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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

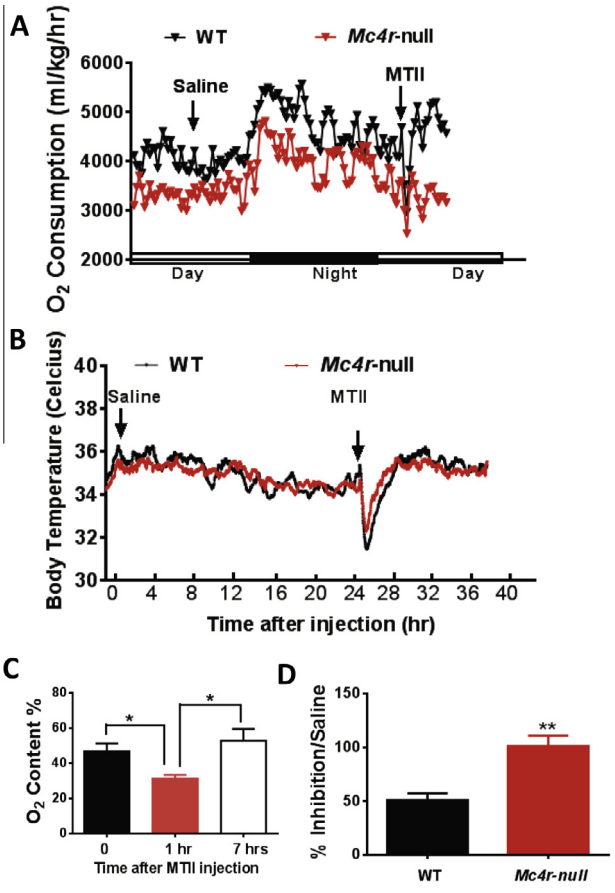

Fig. 1. Profound and rapid reduction in energy expenditure and body temperature by MTII. (A) Effect of MTII (i.p., 200 μg) on O_2 consumption in wild type and *Mc4r-null* mice, compared to that by i.p. saline administered in the previous day. (B) Effect of MTII (i.p., 80 μg) on body temperature in wild 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, $^{*}P < 0.05$, $^{**}P < 0.01$, $n = 4–8$.