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Profound and rapid reduction in body temperature induced by the melanocortin receptor agonists

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ABSTRACT

The melanocortin receptor 4 (MC4R) plays a major role in body weight regulation and its agonist MTII has been widely used to study the role of MC4Rs in energy expenditure promotion and feeding reduction. Unexpectedly, we observed that intraperitoneal (i.p.) administration of MTII induced a rapid reduction in both body temperature and energy expenditure, which was independent of its effect on feeding and followed by a prolonged increase in energy expenditure. The rapid reduction was at least partly mediated by brain neurons since intracerebroventricular (icv) administration of alpha melanocyte-stimulating hormone, an endogenous melanocortin receptor agonist, produced a similar response. In addition, the body temperature-lowering effect of MTII was independent of the presence of MC4Rs, but in a similar fashion to the previously shown effect on body temperature by 5′AMP. Moreover, β-adrenergic receptors (β-ARs) were required for the recovery from low body temperature induced by MTII and further pharmacological studies showed that the MTII's effect on body temperature may be partially mediated by the vasopressin V1a receptors. Collectively, our results reveal a previously unappreciated role for the melanocortin pathway in rapidly lowering body temperature.

1. Introduction

The melanocortin pathway is a well-established neural pathway in metabolic regulation. Melanocortin receptors, especially melanocortin receptors 4 (MC4Rs), are one major regulator in body weight control [1,2]. MC4Rs are broadly expressed in the brain and their action on body weight control is mediated by a distributed neuronal network [3,4]. Studies from last decades, based on both mouse genetics and pharmacology, have revealed important and distinct neural pathways in mediating MC4R action in feeding and energy expenditure. In this regard, the identification of melanotan II (MTII), a synthetic peptide with similar structure to the endogenous ligand, alpha-MSH, as an agonist of MC4Rs (and also MC3Rs), has been instrumental in defining the mechanism underlying the MC4R action on body weight regulation [5,6].

Since its initial identification, MTII has been widely used as a tool in animal models to study food intake and energy expenditure effects regulated by MC4Rs and in electrophysiology to determine the cellular mechanism underlying the MC4R action [6–8]. Since the effect on feeding inhibition of MTII is lost in *Mc4r-null* mice [5,9], it has also been widely used for MC4R-mediated feeding mechanisms. However, MTII is capable of inducing a significant amount of Fos expression in the brain of mice with double knockouts of both *Mc3r* and *Mc4r* [10], suggesting that MTII may activate a subset of brain neurons independent of MC3Rs and MC4Rs and be able to elicit other metabolically important behaviors.

Here, we used a telemetry system to continuously monitor body temperature changes in response to MTII, and surprisingly identified biphasic effects of MTII: initial rapid reduction in body temperature and energy expenditure followed by mild increase in energy expenditure. The first phase is independent of MC4Rs but the second phase is mediated by MC4Rs. Importantly, a similar body temperature-reducing effect was also observed by icv α-MSH, suggesting a role for the melanocortin pathway in reducing body temperature.

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2. Materials and methods

2.1. Materials

MTII and alpha-MSH were purchased from Bachem (Torrance, CA, USA). 5′-AMP, 8-cyclopentyl-1,3-dimethylxanthine (CPT), isoproterenol (Iso), and SR49059 (SR) were purchased from Sigma (St. Louis, MO, USA).

2.2. Animals

FVB mice were purchased from the Jax lab and breeding pairs were maintained to generate FVB study subjects. Mice with deletion of all 3 beta adrenergic receptors were on FVB background and provided by Dr. Bradford Lowell of Harvard Medical School. *Mc4r-null* mice were generated as previously described [9,11]. All animals and procedures were approved by the Animal Welfare Committee of the University of Texas Health Science Center at Houston. Mice were housed at 21–22 °C with a 12 h light/12 h dark cycle with standard mouse chow (Teklad F6 Rodent Diet 8664, 4.05 kcal/g, 3.3 kcal/g metabolizable energy, 12.5% kcal from fat, Harlan Teklad, Madison, WI) and water provided ad libitum.

2.3. Energy expenditure and food intake measurements

Energy expenditure was measured by oxygen consumption using indirect calorimetry. Individually housed mice maintained on chow diet at 7–8 weeks old were placed at room temperature (22–24 °C) in chambers of a Comprehensive Lab Animal Monitoring System (CLAMS, Columbus Instruments, Columbus, OH). Daily food intake was measured for 4 h during the dark period in mice, which have been individually housed for at least 1 week.

2.4. Body temperature and movement measurement

As previously reported [12], precalibrated sensitive transmitters (PDT-4000 G2 E-Mitter sensors, Respironics Inc., Murrysville, PA, USA) were used for performing telemetric measurements. For measuring body temperature and locomotor activity, mice were anesthetized with ketamine/xylazine and then implanted E-Mitters in the space under the skin between the scapulae. For core body temperature monitoring, E-Mitters were placed into the peritoneal cavity. Mice were allowed for 1 week recovery before all data were collected. Signals emitted by the E-Mitter transponders were sensed by a receiver positioned underneath the animal's housing cage and analyzed using VitalView software (Respironics Inc). Locomotor activity counts are recorded as gross motor activity. For all experiments, activity counts and temperature measurements were taken every 1 min. All mice were acclimated for at least three days and then data were collected for 24 h. Multiple series of data at the same collected time point and from the same genotype mice were summed and then averaged to get their mean temperature and movement.

2.5. Blood oxygen content measurement

Mixed arterial and vein blood samples were collected using capillary collection device (ITC, Edison, NJ) from tails of wild-type mice at baseline, 1 h and 7 h after intraperitoneal (i.p.) MTII administration (80 μg/mouse). For measurement of oxygen content, the blood samples were transferred to cuvettes (ITC, Edison, NJ) from the capillary tubes immediately. The oxygen contents were measured using whole blood oximeter (Avoximeter 1000E, ITC, Edison, NJ) [13]. A volume of 50 μl of blood was sufficient to analyze levels of oxygen content for each measurement.

2.6. Statistical analyses

Data sets were presented at mean ± SEM and analyzed for statistical significance using PRISM (GraphPad, San Diego, CA) for two-tailed unpaired Student's *t* tests, or for ANOVA tests using Tukey's multiple comparisons. A *P* value of<0.05 was required for significance.

3. Results

In one of our earlier studies on the role of MC4Rs in energy expenditure regulation (11), we noticed that the MC4R agonist, MTII, used at a routine dose (i.p. 80 μg/animal) for feeding and energy expenditure studies [9,14], induced a rapid reduction in energy expenditure, which lasted around 1 h with the peak at 30 min (Fig. 1A). This reduction was followed by an increase in energy expenditure in wile type mice. Interestingly, the rapid reduction in energy expenditure showed no dependence on the presence of MC4Rs, but the increase did (Fig. 1A). Energy expenditure reduction is normally associated with reduced thermogenesis, which may cause body temperature changes [15,16]. To investigate this, we monitored minute-to-minute body temperature changes using a telemetry system with the E-Mitter implanted in the interscapular cavity. We treated mice on the first day at 6 pm with

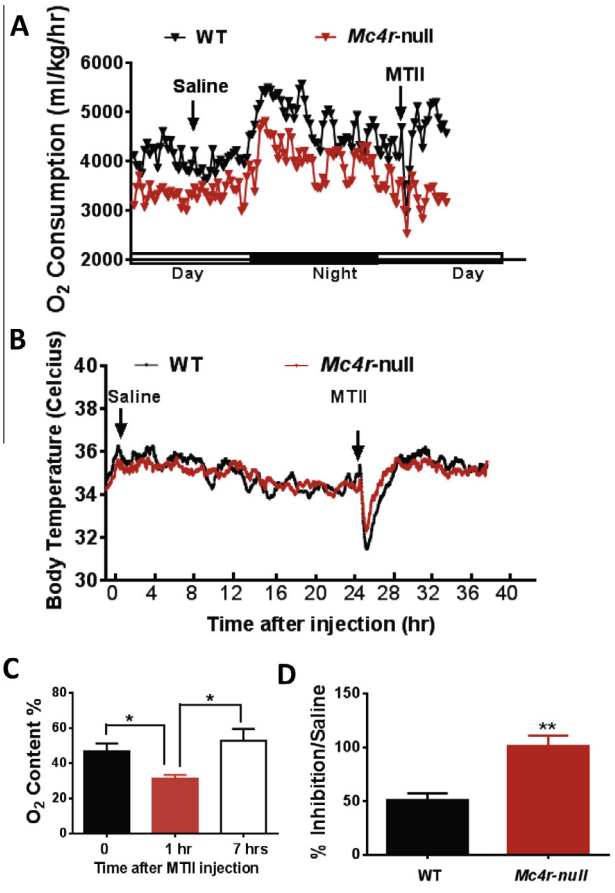

Fig. 1. Profound and rapid reduction in energy expenditure and body temperature by MTII. (A) Effect of MTII (i.p., 200 μg) on O_2 consumption in wild type and *Mc4r-null* mice, compared to that by i.p. saline administered in the previous day. (B) Effect of MTII (i.p., 80 μg) on body temperature in wild type and *Mc4r-null* mice, compared to that by i.p. saline administered in the previous day. (C) Blood O_2 content measured in tail blood taken right before, 1 h and 7 h after MTII administration (i.p., 80 μg). (D) Effect of i.p. MTII at the dose used in (B) and (C) on food intake of wild type control and *Mc4r-null* mice. Data presented as mean ± SEM, $^{*}P < 0.05$, $^{**}P < 0.01$, $n = 4–8$.

saline and at the same time on the second day with MTII (i.p. 80 μg/animal), the dose previously shown to reduce 50% food intake in mice [9]. While saline didnot produce any discernable effects on body temperature, MTII produced a profound and rapid reduction in body temperature (Fig. 1B). Body temperature reduction reached to nadir around 1 h after MTII administration and returned to normal range within 4 h, similarly to the effect on energy expenditure (Fig. 1A), suggesting that reduction in body temperature may be due to reduced energy expenditure. The reduction was profound since it reduced body temperature to below 32 °C at room temperature (Fig. 1B). Interestingly, the reduction in body temperature by MTII was not dependent on the presence of MC4Rs, as a similar reduction in body temperature also occurred in *Mc4r-null* mice by MTII (Fig. 1B). Associated with body temperature reduction, a significant reduction in locomotion was also observed (data not shown).

To explore whether rapid body temperature changes were associated with alterations in blood O_2 content, we measured blood O_2 content in tail blood with mixed arterial and vein blood using an AVOX system. The readings on tail blood were around 50% in wild type mice, as shown in Fig. 1C, prior to MTII administration. Blood O_2 content rapidly dropped to around 30% 1 h after MTII, which returned to control levels 7 h after the MTII administration. The correlation between body temperature reduction and blood O_2 drop suggests that the latter might be the major cause for the rapid body temperature reduction. Since MTII has been widely used in feeding studies [3,5,9,17], we examined whether the rapid body temperature drop could cause secondary effects on feeding. With the same dose used in body temperature studies, MTII had no effects on feeding in *Mc4r-null* mice, but caused 50% reduction in food intake in wild type mice, suggesting that the effect of MTII on feeding is independent of its effects on body temperature (Fig. 1D).

The profound effect of MTII on body temperature triggered us to further examine whether the effect is mediated by brain neurons and whether endogenous melanocortins have a similar effect. We thus administered α-MSH by bolus intracerebroventricular (icv) injection at the amount of 8 μg/2 μl into 6 FVB control mice. Four of them showed obvious and rapid body temperature reduction (Fig. 2A) while the rest two showed no obvious changes in body temperature (data not shown), presumably due to high levels of stress associated with icv injections. This data suggests that the effect on body temperature by i.p. MTII is at least partially mediated by the melanocortin pathway in the brain.

The adrenergic receptors, especially β-adrenergic receptors (β-ARs), play an important role in mediating brain control of energy expenditure [18]. To examine whether β-ARs are involved in mediating the effect on body temperature by MTII, we activated β-ARs with their agonist Isoproterenol (Iso) and used mice lacking all known 3 β-ARs (Beta-less mice [18]). Iso, used at the dose of 1 mg/kg, which is normally used for energy expenditure studies [18], caused no obvious effects on body temperature in wide type or Beta-less mice (data not shown). Surprisingly, Iso, administered together with MTII, largely blunted the effect of MTII on body temperature reduction (Fig. 2B), suggesting that the effect of MTII on body temperature reduction may involve inhibition of β-ARs. To further explore the role of β-ARs, we administered MTII to Beta-less mice. MTII produced a rapid and greater reduction in body temperature in Beta-less mice, compared to control mice (Fig. 2C). The body temperature reduced to nadir at around 26 °C in a room-temperature environment and required approximate 2 times longer duration to return to normal range, compared to that in control mice (Fig. 2C). These data further confirm that β-ARs are activated to minimize the degree of body temperature reduction by MTII and that β-ARs are required for body temperature recovery.

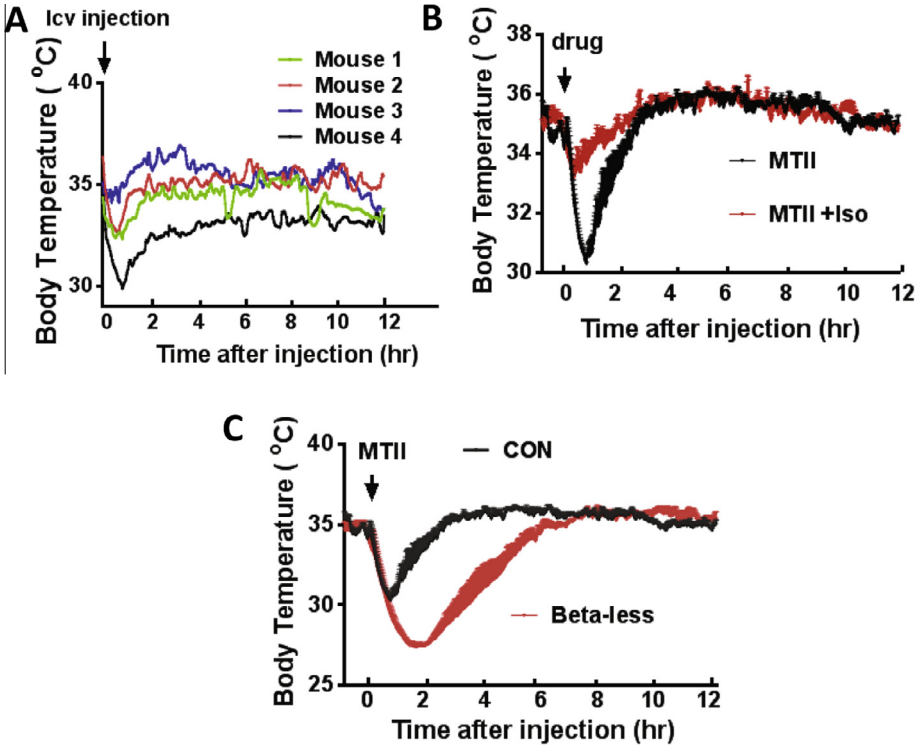

Fig. 2. Effect of central α-MSH on body temperature and role of β-adrenergic receptors. (A) Traces showing body temperature changes following icv injections of α-MSH in four mice tested. (B) Effect of i.p. Isoproterenol (Iso) on body temperature changes in control and mice lacking all 3 β-adrenergic receptors (Beta-less mice). (C) Effect of concurrent administration of MTII and Iso on body temperature changes, compared to that of MTII alone in control (CON) mice. (D) Effect of MTII on body temperature changes in CON and Beta-less mice. Data presented as mean ± SEM except for (A). $n = 4–8$.

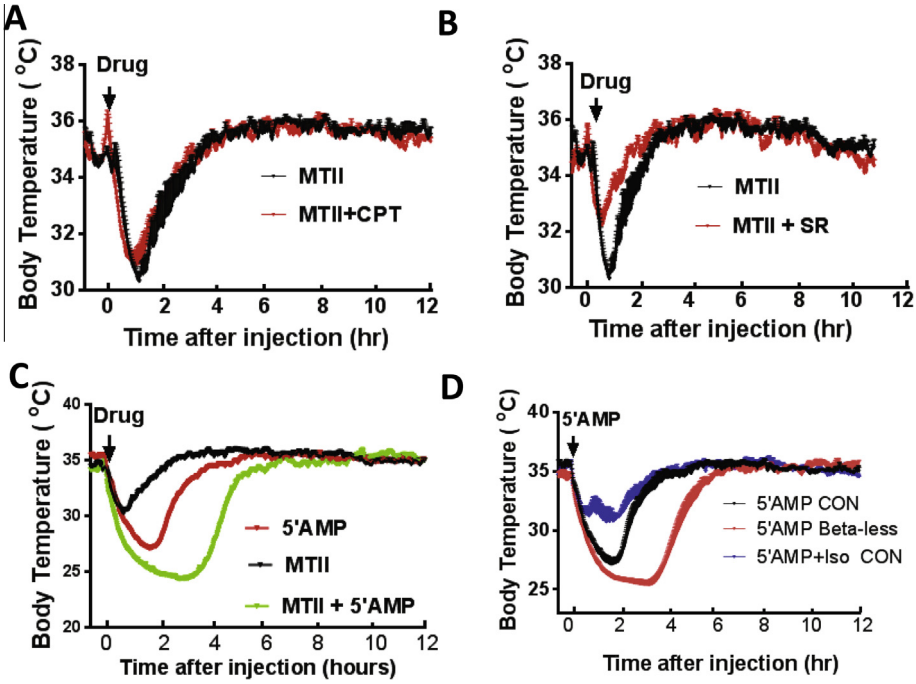

Fig. 3. MTII-mediated hypothermia involved with other body temperature-regulating pathways. (A) Effect of concurrent administration of MTII and CPT on body temperature, compared to MTII alone in control (CON) mice. (B) Effect of concurrent administration of MTII and SR on body temperature, compared to MTII alone in CON mice. (C) Effect of concurrent administration of MTII and 5′AMP on body temperature, compared to MTII alone in CON mice. (D) Effect of 5′AMP on body temperature in CON and Beta-less mice, compared to that of concurrent administration of 5′AMP and Iso. Data presented as mean ± SEM, $n = 4–8$.

Recent reports suggest that both adenosine receptors A1 in the brain and the vasopressin receptor, AVP V1a, regulate body temperature. Activation of A1 receptors and AVP V1a receptors caused rapid body temperature reduction [19,20]. To examine the possibility whether the effect of MTII on body temperature involves A1 or AVP V1a receptors, we concurrently administered MTII with A1 receptor antagonist CPT at the dose of 1 mg/kg or AVP V1a receptor antagonist SR at the dose of 1 mg/kg. Both doses were described previously to sufficiently block A1 and AVP V1a receptors for body temperature regulation [19,20]. Interestingly, while CPT produced no discernable effects on the effect of MTT on body temperature reduction (Fig. 3A), SR significantly blunted the effect (Fig. 3B), suggesting an involvement of activation of AVP V1a receptors in the effect of MTII in body temperature reduction.

The effect of MTII on body temperature is reminiscent of a previous finding on body temperature regulation by 5′AMP, which also induces a rapid body temperature drop [16]. We compared the effects on body temperature between 5′AMP and MTII. Using the doses of 0.5 mg/g (5′AMP) and of 80 μg/animal (MTII), we found that 5′AMP produced more pronounced effects on body temperature drop and longer duration with hypothermia, compared to MTII (Fig. 3C). Surprisingly, the combination of MTII and 5′AMP produced a much greater effect on body temperature reduction, and the drop reached to below 25 °C with 6-h duration for the recovery phase (Fig. 3C). Since MTII used at 80 μg/animal reaches the maximal effect on body temperature reduction in mice [21], this result suggests that MTII and 5′AMP showed a synergistic effect, suggesting a common pathway responsible for the two drugs. To further examine whether β-ARs are also involved in 5′AMP induced hypothermia, we used Beta-less mice. Interestingly, Iso also significantly blunted the hypothermia effect of 5′AMP and 5′AMP also produced a far more pronounced hypothermic effect in Beta-less mice (Fig. 3D), suggesting that β-ARs are also involved in the body temperature regulation by 5′AMP.

4. Discussion

Our results showed an unexpected but profound effect of MTII on energy expenditure reduction, which was followed by a prolonged increase in energy expenditure. Consistent with the effect on energy expenditure reduction, MTII produced a rapid reduction in body temperature. Importantly, central injections of the endogenous agonist, α-MSH, produced rapid body temperature reduction, although at a smaller degree, suggesting that body temperature-reducing effects at least partially mediated by endogenous melanocortin receptors in the brain. However, it appears that the effect is independent of MC4Rs, since MTII also produced similar reduction in both body temperature and energy expenditure in *Mc4r-null* mice, compared to controls. A recent study further showed that the effect of MTII (also other melanocortin receptor agonists) on body temperature reduction was independent of any known melanocortin receptors [21] suggesting that there might be un-identified receptors in the brain that mediate MTII and α-MSH action on body temperature reduction. The notion of body temperature-reducing effects by the melanocortin pathway is supported by previous findings that the melanocortin receptor agonists suppress fever [22–24]. Since brain neural pathways, for example, the leptin neural pathway, have been demonstrated to modulate energy expenditure and therefore body temperature and are involved in episodes of rapid changes in body temperature regulation [12,25], it would be interesting to identify the receptor(s) that mediate the action of the endogenous melanocortin α-MSH and its analogues on energy expenditure/body temperature regulation.

Our data showed that the body temperature reduction by MTII was associated with reduced blood O_2 content, suggesting that reduced blood O_2 delivery might be responsible for the rapidly reduced energy expenditure and therefore rapid body temperature reduction. Reduced O_2 delivery may be due to reduced heart rate

and blood pressure associated with MTII administration (data not shown), which was also demonstrated in the recent study [21]. Reduced O_2 delivery is also associated with the effect of activating brain serotonin neurons on rapid body temperature/energy expenditure reduction [26] and with the effect of 5′AMP in inducing rapid body temperature/energy expenditure reduction [16]. Thus, controlling blood O_2 delivery may be a powerful way to regulate energy expenditure/body temperature.

The effect of both MTII and 5′AMP on body temperature reduction was largely attenuated by β-AR activation and was greatly potentiated in Beta-less mice, suggesting a common β-AR pathway that mediates the effect of MTII and 5′AMP. Since β-ARs are critical to regulate energy expenditure and maintain normal body temperature in cold environment [18], it is conceivable that β-AR-mediated energy expenditure may be disrupted by MTII and 5′AMP, which may be one of the underlying reasons for their effects on rapid reduction in energy expenditure/body temperature. The synergistic effect of MTII and 5′AMP argues that the two reagents act on distinct pathways that converge on the energy expenditure pathway involving β-ARs. Further studies are warranted to investigate how β-ARs affect blood O_2 supply for energy expenditure/body temperature regulation.

Our current observation on the effect of MTII in inducing rapid body temperature reduction seems at odds with the previous reports showing hyperthermia effects by MTII in both brain and periphery [3,9,27–29]. Possible underlying reasons might be due to different doses used or that more attentions were paid to the prolonged hyperthermia effects of MTII in the previous studies. The dose of MTII used in this study was routinely employed in the literature for energy expenditure and feeding regulation [9,14]. Thus, caution must be exercised in using MTII to study the MC4R action on energy metabolism, especially on energy expenditure. Since the prolonged effect on increasing energy expenditure of MTII was specific to MC4R action, focus on the effect during the prolonged phase should be used for MC4R action on energy expenditure. Interestingly, although MTII produced profound effects on body temperature, it didnot alter its MC4R-dependent action on feeding, at least during the 4-h observed period. Thus, MTII still appears to be a good MC4R-dependent feeding modulator. In summary, our current study provides compelling evidence that the melanocortin pathway is capable of rapidly reducing body temperature/energy expenditure, that a commonly shared β-AR pathway is involved in the rapid reduction of body temperature by both MTII and previously shown 5′AMP, and that reduced blood O_2 delivery may be responsible for the rapid reduction in energy expenditure/body temperature by MTII. Given the immerse interest in deep hypothermia for tissue preservation in clinics [15], our current findings are significant in identifying a previously unappreciated role of the melanocortin pathway in producing hypothermia and may lead to further elucidation of the mechanism underlying the rapid and deep hypothermia.

Conflict of interest

None.

Acknowledgments

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