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UCP3 and thyroid hormone involvement in methamphetamine-induced hyperthermia

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Abstract

Here, we determined the extent of hypothalamic-pituitary-thyroid (HPT) axis and uncoupling protein-3 (UCP3) involvement in methamphetamine (METH)-induced hyperthermia. Sprague–Dawley rats treated with METH (40 mg/kg, s.c.) responded with a hyperthermic response that peaked 1 h post-treatment and was sustained through 2 h. After METH treatment, thyroparathyroidectomized (TX) animals developed hypothermia that was sustained for the 3 h monitoring period. In TX animals supplemented for 5 days with levothyroxine (100 μg/kg, s.c.), METH-induced hypothermia was eliminated and the hyperthermic response was restored. Thyroid hormone levels (T3 and T4), measured in euthyroid animals 1 h after METH, remained unchanged. As seen in rats, 1 h post-METH (20 mg/kg, i.p.) treatment, wild-type (WT) mice developed profound hyperthermia that was sustained for 2 h. In marked contrast, UCP3−/− animals developed a markedly blunted hyperthermic response at 1 h compared to WT animals. Furthermore, UCP3−/− mice could not sustain this slight elevation in temperature. Two hours post-METH treatment, UCP3−/− animal temperature returned to baseline temperatures. UCP3−/− mice were also completely protected against the lethal effects of METH, whereas 40% of WT mice succumbed to the hyperthermia. These findings suggest that thyroid hormone plays a permissive role in the thermogenic effects induced by METH. Furthermore, the findings indicate that UCP3 plays a major role in the development and maintenance of the hyperthermia induced by METH. The relationship of these results to the hyperthermia induced by 3,4-methylenedioxymethamphetamine (MDMA) is also discussed.

Keywords: Methamphetamine; Hyperthermia; Uncoupling proteins; Thyroid hormones; 3,4-Methylenedioxymethamphetamine; Thermogenesis

1. Introduction

The abuse of methamphetamine (METH, Speed, Ice) has reached epidemic proportions in many parts of the world because of its relative abundance and simplicity to manufacture [1]. One of the most acute and life-threatening toxicological responses to METH is hyperthermia leading to rhabdomyolysis, intravascular coagulation, multiorgan failure, and in some rare cases even death [2–4]. Other sympathomimetic agents, such as ephedrine, amphetamine, 3,4-methylenedioxymethamphetamine (MDMA, ecstasy) and cocaine are also commonly associated with hyperthermia [4]. To date, most studies have focused on the central triggers of thermogenesis induced by METH, but not on the peripheral mediators.

Despite their obvious importance, the actual molecular generators of heat production during severe hyperthermic syndromes are only beginning to be examined. For example, bacteria- or other pathogen-induced fevers can be life-threatening in susceptible individuals, yet we know of no molecule that is invoked to produce heat during fever in humans. Likewise, thermogenesis is associated with diseases such as pheochromocytoma, thyroid storm, and iatrogenic thermogenic syndromes such as strychnine poisoning, serotonin syndrome, neuroleptic malignant syndrome, and malignant hyperthermia, all of which can also lead to rhabdomyolysis, intravascular coagulation, multiorgan failure, and death [5].

Abbreviations: METH, methamphetamine; MDMA, 3,4-methylenedioxymethamphetamine; SNS, sympathetic nervous system; HPT, hypothalamic-pituitary-thyroid; UCP, uncoupling proteins; RyR, ryanodine receptor; WT, wild-type; TX, thyroparathyroidectomized

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To date in mammals, only three molecules appear to produce rapid or inducible heat production, and each one biochemically mediates a futile cycling of ions across an electrochemical gradient. (1) Malignant hyperthermia occurs in response to general anesthetic agents in susceptible individuals bearing mutations in the skeletal muscle protein ryanodine receptor (RyR), which is involved in calcium cycling in the sarcomere [6]. (2) Uncoupling protein-1 (UCP1, thermogenin) is a fatty-acid activated mitochondrial protein highly expressed in brown fat [7]. However, adult humans have very little or no brown fat, and thus there is active interest in determining the molecular origin of inducible or adaptive thermogenesis in man. (3) We recently demonstrated that skeletal muscle UCP3, which was previously not thought to be thermogenic, largely mediates increases in skeletal muscle and core temperatures in mice evoked by MDMA [8].

Although pharmacologically and structurally similar, METH and MDMA produce differing degrees of effects on biogenic amine release and as a consequence also display distinct patterns of behavioral responses [9,10]. Furthermore, the neurotoxicity of METH exhibits a pattern of striatal dopaminergic toxicity in the rodents and primates [11], whereas MDMA neurotoxicity appears largely to cause serotonergic toxicity in mammals, except in mice where dopaminergic neurotoxicity appears to be more prevalent [12].

Central control of thermoregulation occurs within the preoptic/anterior hypothalamus, and recent studies on the cellular mechanisms involved in the thermogenesis induced by MDMA indicate a pivotal role for the hypothalamic-pituitary-thyroid (HPT) axis [13], sympathetic nervous system (SNS) and skeletal muscle UCP3 [8]. MDMA mediated activation of the SNS is speculated to increase α_1- and β_3-adrenoreceptor regulation of UCP3 activity [13]. The pharmacologic and toxicologic differences between the METH and MDMA suggest that their thermogenic mechanisms may not be identical. In order to better understand METH-induced hyperthermia and its peripheral mechanisms, we examined the role of the HPT axis and uncoupling protein-3 (UCP3) in the peripheral hyperthermia induced by METH.

2. Materials and methods

All procedures within this study were carried out in accordance with protocols approved by the Ohio Northern University Animal Care and Use Committee.

2.1. Animals

Sham, thyroparathyroidectomized (TX), or euthyroid male Sprague–Dawley rats were used. Animals were obtained from Harlan (Indianapolis, IN) and weighed 175–200 g (7–8 weeks of age) at the time of delivery. All animals were housed three per cage (size: 21.0 cm × 41.9 cm × 20.3 cm), provided ad libitum access to food and drinking water and maintained on a 12:12 h lightdark cycle at a room temperature of 22–24 °C. TX animals were given 2–4% calcium lactate as their only fluid source throughout the experiment for calcium supplementation. The method of thyroparathyroidectomy has been previously described [13].

The characterization of UCP3−/− and wild-type (WT) mice has been reported previously [14] and these animals were a generous gift of Dr. Marc Reitman. The original UCP3−/− mice were backcrossed ten times into a C57BL/6J background and wild-type C57BL/6J littermate mice were used as controls. All mice were maintained on a diet of 9% fat. All mice (weighing 20–25 g; 7–8 weeks of age) were group housed five per cage (size: 17.2 cm × 27.9 cm × 12.7 cm) with microisolator lids. All studies were begun at 08:00 h and performed in an ambient temperature of 23.9 ± 0.05 °C in a room with relative humidity of approximately 22%.

2.2. The effects of METH on hyperthermia

Twelve sham and eighteen TX Sprague–Dawley rats were divided into five groups based on designated treatments: sham-saline, sham-METH (40 mg/kg, s.c.), TX-saline, TX-METH, TX-levothyroxine (100 μg/kg, s.c. × 5 days)-METH. In TX animals, the thyroid and parathyroid glands were removed 7-days prior to METH administration. Rectal temperatures were measured in all animals using a Physiotemp Thermalert TH-8 thermocouple (Physitemp Instruments, Clifton, NJ) attached to a RET-2 (rat) or RET-3 (mice) rectal probe coated with white petrolatum prior to insertion. RET-2 probes were inserted 5 cm and RET-3 probes 2 cm into the rectum, where they remained for at least thirty seconds, until a stable temperature was obtained. Skeletal muscle temperatures were measured in the biceps femoris using a Type MT-23/3 (rat) or MT-29/2 (mice) hypodermic needle microprobe attached to the thermocouple. Rectal and skeletal muscle temperatures were determined at the time of METH or saline (1 mL/kg, s.c.) injection as the baseline temperature and at time points 1, 2, and/or 3 h post-injection. Because of the high degree of mortality (six out of six sham animals) with a 40 mg/kg dose of METH in the rat model, UCP3−/− and WT mice were subsequently treated with a 20 mg/kg dose of METH.

2.3. The effects of METH on T3 and T4 levels

Twelve Sprague–Dawley rats were randomly divided into two treatment groups. Rectal temperatures were monitored for 2 h prior to treatment, at the time of METH (40 mg/kg, s.c.) or saline (1 mL/kg, s.c.) administration and at 1 h post-injection. At the time of the last temperature

reading, 1 mL of trunk blood was obtained. Blood samples were allowed to clot at room temperature for 30 min and centrifuged at 10,000 × *g* at 4 °C for 15 min. Serum supernatants were stored in eppendorf tubes (−80 °C) until analysis.

2.4. RIA

Total T3 and T4 levels were measured in 25 and 100 μL of serum, respectively, in duplicate determinations by radioimmunoassay (ICN Diagnostics, Cosa Mesa, CA).

2.5. Drugs and chemicals

METH was purchased from Sigma–Aldrich (St. Louis, MO). Levothyroxine was obtained from Amerisource Bergen (Toledo, OH).

2.6. Statistical analysis

Rectal temperatures were compared within each treatment group with an ANOVA and a Dunnett's post-hoc test to determine significant differences from baseline levels. Between treatment groups, rectal temperatures were compared by ANOVA with a Student–Newman–Kuels post-hoc test. Statistical significance was set a priori at $P \leq 0.05$.

3. Results

3.1. The effects of METH on rat rectal and skeletal muscle temperature

Alone, METH induced a statistically significant rise in both rectal and skeletal muscle temperatures in sham animals ($P < 0.001$). In contrast, METH-induced an approximate 2 °C hypothermic response ($P < 0.001$) in TX animals compared to baseline rectal and skeletal muscle temperatures. Furthermore, in TX animals, 5 days of supplementation with levothyroxine reversed the hypothermic effects of thyroparathyroidectomy and restored the hyperthermic response ($P < 0.05$) to METH. The baseline temperatures for all TX animals were significantly lower than the baseline readings for the sham treatment groups (37.6 ± 0.25 °C; $P < 0.05$). All sham rats administered METH died before the 3 h temperature measurements as a result of extreme hyperthermia (Fig. 1) and all TX animals supplemented with levothyroxine died within 24 h of treatment whereas none of the TX-METH animals died.

3.2. The effects of METH on T3 and T4 levels

There was no significant difference in either T3 or T4 levels 1 h after METH administration. T3 in saline control animals was 68.2 ±2.1 ng/dL and after METH was 67.0 ±3.1 ng/dL. Similar to T3, T4 in controls was 3.7 ± 0.4 μg/dL compared to 4.0 ± 0.3 μg/dL 1 h after METH (data not shown).

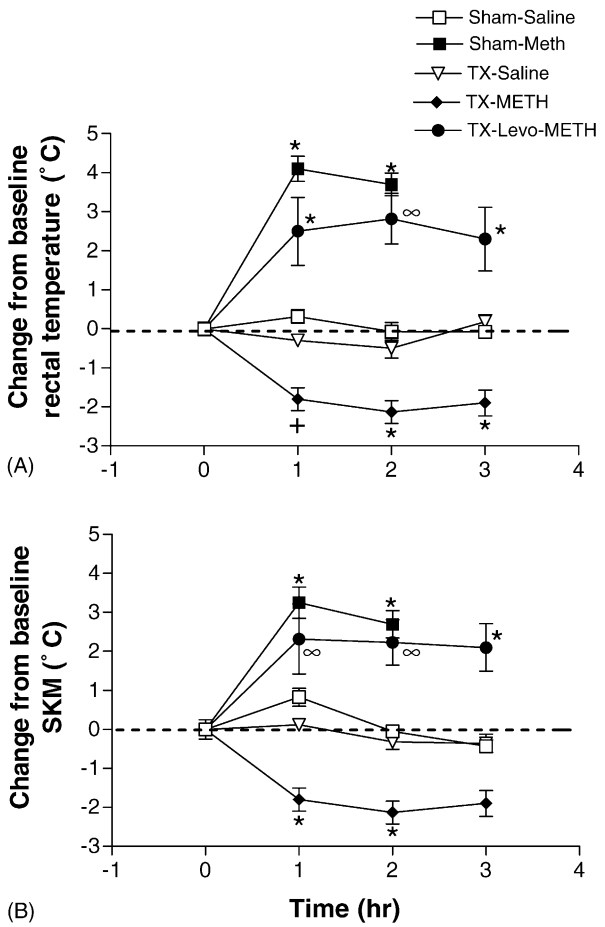

Fig. 1. Effects of levothyroxine (100 μg/kg s.c. × 5 days) supplementation in TX animals on METH (40 mg/kg s.c.) induced hyperthermia. Each value is the mean ± S.E.M. ($n = 5$–6) for rectal (A) and skeletal muscle (SKM) (B) temperatures: (∗) significantly different from all other treatment groups ($P < 0.05$); (+) significantly different from sham-METH and TX-METH-Levo treatment groups ($P < 0.001$); (∞) indicates significantly different from all other treatment groups except sham-METH ($P = 0.001$).

3.3. The effects of METH on WT and UCP3V-mice rectal and skeletal muscle temperatures

UCP3−/− mice treated with METH (20 mg/kg, i.p.) showed a significantly blunted rise in rectal and skeletal muscle temperatures compared to their WT controls. One hour post-METH treatment, WT animals displayed a 2.22 ± 0.26 °C rise in rectal temperature compared to a 1.14 ± 0.18 °C in the UCP3−/− animals. Baseline rectal temperature were 38.1 ± 0.1 °C for WT and 38.9 ± 0.1 °C for UCP3−/−. Two hours post-treatment, the differences between the WT and UCP3−/−animals was most dramatic, with the WT mice sustaining a 2.02 ± 0.28 °C elevation over baseline in rectal and a 1.8 ± 0.3 °C

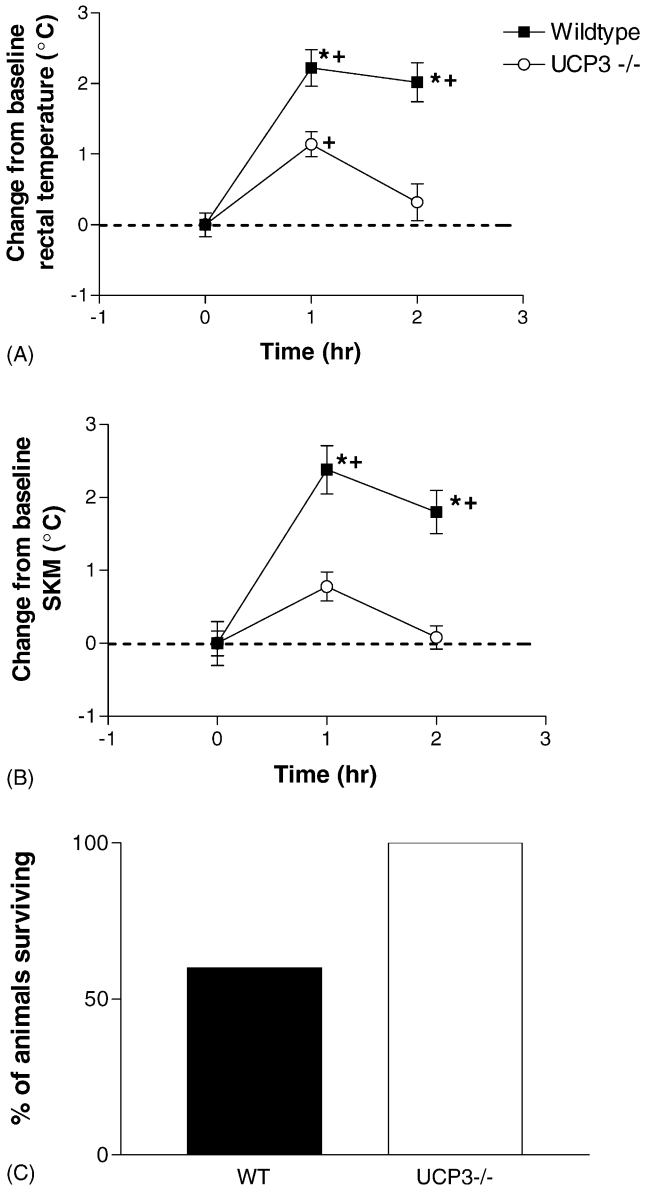

Fig. 2. UCP3 −/− mice are protected from the hyperthermic effects of methamphetamine (20 mg/kg, i.p.). Change for baseline (A) rectal or (B) skeletal muscle (SKM) temperature was assessed for 2 h post-treatment, indicates significantly different from UCP3 −/− group ($P < 0.001$): (+) indicates significantly different from that treatment groups corresponding baseline ($P < 0.01$); (∞) indicates significantly different from that treatment groups corresponding baseline ($P = 0.05$). Each value is the mean ± S.E.M. ($n = 5$). (C) Percent of animals surviving 24 h post-treatment was also monitored.

elevation in skeletal muscle temperatures compared to a 0.32 ± 0.26 °C elevation in rectal and a 0.08 ± 0.16 °C elevation in skeletal muscle temperatures in the UCP3−/− animals. Baseline skeletal muscle temperatures were 37.8 ± 0.2 °C for WT and 38.5 ± 0.1 °C for UCP3−/− animals. UCP3−/− were also more resistant to the lethal effects of METH, where none of the UCP3−/− animals died following METH administration, compared to 40% lethality in the WT (Fig. 2).

4. Discussion

In 2002, amphetamines, methamphetamines and MDMA represented the fifth, sixth, and seventh most commonly mentioned drugs of abuse associated with emergency department visits, with only alcohol, cocaine, heroin and marijuana being mentioned more frequently [15]. All three stimulants present with a similar clinical syndrome characterized by hyperadrenergic activity, sometimes resulting in hyperthermia and rhabdomyolysis. Of the many clinical effects from stimulants, hyperthermia is most life-threatening. Elevated temperatures directly correlate with mortality after MDMA [16] and are commonly reported in METH and amphetamine related deaths [2,3]. Despite the importance of hyperthermia in deaths associated with stimulants, there is still a significant lack in our understanding of how this hyperthermia occurs.

Previous studies have shown the importance of both the thyroid gland [13] and UCP3 [8] in MDMA mediated hyperthermia. Our current study demonstrates an endocrine-molecular pathway in METH-mediated hyperthermia. Surgical removal of the thyroid gland not only eliminates the hyperthermic response typically induced by METH in rodents, but actually results in a significant hypothermic response and increased survival. Subchronic replacement with thyroid hormones in TX rats restored the typical hyperthermic response, indicating a dependence upon thyroid status for amphetamine-induced thermoregulatory derangements. Even decades ago, it was appreciated that hyperthyroid animals were remarkably more susceptible to the thermogenic and lethal effects of amphetamines [17], while hypothyroidism protected animals from amphetamine-induced hyperthermia [18]. In contrast to our previous observations with MDMA [13], METH did not induce a change in T4 levels 1 h post-treatment. The lack of an acute change by METH in the present studies suggests that T3 and T4 play a permissive role in the hyperthermia induced by METH, in other words, thyroid hormones must be present for METH-mediated hyperthermia, but need not be acutely increased. These findings correspond with work by Ribeiro et al. [19] showing the permissive role for thyroid hormones in norepinephrine mediated hyperthermia.

Thyroid hormones regulate UCP expression and activity. Specifically, hypothyroid animals have lower amounts of skeletal muscle UCP3 and hyperthyroid animals have significantly greater amounts of UCP3 compared to euthyroid animals [7,20–22]. Supplementing hypothyroid animals with T3 (100 μg/kg, i.p. × 4 days) returns UCP3 expression in skeletal muscle although this effect was blunted in brown adipose tissue [7]. Using both rat and UCP3−/− mice, Cunningham et al. [22] illustrated that euthyroid rats made hyperthyroid had a 2.3-fold increase in isolated skeletal muscle UCP3 and that UCP3−/− mice were indeed deficient in UCP3 protein levels [22]. Based upon these results, we would predict that in our TX animals

supplemented with levothyroxine for 5 days, UCP3 expression would return to near normal, and as a consequence, METH-induced hyperthermia would be restored.

The results of our study clearly show that hyperthermia induced by METH is largely dependent upon UCP3. UCP mediates thermogenesis by uncoupling proton transfer from ATP synthesis, essentially ''wasting'' the electrochemical gradient to produce heat [7]. In the present study, UCP3−/− mice were less responsive to the hyperthermic and lethal effects of METH, as compared to their WT littermates, of which two of five succumbed to hyperthermia following METH administration. The involvement of UCP3 in METH lethality is similar to that seen following MDMA in UCP3−/− mice [8]. While the role of UCP1 in non-shivering thermogenesis is well described, the relative lack of this protein in adult humans casts much doubt on its significance in hyperthermic syndromes. The more recent discovery of the UCP homologues UCP2 and UCP3, along with their distribution in tissues such as liver (UCP2) and skeletal muscle (UCP3) makes tempting the speculation that they participate in human disease and toxicity states. Our finding that both METH and MDMA depend largely upon UCP3 to produce hyperthermia and death, supports the notion that derangements in the levels or activity of UCP3 in humans may contribute to varying degrees of susceptibility to thermogenic crises. Drugs that block UCP activity may therefore represent novel treatment options for sympathomimetic-mediated hyperthermia. In conclusion, the results of previous findings with MDMA [8,13] and the present findings with METH suggest that the hyperthermia induced by substituted amphetamines is highly dependent upon thyroid function and UCP3 activity.

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