Centrally administered MTII affects feeding, drinking, temperature, and activity in the Sprague-Dawley rat

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Murphy, B., C. N. Nunes, J. J. Ronan, M. Hanaway, A. M. Fairhurst, and T. N. Mellin. Centrally administered MTII affects feeding, drinking, temperature, and activity in the Sprague-Dawley rat. J Appl Physiol 89: 273–282, 2000.—MTII, an agonist of melanocortinergic receptors, is a well-documented anorexigenic agent in rats. Many investigators have reported its effects on feeding without considering concurrent alterations in other behaviors. Accordingly, we performed studies to simultaneously measure nocturnal feeding, drinking, activity, and temperature of rats after intracerebroventricular (third ventricle) administration of a wide dose range of MTII (0.05–500 ng). We observed that MTII modulates these physiological parameters in a dose-dependent manner. Low doses of MTII (0.05 ng) caused reductions in feeding without alterations in body temperature, drinking, or activity. In contrast, hyperthermia and disrupted drinking patterns, along with food intake reductions, were evident at doses exceeding 50 ng. The fact that low doses altered only feeding, whereas higher doses affected a range of parameters, suggests that certain melanocortin-induced behavioral changes may be mediated by distinct populations of melanocortin receptors with varying affinities or that those changes seen at higher doses may be nonspecific in nature.

obesity; telemetry; third ventricle; continuous physiological monitoring; melanocortin

ENERGY BALANCE IS CONTROLLED by a complex array of overlapping physiological mechanisms. Maintenance of body weight occurs when energy input equals that of energy output. A metabolic state that favors energy storage, as opposed to energy expenditure, can lead to obesity. Stimulation of energy expenditure by manipulating thermogenesis directly or via one of the neuronal pathways involved in controlling energy flow could potentially alleviate many obesity-induced pathologies.

An alternative approach to restoring energy balance in the obese individual is to reduce food consumption with anorexigenic agents. An attractive assumption is that drug-induced reductions in food intake (FI) result from inhibition of the natural process of hunger or stimulation of satiety. An often-overlooked possibility is that FI reductions may be the result of nausea, malaise, sedation, or one of many of other phenomena that are unrelated to the proposed anorexic mechanism of the test compound (6, 10, 36). Simply recording the amount of food consumed over a specified time period does not reveal the exact mechanism by which FI is being reduced. An in-depth analysis of the feeding microstructure, as well as observation of other physiological parameters, is required to provide a more complete picture (33).

Melanocortins are one of many neuronal components that can regulate FI and body weight. Genetic mutations involving the melanocortin system have been linked to obesity and diabetes in both laboratory rodents and humans (7, 23, 24, 29, 34, 40, 41, 58, 66). Both peripheral and central injections of melanocortin-receptor (MCR) agonists and antagonists result in dramatic alterations in food consumption when given to mice and rats (5, 11, 16, 19, 25–27, 43, 44, 65, 67). MTII, an agonist analog of α-melanocyte-stimulating hormone (α-MSH), has been shown by our laboratory and others to be a particularly potent inhibitor of FI and body weight gain in rodents (11, 19, 44). However, little is known about the mechanisms underlying the anorectic action or its effect on other behavioral responses. The purpose of this study was to investigate physiological changes associated with MTII by continuously monitoring the core body temperature, gross motor activity, and drinking and feeding behavior of MTII-treated rats.

MATERIALS AND METHODS

Animals and Diet

Male Sprague-Dawley rats (Harlan, Indianapolis, IN) weighing 250–350 g were maintained in a temperature- and humidity-controlled facility with a 12:12-h light-dark cycle (4:00 AM lights on). Before surgery, the rats were fed standard rodent pellets (Purina 5008). Postsurgery, they were fed a semiliquid diet consisting of a 70:30 (wt/wt) mixture of Carnation sweetened condensed milk and ground rodent chow (Purina 5008). Fresh diet was provided daily. Both food and water were supplied ad libitum throughout the pre- and postoperative periods.

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postsurgery periods. All procedures were approved by the Merck Animal Use and Care Committee.

Surgical Procedures

All surgeries used ketamine (80 mg/kg) and xylazine (20 mg/kg) anesthesia and aseptic technique. Each rat received an intraperitoneal radio transmitter (Minimitter, Sunriver, OR) and a permanent, indwelling, 26-gauge guide cannula (Plastics One Roanoke, VA). Procedures for implanting guide cannulas were reported previously (44, 46).

Acclimation

Beginning the day after surgery, each rat was acclimated to the handling, injection procedure, and feeding regimen. At ~10:00 AM, all rats were presented with freshly prepared condensed milk and ground chow diet. Rats were allowed free access to the fresh diet for 1 h to induce satiation (32). At the end of the satiation period, each rat was handled with minimal restraint, and each dummy cannula was removed to maintain cannula patency and simulate the injection procedure. Daily handling familiarized the rats with the injection procedure and minimized associated stress, an important consideration in feeding behavior studies in which stress can evoke hormonal changes that can effect FI (1, 55). FI was considered in feeding behavior studies in which stress can evoke hormonal changes that can effect FI (1, 55). FI was considered in feeding behavior studies in which stress can evoke hormonal changes that can effect FI (1, 55). FI was considered in feeding behavior studies in which stress can evoke hormonal changes that can effect FI (1, 55). FI was considered in feeding behavior studies in which stress can evoke hormonal changes that can effect FI (1, 55). FI was considered in feeding behavior studies in which stress can evoke hormonal changes that can effect FI (1, 55). FI was considered in feeding behavior studies in which stress can evoke hormonal changes that can effect FI (1, 55). FI was considered in feeding behavior studies in which stress can evoke hormonal changes that can effect FI (1, 55). FI was considered in feeding behavior studies in which stress can evoke hormonal changes that can effect FI (1, 55). FI was considered in feeding behavior studies in which stress can evoke hormonal changes that can effect FI (1, 55). FI was considered in feeding behavior studies in which stress can evoke hormonal changes that can effect FI (1, 55). FI was considered in feeding behavior studies in which stress can evoke hormonal changes that can effect FI (1, 55). FI was considered

Dose preparation and injection. Test substances were dissolved in sterile pyrogen-free artificial cerebrospinal fluid (CSF; Harvard Apparatus, Holliston, MA). An injector was constructed on the basis of that described by Stanley and co-workers (60). The injection procedure was the same as that reported previously (44).

Confirmation of cannula placement. Once the rats were fully recovered from the surgery, guide cannula placement was confirmed by evaluating neuropeptide Y (NPY)-induced FI. Human NPY (5 μg; Peninsula Laboratories, Belmont, CA) was injected after a 1-h satiation period and 2-h postinjection FI was recorded for each rat. The guide cannula was considered to be in the correct location if the NPY-induced FI was at least twice that of the 2-h postsatiation FI recorded the previous noninjection day. Only those rats shown to be NPY responsive were used in any experimental protocols. Cannula placement was reconfirmed after each MTII injection.

MTII injections. NPY-responsive rats were first injected intracerebroventricularly (ICV) with CSF to determine basal overnight FI (n = 35). The nonspecific peptide melanocortin agonist MTII (Peninsula Laboratories) was used to evaluate MCR involvement in temperature, activity, feeding, and drinking. Rats were randomly assigned to groups that received MTII doses of 0.05 ng (n = 11), 0.5 ng (n = 11), 5 ng (n = 18), 0.5 ng (n = 17) or 0.05 ng (n = 15) between 3:00 PM and 4:00 PM. Rats received more than one, but not necessarily all, doses of MTII. NPY responsiveness was confirmed between test injections. A minimum time of 72 h separated each NPY or MTII injection.

Measurement of Physiological Parameters

Temperature and activity. Core body temperature and gross motor activity were measured continuously using a telemetry system (Minimitter). A transmitter was placed in the peritoneal cavity of each rat before ICV cannulation. A receiver placed under each cage monitored transmitter output. Cumulative data for the 17-h period (3:00 PM–8:00 AM) was measured for each rat. Feeding duration (FD) was continuously measured over the 17-h test period by using an infrared monitor attached to the feed cup. The monitor generated an infrared beam that was broken each time the rat placed its head into the cup. The time that the beam was broken (FD) was recorded. Data were collected at 1-min intervals by using the VitalView data-acquisition system (Minimitter). Cumulative FD was determined over time for each rat.

Water intake and lick frequency. Water intake (WI) over a 17-h period (3:00 PM–8:00 AM) was also measured for each rat. The number of water licks or lick frequency (LF) was continuously measured over the 17-h test period via a lick sensor attached to the water bottle and metal floor of each cage. Each lick of the water bottle completed an electrical circuit. The number of completed electrical circuits corresponding to the number of licks. Data were collected at 1-min intervals by using the VitalView data-acquisition system (Minimitter). Cumulative LF over time was determined for each rat.

Data Analysis

All physiological parameters were recorded for individual rats at different times and under different treatment regimens according to a crossover-type design. In this paradigm, each rat received vehicle and one or more test doses of MTII. Percent changes in FI and WI of individual MTII-treated rats were calculated relative to the average intake of the vehicle group. All results are expressed as means ± SE.

Cumulative data. The 17-h values of FI and WI from treatment groups were compared with the vehicle group by using a one-way ANOVA followed by Scheffé’s F-procedure for post hoc comparisons. Cumulative FD and LF, core body temperature change, and gross motor activity change of treated rats were compared with vehicle by using a repeated-measures ANOVA followed by Scheffé’s F-procedure for post hoc comparisons, when appropriate.

Time intervals. The test session beginning at 4:00 PM was divided into four equal time intervals of 4 h to assess the kinetics of MTII effects on feeding and drinking. The first hour of testing from 3:00 to 4:00 PM was not included so that each interval would be the same length. The time periods were selected to reflect the fact that rats consume the majority of their food at the beginning and end of the dark period (6). Thus 4:00 PM to 8:00 PM was designated as period 1, 8:01 PM to 12:00 AM as period 2, 12:01 AM to 4:00 AM as period 3, and 4:01 AM to 8:00 AM as period 4. Cumulative FD and LF were determined for each rat in each time period on each test day. Mean cumulative FD and LF for treated rats
were compared with vehicle by using a one-way ANOVA followed by Scheffe’s F-procedure for post hoc comparisons.

RESULTS

Effects of MTII on Rat Feeding Behavior

ICV-administered MTII inhibited cumulative overnight FI in a dose-dependent manner (Fig. 1A; P < 0.01). MTII had similar effects on FD (Fig. 1B). Rats injected with the three highest doses of MTII (i.e., 500, 50, and 5 ng) had highly significant reductions in FD over time (P < 0.01). FD was significantly reduced in rats injected with 0.5 and 0.05 ng of MTII (P < 0.05).

Effects of MTII on Rat Drinking Behavior

MTII reduced WI at doses >50 ng (Fig. 2A). Rats receiving both 500 and 50 ng of MTII demonstrated significantly decreased WI compared with controls (P < 0.01).

Effects of MTII on water licking and total water consumed were similar (Fig. 2B). Statistical analysis showed that MTII had a significant effect on LF over the entire 17-h test period (P < 0.05). Rats injected with both 50 and 500 ng of MTII had depressed LF throughout the entire observation period (P < 0.05). LF of rats receiving the 5-ng dose of MTII was depressed up to 11:00 PM, after which they appeared to recover, as indicated by the slope of the LF curve. Rats receiving the 0.5- and 0.05-ng doses had a LF similar to that of CSF controls.

Kinetics of MTII Action on 4-h Feeding and Drinking Time Intervals

When analyzed over the entire 17-h test period, MTII significantly reduced feeding over the entire dose range. WI was marginally reduced in rats treated with MTII at 5 ng, but a more definitive effect was observed at the 50- and 500-ng doses. To discern the kinetics of MTII action on feeding and drinking, the test session beginning at 4:00 PM was divided into four equal time intervals. Mean cumulative FD and LF of each treatment group over each time interval were used for comparison.

Interval 1 (4:00 PM–8:00 PM). During the first 4 h of the nocturnal feeding cycle, MTII effects on FD were dose dependent (Fig. 3A). FD was significantly reduced at all dose levels of MTII tested (P < 0.05). During the same time interval, no discernible relationship between dose of MTII and LF was observed (Fig. 3B).

Interval 2 (8:00 PM–12:00 AM). During the first 4 h of the test session, MTII effects on FD were dose dependent (Fig. 2A). FD was significantly reduced at all dose levels of MTII tested (P < 0.01). During the same time interval, no discernible relationship between dose of MTII and LF was observed (Fig. 3C).

Interval 3 (12:00 AM–4:00 AM). During the first 4 h of the test session, MTII effects on FD were dose dependent (Fig. 2A). FD was significantly reduced at all dose levels of MTII tested (P < 0.01). During the same time interval, no discernible relationship between dose of MTII and LF was observed (Fig. 3C).

Interval 4 (4:00 AM–8:00 AM). During the first 4 h of the test session, MTII effects on FD were dose dependent (Fig. 2A). FD was significantly reduced at all dose levels of MTII tested (P < 0.01). During the same time interval, no discernible relationship between dose of MTII and LF was observed (Fig. 3C).
reduced FD (Fig. 3A). Variation of FD responses within these treatment groups account for the lack of statistical significance. A possible reason for the observed variation could be that the anorexigenic effect of MTII is beginning to subside in some, but not all, rats.

The tendency of MTII to reduce LF was more evident in interval 2 (Fig. 3C). All rats injected with MTII showed reduced LF. Rats injected with 0.5- to 50-ng doses showed significant reductions in LF (P < 0.05), whereas the LF of the 500-ng treatment group showed a highly significant decrease (P < 0.01).

Interval 3 (12:00 PM–4:00 AM). During this period, statistically significant reductions in FD were maintained at the two highest doses of MTII (50 and 500 ng; P < 0.01; Fig. 3B).

LF of MTII-treated rats began to return to control levels, but variability in the data makes it impossible to draw a definitive conclusion (Fig. 3D).

Interval 4 (4:00 AM–8:00 AM). The FD pattern observed was similar to that seen in interval 3 (Fig. 3B). Once again, rats receiving the 50- and 500-ng doses showed substantial reductions in FD (P < 0.05).

As was noted for interval 1, no obvious relationship between dose of MTII and LF was discernable in interval 4. Once again, variation and the fact that control animals had low LF made detecting a causal relationship between MTII and LF during this time interval difficult.

Evaluation of combined FD and LF in 20-min time intervals. Eating and drinking are closely integrated in the rat (8, 27). To determine whether MTII had an effect on the feeding-drinking relationship a direct comparison of LF and FD was performed (Fig. 5). The pattern observed in CSF-treated rats was much like the basal ingestive pattern described by Kisseleff (28). In these published studies, rat feeding and drinking showed a close temporal relationship in which there was a positive correlation between sizes of the associated feeding and drinking bouts. A similar relationship was observed in rats treated with

MTII at 50 ng caused significant reductions in both total food consumed (−61 ± 10%; P < 0.01) and cumulative FD (−51 ± 8%; P < 0.01). A closer examination of feeding patterns (Fig. 4C) showed that 50-ng-treated rats ate in fewer bouts separated by longer periods of nonconsumption in comparison to CSF-injected controls (Fig. 4A).

In contrast, injections of 0.05 ng of MTII resulted in unchanged 17-h cumulative FI and FD compared with controls. It could be assumed that this dose had no effect on feeding, but a closer examination of the feeding pattern showed this to be untrue (Fig. 4B). Initially, the feeding patterns of 0.05-ng-treated rats were similar to 50-ng-treated rats. The transient decrease in FD lasted for approximately the first 3 h of the test session. At ~7:00 PM, these rats resumed feeding but in a pattern different from that of control animals. These animals ate for very long periods of time, separated by intervals of little or no feeding. There are four intervals in which this pattern is particularly apparent (indicated by arrows).

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0.05 ng of MTII (Fig. 5B). However, a different pattern was observed in rats injected with 50 ng of MTII. Although the temporal relationship between eating and drinking was maintained, the correlation between the length of time spent feeding and the number of water licks was not. This observation was most apparent between 11:00 PM and 4:00 AM. These rats drank more water in relation to the extent of food consumption than rats treated with either 0.05 ng of MTII or CSF (Fig. 5C).

**Effect of MTII on Core Body Temperature**

*Full test period.* The effect of MTII on core body temperature appeared to be biphasic in nature (Fig. 6). All core body temperature measurements are expressed as degrees Celsius change from baseline (refer to MATERIALS AND METHODS). Initial increases in temperature were observed in rats injected with the four higher doses of MTII (Fig. 6, B–E). Temperature remained elevated for ~90 min into the dark cycle. At this time, temperature of control rats began to increase, a phenomenon that is associated with the normal physiological nocturnal temperature rhythm [see Temperature change (4:00 PM–6:00 PM) for a more in-depth analysis]. Temperature of MTII-treated rats remained comparable to that of controls throughout the remainder of the test session, with the exception of those injected with MTII doses of 50 and 500 ng (Fig. 6, D and E). In these two treatment groups, temperature began to increase relative to controls at ~12:00 AM, 8 h into the dark cycle. In the group given 500 ng MTII, group temperature remained increased for ~3 h before returning to control levels (Fig. 6E). In contrast, temperature of animals receiving the 50-ng dose (Fig. 6D) remained elevated, even after the start of the light cycle at 4:00 AM.
Temperature change (4:00 PM–6:00 PM). Temperature changes during the first 2 h of the test session were evaluated because it was during this time that the anorexigenic effects of MTII were the most robust. Mean temperature changes during each 20-min sample interval were evaluated to determine whether there was a connection between temperature and FD (Fig. 7). Initially, all rats injected with MTII (0.5–500 ng) showed an increased core body temperature compared with CSF controls. By 100 min, core body temperature of all rats returned to control levels. Rats given 0.05 ng of MTII demonstrated temperature...

Fig. 6. Effect of MTII on core body temperature in Sprague-Dawley rats. Body temperature was expressed as change from baseline over time in rats treated with 500 ng (E; n = 11), 50 ng (D; n = 11), 5 ng (C; n = 18), 0.5 ng (B; n = 17), or 0.05 ng (A; n = 15) of MTII. Control rats were injected with an equal volume of CSF (n = 35).

Fig. 7. Effect of MTII on temperature 2-h postdose (4:00 PM–8:00 PM). Mean temperature change from baseline was calculated for each 20 min interval. Sprague-Dawley rats were treated with 500 ng (n = 11), 50 ng (n = 11), 5 ng (n = 18), 0.5 ng (n = 17), or 0.05 ng (n = 15) of MTII. Control rats were injected with an equal volume of CSF (open bars; n = 35).
changes that were similar to those of controls throughout the 2-h sample time.

**Effect of MTII on Gross Motor Activity**

A repeated-measures ANOVA showed that MTII treatment had no apparent effect on activity (Fig. 8).

**DISCUSSION**

The purpose of this study was to evaluate the effect of exogenous MTII on various physiological parameters. Melanocortins have a wide range of physiological effects (9, 17, 20, 22, 37, 61), and it is not surprising that we observed changes in drinking and body temperature in addition to reduced FI. The observed changes in drinking and temperature evoked by MTII administration have not been previously reported. Additionally, continuous monitoring of these physiological parameters enabled us to demonstrate MTII-in-

![Fig. 8. Effect of intracerebroventricular MTII on gross motor activity in Sprague-Dawley rats. Gross motor activity expressed as change from previous non-test day over time in rats treated with 50 ng (C; n = 11), 5 ng (B; n = 18) or 0.05 ng (A; n = 15) of MTII. Control rats were injected with an equal volume of CSF (n = 35).](image)

duced changes in both feeding and drinking patterns. Finally, we confirmed the observation of Fan and colleagues (11) that MTII injections have no effect on gross motor activity. Thus, like many other appetite-controlling agents, MTII influences processes other than food consumption (3, 4, 6, 43, 47), an indication that melanocortins may affect multiple systems that impact a variety of physiological parameters.

The observation that ICV MTII reduces FI confirms previous observations that MCRs play a role in modulating feeding. Significant reductions in cumulative 17-h FI and FD were both observed at doses of MTII as low as 0.05 ng. Effects on feeding after administration of very low doses of MTII were also observed by Grill and colleagues (19). The ability of such small doses of MTII, those that achieve endogenous plasma α-MSH levels (65), to alter feeding behavior emphasizes the importance that melanocortinergic neurons play in controlling feeding (1, 32).

Many investigators have stressed the importance of considering more than just the amount of food consumed when evaluating appetite-altering substances. Assessing changes in feeding patterns also provides useful information concerning the mechanism by which the drug is working (5, 6, 14, 33, 36). This type of analysis becomes particularly important when the total amount of food consumed over an extended test session is very small, as is seen with 0.05 ng of MTII. At this dose, initial reductions in FD diminished as the anorexigenic effect of the drug waned, with the net result being marginal reductions in FI. In contrast, the effect of the 50-ng dose of MTII was sustained throughout the entire test session. Decreased FD, coupled with increased time between actual feeding events, accounts for the suppressed FI in this instance. More detailed kinetic evaluation of the 0.05-ng MTII data showed a feeding pattern very similar to that of the 50-ng dose over the first 4 h. Transient suppression of FD suggests that the anorexigenic effect of the lower dose is diminishing with time.

A similar pattern of initial depressed feeding with a later compensatory increase in feeding was observed in rats treated with low doses of dexfenfluramine (6). Although rats injected with 0.05 ng of MTII did not demonstrate a compensatory increase per se, they did exhibit an altered feeding pattern composed of spikes in FD separated by long periods of little or no feeding. This profile is similar to that seen in rats fed after a period of food restriction (35, 63). Quite possibly, the rats treated with 0.05 ng of MTII are compensating for low FI during the first few hours of the dark cycle. In the short term, rats will increase the sizes of feeding bouts, as opposed to the number of feeding bouts, to compensate for food deficits (63). The concept of increasing feeding after periods of decreased intake, as seen in the 0.05-ng MTII group, is in line with the notion that feeding is a homeostatic mechanism (33, 56). However, FD of rats receiving the 50-ng dose of MTII was still depressed at the end of the test session. This suggests that the effect of MTII can be sustained.
and that a period of time longer than 17 h may be required for FD of these rats to return to normal.

Changes in FI are often associated with concurrent changes in WI. Rats with restricted access to either food or water will reduce the intake of the one that is freely available (28, 35, 60). This observation, in combination with the observation that α-MSH can suppress drinking (67), led us to investigate the effects of MTII on WI. In these experiments, only those rats injected with the highest doses of MTII showed suppressed WI. The fact that reduced WI was only observed in rats injected with high concentrations of MTII suggests that dipsogenesis may be regulated by another MCR with a lower affinity for MTII than the MCR affecting feeding (11, 16, 19, 23, 25, 40). Additionally, the possibility that reduced drinking may be a nonspecific phenomenon cannot be ruled out.

Simultaneous recordings of feeding and drinking show a strong temporal relationship between FI and WI in the rat. When food and water are provided ad libitum, 70% of drinking is directly associated with feeding and the amount of water consumed is proportional to the amount of food consumed over any given time (28). This pattern of feeding and drinking was unaltered in the rats treated with 0.05 ng of MTII. In contrast, the feeding–drinking relationship apparently was disrupted in animals treated with 50 ng of MTII. Although the temporal relationship is maintained, the rats display an exaggerated drinking response that is particularly evident between 12:00 AM and 4:00 AM. Even though rats receiving 50 ng of MTII drank significantly more than control animals during this 4-h time period, their overall WI for the entire test session was still decreased. One possible explanation for this observation is that these rats are compensating for lack of drinking at the earlier time points. Another possibility is that the effects of MTII on drinking and feeding are mediated via two different mechanisms. There is evidence that FI and WI are related but are not necessarily dependent on one another. Both activities occur with a similar circadian rhythm, and feeding and drinking can be separated as a result of experimental manipulation (61). In addition, the fact that α-MSH-induced reductions in FI, but not WI, are reversed in the presence of the MCR antagonist HS104 and that the MCR agonist [Nle³,D-Phe⁶] (NDP-α-MSH reduced FI but had no change on WI further supports the idea that melanocortin modulation of these behaviors may be mediated by different MCRs (68).

The role of melanocortins in body temperature regulation is complex. The effects appear to be species specific and are dependent on experimental conditions (20–22, 39, 48, 49). In the present study, we observed that MTII caused both immediate and long-term elevations in core body temperature. To date, there are no published reports of MTII causing changes in body temperature. The data concerning the effects of α-MSH on body temperature are contradictory (20, 48, 49, 68). In the short term, MTII exhibited a bell-shaped dose–response relationship. A similar dose relationship has been reported with anterior hypothalamic preoptic area injections of α-MSH and NDP-α-MSH (48). In the present studies, temperature of MTII-treated rats returned to control levels by 100 min postinjection. A similar time course was reported with NDP-α-MSH, the peptide from which MTII is derived (48).

The observed MTII-induced hyperthermia is in contrast with the well-documented antipyretic property of melanocortin peptides (20, 22, 39). We propose that central MTII temperature effects are mediated by MCR 4. We believe that melanocortin temperature effects are mediated by different receptors centrally and peripherally. A possible explanation for the contradiction in the literature is that, in the CNS, melanocortins act to elevate core temperature acting via MC4R, whereas, in the periphery, they act via another receptor, possibly MCR 1, to block the effects of pyrogens. Studies evaluating the effect of specific MCR agonists and antagonists on factors that mediate fever (30, 52, 56) will help to further resolve this issue.

Previous evaluations of the melanocortins in thermal regulation occur over short periods of time (i.e., 1–2 h). In this study, we observed both delayed and prolonged increases in body temperature in rats treated with higher doses (50 and 500 ng) of MTII. The length of time that temperatures were elevated in rats treated with these two doses far exceeded the 1- to 2-h observation periods used by other investigators. Therefore, it is impossible to make direct comparisons between our research and that of others.

Numerous factors can influence body temperature. Those associated with temperature elevation include thermogenesis, activity, and pyrexia. One possible explanation for the MTII-induced hyperthermia is increased metabolic rate and thermogenesis. This hypothesis is supported by the fact that many proopiomelanocortin peptides have been implicated in metabolic regulation (52, 54, 56, 69). The results of these studies and of others (11) show that there were no changes in activity of MTII-injected animals. Thus increased physical activity cannot account for the observed hyperthermia. Some investigators have reported that administration of proopiomelanocortin products causes increases in grooming behavior (18). This behavior was not observed in the present studies. Nonspecific pyrogenic effects can be ruled out as cause of increased temperature by evaluating the effect of ICV MTII in combination with antipyretic agents.

In summary, MTII-induced reductions in FD, induced by a low dose of MTII (0.05 ng), appear to be specific and are unaccompanied by changes in WI, temperature, or activity. Taste aversion or nausea could also cause reduced feeding. Indeed, others have shown that MTII can cause conditioned taste aversion, but a much higher dose is needed for this effect (64). In contrast, animals treated with MTII at higher doses (500 and 50 ng) show alterations in several physiological parameters in addition to feeding. These include an altered drinking pattern and a long-lasting hyperthermia that may contribute to the sustained anorexia-genesis. These data suggest that, in studies of MTII or other potential anorexigenic agents, it is important to
consider both the dose and the effects on other behaviors and physiological parameters when interpreting the significance of drug-induced reductions in feeding.

**Perspective**

The fact that endogenous MTII can affect multiple physiological parameters reinforces the concept that FI is not an isolated event but, rather, is one that should be regarded in combination with other behaviors. The results of these studies indicate that the melanocortin system may play a physiological role in regulating FI and body weight. We showed that reductions in feeding were still evident with doses of MTII that had no effect on drinking, body temperature, or activity levels. It is important to note that the suppression of feeding evoked by this low dose of MTII would have been missed if continuous monitoring had not been performed. In contrast, a much higher dose of MTII caused marked changes in not only feeding but also drinking and body temperature. It is possible that the effects on feeding of higher doses of MTII may be nonspecific in nature. The reductions in feeding may be a direct result of the elevated temperature observed in these rats. Altered drinking patterns indicate that the effects on drinking may be indirect. Thus continually monitoring several physiological parameters gives us valuable information concerning ancillary mechanisms that may contribute to the observed decreases in FI.

There is still much to be learned about the role of melanocortinergic neurons in regulating physiological processes. Specific agonists and antagonists of each MCR will be required to attribute specific functions to individual receptors.

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**REFERENCES**

44. Murphy B, Nunes CN, Ronan JJ, Harper CM, Beall MJ, Hanaway MJ, Fairhurst AM, Van Der Ploeg LTH, MacIn-...