food-deprived and ad libitum-fed hamsters. We then tested whether the suppression of food intake by MTII differed between obese [long photoperiod (LP)] and lean (SP) hamsters, predicting that if increased activity of the endogenous melanocortin system contributes to the generation and maintenance of this catabolic state in SP, then we would observe a diminished response to MTII in SP.

MATERIALS AND METHODS

Animals and housing. A colony of Siberian hamsters (Phodopus sungorus) was maintained in a temperature-controlled room (21 ± 1°C) on a 16:8-h light-dark cycle (LP; lights on from 0000 to 1200) with red light (~10 lx) during the dark period. At a minimum age of 8 wk, gonadally intact male Siberian hamsters weighing 35–45 g were separated into single transparent cages and kept individually during all experimental treatments. Two groups of animals used in experiments 1 and 2 stayed in LP. A third group of hamsters used in experiment 3 was transferred to a short photoperiodic room on a 8:16-h light-dark cycle (SP; lights on from 0400 to 1200) with red light (~10 lx) during the dark period. Lab chow and water were allowed ad libitum until the start of the dark phase and during the dark period (1115 to 1200). Infusions were carried out over 1 min in conscious unrestrained hamsters via a 29-gauge injection cannula inserted in the guide cannula, connected to a 10-μl Hamilton syringe with fine polyethylene tubing. A Harvard infusion pump delivered the vehicle or test substance at a rate of 1 nl/min. The injection cannula was left in place for 3 min to allow diffusion of the injected solution. Animals were injected with 1 μl of vehicle (PBS) or MTII. The cannula stylet was replaced immediately after withdrawal of the infusion cannula. Each hamster received all the treatments in random order; those that did not receive all the treatments were excluded from the study. Each hamster did not receive more than one injection per week.

In experiment 1 (n = 12), hamsters maintained in LP were food deprived for a 24-h period and infused with 1 μl of vehicle (PBS) or MTII (5 μg/μl). In experiment 2 (n = 10), ad libitum-fed Siberian hamsters in LP were injected with 1 μl of vehicle or MTII (0.3, 0.6, 1.25, 2.5, or 5 μg/μl). In experiment 3 (n = 12), ad libitum-fed Siberian hamsters in SP were injected with 1 μl of vehicle or MTII (0.3, 1.25, or 5 μg/μl), and all injections were carried out between week 9 and week 13 in SP.

Measurement of food intake. Immediately before drug infusion, the used sawdust was replaced in each cage to eliminate any food hoards. To reduce novelty-induced stress, we kept the used paper bedding in each cage. Food was removed from the hopper, and dry food was soaked in tap water for 30 min. Wet food has the advantage of higher palatability than dry pellets, while its consistency reduces spillage and prevents hamsters from storing food in their cheek pouches (Schuhler and Ebling, unpublished observations). To estimate the reduction in weight of the test meal through water evaporation, preweighed dishes containing wet pellets were also placed in control cages alongside the experimental hamsters. All studies were carried out at the beginning of the dark phase (1200). Six hours later (at 1800), all dishes containing the food were collected and weighed. The calculation of the amount of food taken by each hamster during 6 h included the deduction of the mean evaporation of water of four controls adjacent to the experimental cages. In experiments 2 and 3, we used this protocol on the injection day (day 1; D1) and on the 2 following days (day 2 (D2) and day 3 (D3))

Behavioral scores. On the injection day, under red light of ~10 lx at cage level, each hamster was observed in its home cage for 5 s in every minute for periods of 30 min, as indicated below. Each behavior was scored as being present or absent in the 5-s period and recorded in a table. Six hamsters were observed in experiment 2 (ad libitum-fed hamsters in LP) and eight hamsters in experiment 3 (ad libitum-fed hamsters in SP). Five behavioral categories (whose definitions are based on Ref. 15) were used in this study: 1) feeding (biting, gnaw-
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Fig. 2. Food intake over 6 h after icv injection of MTII (5 μg/μl) or vehicle in ad libitum-fed Siberian hamsters in long photoperiod at the start of the dark phase (n = 10). **P < 0.01 vs. vehicle. D1, day of injection; D2, 2nd day after injection; D3, 3rd day after injection.

Fig. 3. Dose-response relationship between doses of MTII and the amount of food taken on the 6-h posttreatment period in ad libitum-fed Siberian hamsters in long photoperiod at the start of the dark phase (n = 10). **P < 0.01 vs. vehicle.

RESULTS

Experiment 1: effect on food intake of intracerebroventricular injection of MTII in 24-h food-deprived Siberian hamsters in LP. Food intake in the 6-h postinfusion period was significantly reduced by 55% after intracerebroventricular injection of 5 μg/μl of MTII compared with vehicle injection (Fig. 1; t-test: P < 0.0001).

Experiment 2: effect on food intake of intracerebroventricular injection of MTII in ad libitum-fed Siberian hamsters in LP. On D1, MTII at the dose of 5 μg/μl significantly reduced food intake by 83% relative to the vehicle control treatment (Fig. 2; P < 0.01). Mean food intake was 18% less than the vehicle control treatment on D2 and was 24% higher than the control day on D3 (Fig. 2). MTII inhibited food intake in a dose-dependent manner (Fig. 3). Four doses of MTII (0.6, 1.25, 2.5, and 5 μg/μl) led to a significant reduction in food intake (49, 68, 79, and 83%, respectively; P < 0.01). The lowest dose (0.3 μg/μl) did not significantly reduce food intake (31% less than vehicle treatment).

Experiment 3: effect on food intake of intracerebroventricular injection of MTII in ad libitum-fed Siberian hamsters in SP. All intracerebroventricular injections were carried out between week 9 and week 13 in SP, at a time when the hamsters’ body weight had decreased. A two-factor ANOVA revealed an overall significant effect of the MTII dose (F = 41.2, P < 0.001), but no significant effect of the photoperiod or any interaction between dose and photoperiod was observed. Post hoc tests revealed that MTII at the dose of 5 μg/μl significantly decreased food intake by 70% relative to the vehicle treatment (Fig. 4; P < 0.01). Compared with vehicle, the mean food intake was 19% lower on D2 and 8% higher on D3, but these values were not significantly different from the control period. MTII inhibited food intake in a dose-dependent manner (Fig. 5). MTII at doses of 5 and 1.25 μg/μl led to significant reductions in food intake (70 and 61%, respectively; P < 0.01). At
a dose of 0.3 μg/μl, MTII was also able to decrease food intake significantly (32%; $P < 0.05$).

**Behavioral scores.** In the first period of behavioral observation shortly after treatment (Fig. 6A), two-factor ANOVA revealed an overall significant effect of the MTII dose ($F = 6.19, P < 0.001$) and of the photoperiod ($F = 5.92, P < 0.05$) on the proportion of time spent feeding (Fig. 6A). However, there was no interaction of the two main effects; thus the dose-dependent suppression of time spent engaged in feeding behavior did not differ between long and short days. No drinking behavior was observed when hamsters were provided with a test meal, i.e., hamsters did not approach their water bottles. MTII dose dependently increased the time spent grooming ($F = 7.82, P < 0.001$), but no significant effect of the photoperiod or any interaction between dose and photoperiod was observed on grooming (Fig. 6A). MTII dose dependently reduced the proportion of time spent resting ($F = 10.8, P < 0.0001$), but no significant effect of the photoperiod was observed. MTII did not significantly affect the proportion of time that the hamsters were engaged in other types of activity (Fig. 6A).

In the second period of behavioral observation, ~3 h after treatment (Fig. 6B), two-factor ANOVA revealed that MTII dose dependently decreased the proportion of time spent feeding ($F = 3.28, P < 0.05$). No significant effect of the photoperiod or any interaction between dose and photoperiod was observed on the time spent feeding. MTII dose dependently increased the time spent grooming ($P < 0.05$), but there was no significant effect of the MTII treatment on the proportion of time spent engaged in other activity or resting, during this observation period (Fig. 6B).

In the third period of behavioral observation, ~6 h after treatment (Fig. 6C), MTII had no significant effect.
on the proportion of time spent feeding or grooming, and there were no significant effects of photoperiod on these two parameters, nor were there significant interactions between MTII treatment and photoperiod. However, there was a significant effect of MTII on the proportion of time spent engaged in other activity ($F = 5.43, P < 0.005$) and conversely on the proportion of time spent resting ($F = 3.75, P < 0.05$). There was also a significant overall effect of photoperiod on these two parameters, but no significant interaction between dose and photoperiod, indicating that the effect of the MTII treatment on general activity and rest did not differ between the photoperiodic conditions.

**DISCUSSION**

Our results show that intracerebroventricular administration of MTII (a MC3/4-R agonist) was able to exert a potent anorexigenic effect in Siberian hamsters in the three conditions tested. When the animals were in a hungry state after being deprived of food for 24 h, MTII at the dose of 5 $\mu$g/$\mu$l significantly decreased food intake by 55%. Although it is common practice to test the anorectic potential of compounds in food-restricted rodents, hypothalamic gene expression is modified by short-term food restriction in Siberian hamsters as in other species (1, 23, 24, 28, 29), so in subsequent experiments we chose to investigate the melanocortin system in animals fed ad libitum. Hamsters were studied at the start of the dark phase when their circadian system would dictate a hungry state, a more physiological condition of hunger in which short-term compensatory responses to complete dietary restriction would not be able to influence the response to a melanocortin agonist. In such ad libitum-fed hamsters maintained in LP, food intake after vehicle injection was lower than in the food-restricted group, and the significant decrease of food intake induced by 5 $\mu$g/$\mu$l of MTII was greater than in the first experiment (85%). In ad libitum-fed hamsters maintained in SP, food intake after vehicle injection was slightly higher over the first 6 h of the dark phase than in ad libitum-fed hamsters maintained in LP and was similar to the food intake in the restricted group in LP (experiment 1). It is our previous experience that food intake over 24 h is reduced by ~20% in hamsters in SP, either when measured chronically (10) or in acute food intake trials (28). Nevertheless, MTII at the dose of 5 $\mu$g/$\mu$l was able to decrease significantly food intake by 70%. In both ad libitum-fed groups (LP and SP), the food intake on D2 tended to be lower than the control period, which may indicate that MTII has a prolonged duration of action and is consistent with previous studies in rats (11, 32).

In ad libitum-fed hamsters in both LP and SP, MTII led to a dose-dependent reduction in food intake with doses as low as 0.3 $\mu$g/$\mu$l in SP and 0.6 $\mu$g/$\mu$l in LP. ANOVA revealed no significant interaction between dose and photoperiod. We therefore infer that there is no differential sensitivity between hamsters in LP and SP. Although the post hoc tests indicate that the effect of 0.3 $\mu$g/$\mu$l treatment was not significant in LP, the magnitude of the effects of MTII is broadly similar in LP and SP (reduction of 31 and 32%, respectively) as is the case for 1.25 $\mu$g/$\mu$l MTII (reduction of 68% in LP and 61% in SP).

Suppression of food intake can occur for many reasons, including nausea, malaise, sedation, or induction of other behaviors that conflict with ingestion. In rats, administration of MTII into the third ventricle was found to induce conditioned taste aversion (32). This effect is believed to be due to the action of MTII on MC3-R rather than on MC4-R (6). In hamsters, we cannot exclude that the reduction of food intake induced by MTII may be a consequence of a taste aversion. In rats, MTII significantly reduced water intake (27). In our study, we never observed any drinking behavior in vehicle-treated or MTII-treated hamsters. Hamsters are omnivores that have evolved under arid conditions and are rarely observed drinking from water bottles, particularly when their food supply has a significant water content. The moisture content of the test meal may have masked any effect of MTII on drinking per se but represents a biologically relevant test. The reduction of food intake by MTII may well relate to the induction of grooming by this agonist, because in the first and the second periods of behavioral observation, MTII significantly increased the proportion of time spent grooming. As a probable consequence of this increase of grooming, the time spent resting was decreased significantly. The induction of grooming behavior by melanocortin receptor agonists was also reported in rats (2). Indeed, excessive grooming can be induced by intracerebroventricular injections of $\alpha$-MSH in the rat, and this effect can be blocked by MC4-R antagonists, suggesting that melanocortin-induced grooming is mediated by the MC4-R (2). Intravenous injections of MTII were also able to induce grooming in the rat, and this effect was blocked by SHU-9119, a MC3/4-R antagonist (2). The significance of MC4-R activation for physiologically evoked grooming is still unclear.

The potency of administration of MTII into the third ventricle of hamsters is consistent with an intrahypothalamic site of action of MC3/4-R agonists. In mice and rats, MTII or $\alpha$-MSH causes significant and long-lasting reductions in food intake when injected in the third ventricle (11, 32) or into the paraventricular nucleus of the hypothalamus (9, 12, 21, 34). Moreover, in the rat, intracerebroventricular injections of MTII induced c-Fos expression in the paraventricular nucleus of the hypothalamus and the central nucleus of the amygdala (32), and MC4-R mRNA was detected in the ventromedial, lateral, dorsomedial, and paraventricular nuclei (26). Fibers containing POMC are also found in these nuclei (16). In Siberian hamsters, Mercer et al. (24) detected MC3-R mRNA in the arcuate nucleus and in the ventromedial nucleus, and MC4-R gene expression was found in the paraventricular nucleus of the hypothalamus.

In addition to actions within the hypothalamus, our results do not rule out the possibility that MTII may also act in lower brain stem sites, as compounds delivered into the third ventricle will clearly reach regions adjacent to the fourth ventricle. Indeed, MC4 receptors are widespread in the caudal brain stem (26), and melanocortin receptor ligands delivered to the caudal brain stem have clear effects on food intake in rats (13, 14).
In conclusion, the current studies demonstrate that the melanocortin agonist MTII is a potent suppressor of food intake in the Siberian hamster in LP (after 24-h food deprivation and ad libitum fed) and in SP (ad libitum fed). Our results do not provide clear evidence of differential response or sensitivity in the obese (long day) vs. lean (short day) states. The behavioral observations show that MTII influences processes other than food intake, including grooming, which is consistent with the view that in addition to direct effects on satiety, melanocortins affect multiple systems that ultimately influence energy balance.

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REFERENCES

8. Brown KS, Gentry RM, and Rowland NE. Central injection of 

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