Hypothalamic-Pituitary-Thyroid Axis and Sympathetic Nervous System Involvement in Hyperthermia Induced by 3,4-Methylenedioxymethamphetamine (Ecstasy)

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ABSTRACT
An acute and potentially life-threatening complication associated with the recreational use of the 3,4-methylenedioxymethamphetamine (MDMA, Ecstasy) is hyperthermia. In the present study, Sprague-Dawley rats treated with MDMA (40 mg/kg s.c.) responded with a significant increase (maximal at 1 h) in rectal and skeletal muscle temperatures that lasted for at least 3 h post-treatment. Hypophysectomized (HYPO) and thyroparathyroidectomized (TX) animals treated with MDMA (40 mg/kg s.c.) did not become hyperthermic and in fact displayed a significant hypothermia. The HYPO and TX animals were also resistant to the serotonergic neurotoxic effects of MDMA assessed by serotonin measurements 4 to 7 days later in the striatum and hippocampus. MDMA (40 mg/kg s.c.) induced a significant increase in thyroxine levels 1 h post-treatment. Thyroid hormone replacement in TX animals returned the hyperthermic response seen after MDMA. Prazosin, an α1-antagonist (0.2 mg/kg i.p.), administered 30 min before MDMA significantly attenuated the MDMA-induced increase in rectal temperature, but had no effect on skeletal muscle temperature. Cyanopindolol, a β2-antagonist (4 mg/kg s.c.), administered 30 min before MDMA (40 mg/kg s.c.) significantly attenuated the increase in skeletal muscle temperature, but had no effect on the rise in rectal temperature. The combination of prazosin and cyanopindolol resulted in an abolishment of MDMA-induced hyperthermia. The mechanisms of thermogenesis induced by MDMA seem to result from an interaction between the hypothalamic-pituitary-thyroid axis and the sympathetic nervous system, wherein mechanisms leading to core and skeletal muscle hyperthermia after MDMA exposure seem to be differentially regulated by α1- and β2-adrenergic receptors.

The substituted amphetamine 3,4-methylenedioxymethamphetamine (MDMA, ecstasy) is commonly associated with an increase in body temperature in both humans (Dar and McBrien, 1996; Mallick and Bodenham, 1997) and rodents (Gordon et al., 1991). Because of its association with weekends and rave parties, emergency room personnel often refer to severe forms of this hyperthermia as “Saturday Night Fever” (Williams et al., 1998), which can be associated with rhabdomyolysis, multiorgan failure, and death (Walubo and Seger, 1999). Although deaths from overdose remain rare, the prevalence and especially hospitalizations resulting from MDMA exposure have dramatically increased from 250 hospitalizations in 1994 to over 2850 in 1999 (Drug Abuse Warning Network, 2000). Much evidence from rodent and nonhuman primate studies suggests that MDMA also induces long-term serotonergic neurotoxicity that seems to be ostensibly linked to hyperthermia (Broening et al., 1995; Farfel and Seiden, 1995; Malberg et al., 1996). Although the importance of their elucidation cannot be overstated, the fundamental biological mechanisms involved in heat production and progression to hyperthermia after MDMA exposure are unknown. Furthermore, we do not understand clearly the associations between hyperthermia and many of the pathological changes induced by MDMA.

Gordon et al. (1991) proposed that MDMA induces a dysfunction in central nervous system thermoregulatory mechanisms that are influenced by ambient temperature. Several laboratories have shown that when MDMA is given to rats in a 24°C or greater environment, hyperthermia results (Schmidt et al., 1990; Gordon et al., 1991). If the ambient temperature is lowered to 10°C, however, a hypothermic response occurs (Gordon et al., 1991). The set point for either a hyperthermic or hypothermic response seems to be above or below 20–22°C (Gordon et al., 1991; Malberg and Seiden, 1998) with most neurotoxicity studies being conducted at 23–24°C.

In addition to evidence suggesting that MDMA acutely...
disturbs central nervous system thermoregulatory functions, MDMA-induced activation of the sympathetic nervous system (SNS) and subsequent alterations in vascular hemodynamics may also play an important role in heat production and redistribution. Peripheral heat production in organs such as brown and white fat and skeletal muscle is regulated in part by norepinephrine (Bianco et al., 1988; Rubio et al., 1995a,b). Recently, Pedersen and Blessing (2001) showed that MDMA induces cutaneous vasoconstriction and that this cutaneous restriction in blood flow contributes to the increase in core body temperature seen after treatment with MDMA. These authors also showed that sympathectomy only partially attenuated the hyperthermic response seen after MDMA. Recently, Fernandez et al. (2002) showed that ganglionic blockade only partially attenuated the hyperthermic effect induced by MDMA. Taken together, these studies suggest that the hyperthermic response to MDMA involves more than merely the activation of the SNS and changes in regional blood flow.

Thyroid hormone is the primary endocrine regulator of metabolism and thermogenesis. Surprisingly, there seem to be no studies that have directly examined the role of thyroid hormone in the hyperthermic response to MDMA. Three lines of evidence suggest that thyroid hormone may be linked to the hyperthermic response to MDMA. 1) Fekete et al. (2000) observed that amphetamines induce the cocaine- and amphetamine-regulated transcript in the hypothalamic paraventricular nucleus, resulting in an increased biosynthesis of thyrotropin-releasing hormone. This study predicts that MDMA may also increase thyroid hormone levels. 2) At the cellular level, thyroid hormone seems to play both a permissive and synergistic role in norepinephrine- or SNS-mediated thermogenesis (Bianco et al., 1988; Rubio et al., 1995a,b). According to recent evidence, these effects may be mediated by a family of mitochondrial uncoupling proteins (UCP), members of which mediate nonshivering thermogenesis (Argyropoulos and Harper, 2002). 3) The synergism between thyroid hormone and norepinephrine-dependent mechanisms of UCP (Gong et al., 1997), seems to be mediated specifically by the thyroid hormone receptor and α1- and β2-adrenergic receptors (Silva, 1995).

Because the role, if any, that thyroid hormone plays in MDMA-induced hyperthermia is not known, we examined the role of and the interactions between the SNS and the hypothalamic-pituitary-thyroid (HPT) axis in the development of the hyperthermia induced by MDMA. We hypothesized that MDMA administration would induce an increase in thyroid hormone levels, ultimately facilitating norepinephrine-mediated thermogenesis. Furthermore, we predicted, as evidence would suggest, that acute hyperthermia plays a prominent role in MDMA-induced chronic neurotoxicity.

**Materials and Methods**

The present study was carried out in accordance with protocols approved by the Ohio Northern University Animal Care and Use Committee.

**Animals.** Sham, hypophysectomized (HYPO), and thyroparathyroidectomized (TX) adult male Sprague-Dawley rats (weighing 175–200 g) were obtained from Harlan (Indianapolis, IN). All animals were housed in groups of three and given ad libitum access to food and drinking water. Housing conditions were maintained at a constant temperature of 23°C and a 12:12-h light/dark cycle. For hypophysectomy, anesthetized animals were placed in a ventral recumbency in a Hoffman-Reiter hypophysectomy instrument. A 19-gauge needle was inserted through the hollow right ear bar. The needle was pushed through the bone with the bevel side down until the needle stopped made contact with the ear bar. The needle was rotated in a semicircle two or three times. The needle was then rotated so the bevel pointed downward and the pituitary was slowly aspirated into the water-filled syringe. The pituitary was then examined to ensure complete removal. Once confirmed, HYPO animals were given 5% sucrose solution as a drinking source for 5 days postsurgery. For thyroparathyroidectomy, anesthetized animals had the ventral cervical area shaved and swabbed with surgical scrub. A 1- to 1.5-cm midventral incision was made from just caudal to the pharynx to the cranial edge of the pectoral muscle cutting through the underlying fat until the sternohyoid muscle was exposed. The trachea was exposed and the thyroid gland located. The isthmus was then held and the thyroid gland was separated from the trachea. TX animals were given 2 g of 4% calcium lactate solution as a drinking source for the duration of the experiment. HYPO animals were administered MDMA (40 mg/kg s.c.) or saline 72 h postsurgery. TX and their corresponding shams were treated with MDMA (40 mg/kg s.c.) or saline 1-week postsurgery.

**Drugs and Chemicals.** Cyanopindolol, a β1-receptor antagonist, was purchased from Tocris Cookson, Inc. (Ellisville, MO). MDMA was generously donated by Dr. David E. Nichols (Purdue University, West Lafayette, IN). Prazosin, an α1-receptor antagonist, and all other reagents were purchased from Sigma-Aldrich (St. Louis, MO) or YWR Scientific-Products (Columbus, OH).

**MDMA Effects on Thermogenesis.** Basal skeletal muscle and/or rectal temperatures were taken in all animals before administering MDMA or saline. Skeletal muscle temperatures were taken in the biceps femoris using a thermocouple with the electrode inserted into an 18-gauge needle. Rectal temperatures were taken with a rectal probe (Physitemp Instruments, Clifton, NJ) attached to the thermocouple and white petrolatum was applied to the probe before insertion. Skeletal muscle and rectal temperatures were taken at 1, 2, and 3 h post-treatment. Skeletal muscle temperatures were not monitored in TX animals due to the stress of the surgeries in general.

**Assessment of MDMA-Induced Serotonergic Neurotoxicity.** To assess neurotoxicity, 5-HT levels were measured from each hemisphere of the striatum and hippocampus 7 days after treatment. TX animals were assessed 4 days post-treatment. Samples were collected by brain dissection of the specific brain regions over ice. Tissue samples were then frozen in liquid nitrogen and stored at −80°C until analysis could be performed. These samples were subsequently sonicated, using a sonic dismembrator (Fisher Scientific Co., Pittsburgh, PA), for 15 s at a setting of approximately 4 while suspended in 100 µl of mobile phase.

High-performance liquid chromatography (Waters) was conducted to determine 5-HT levels. The mobile phase (consisting of 0.05 M sodium phosphate, 0.03 M citric acid buffer with a pH range of 2.1–3.9, 0.1 mM EDTA, 0–0.05% sodium octyl sulfate, and 0–30% methanol) was pumped through a Nova-Pak C18 (5 µm 3.9 × 300 mm) reverse-phase column at a flow rate of 0.7 ml/min with the potential set at 1 nA and an E of +750 mV. Millennium software was used to integrate and analyze the raw data for the determination of 5-HT levels compared with internal standard curves. The detection limits were 5 pg/µl.

**MDMA Effects on Thyroxine (T4) Levels.** Twelve Sprague-Dawley rats were assigned to receive saline or MDMA (40 mg/kg s.c.) to assess the effects of MDMA on T4 levels 1 h post-MDMA administration. The 1-h time point was selected based on previous results that showed MDMA-induced hyperthermia to be most robust at the 1-h time point. Animals were anesthetized using chloroform and blood was subsequently drawn from the left ventricle. Measurement
of T₄ levels was conducted with a Snap T₄ Test. Snap T₄ test is an enzyme linked immunosorbent assay for the quantitative measurement of total T₄ in serum. The Snap T₄ test uses a competitive enzyme immunoassay format.

In the test procedure, the serum sample is first incubated with an anti-T₄ antibody-enzyme conjugate. During incubation, T₄ present in the serum sample will bind to the conjugate. The T₄ concentration is then calculated from the ratio of T₄ test stripe color to reference stripe color developed from standard curves ($R^2 = 0.95$) with detection limits of 0.4 μg/dl.

**Statistical Analysis.** Data were analyzed by analysis of variance with a Student-Newman-Keuls post hoc test or a t test. Significance was set at $p \leq 0.05$. All biochemical measurements were based on tissue wet weight and are represented as percentage of saline control for ease of presentation. Control groups in all studies were treated with saline only. All figure legends contain the control values and sample size.

**Results**

**Effects of MDMA on Rat Rectal and Skeletal Muscle Temperature.** MDMA induced a statistically significant increase ($p < 0.01$) in rat rectal (Fig. 1A) and skeletal muscle (Fig. 1B) temperature at the 1-, 2-, and 3-h time points.

**Effects of Hypophysectomy on MDMA-Induced Hyperthermia.** MDMA produced a significant ($p < 0.001$) increase in rectal temperature at the 1-, 2-, and 3-h time points. Treatment of HYPO animals with the same dose of MDMA resulted in a significant ($p < 0.001$) decrease in skeletal muscle temperatures at the 1-, 2-, and 3-h time points.

**Effects of Hypophysectomy on MDMA-Induced Serotonergic Neurotoxicity.** Striatal 5-HT concentrations were significantly ($p < 0.03$) decreased in the MDMA treatment group compared with sham only (Fig. 3A). Hippocampal 5-HT concentrations were also significantly ($p < 0.01$) decreased in the MDMA treatment group compared with all other treatment groups (Fig. 3B). Hypophysectomy attenuated this decrease in 5-HT levels seen in both regions.

**Effects of MDMA on T₄ Levels.** MDMA induced a significant ($p < 0.007$) increase in T₄ levels 1 h post-treatment. Theses results are shown in Fig. 4.

**Effects of Thyroparathyroidectomy on MDMA-Induced Hyperthermia.** As was seen in the HYPO animals, thyroparathyroidectomy resulted in a hypothermic response ($p < 0.01$) after treatment with MDMA. MDMA alone induced a significant ($p < 0.01$) elevation in rectal temperature. The thyroparathyroidectomized treatment group began the study with a baseline temperature that was significantly ($p < 0.05$) less than the sham control group (Fig. 5A).

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**Fig. 1.** Effects of MDMA (40 mg/kg s.c.) on rat rectal (A) and skeletal muscle (B) temperature. Each value is the mean ± S.E.M. ($n = 4$). *, significantly different from the corresponding control group ($p < 0.01$).

**Fig. 2.** Effects of MDMA (40 mg/kg s.c.) on rectal (A) and skeletal muscle (B) temperature in HYPO rats. Each value is the mean ± S.E.M. for rectal temperatures ($n = 12$) and for skeletal muscle ($n = 6$) temperatures. *, significantly different from all treatment groups ($p < 0.001$). Sham and HYPO treatment groups received saline (s.c.). MDMA only treatment groups also received sham surgeries.
Effects of Levothyroxine Supplementation on MDMA-Induced Thermogenic Response in TX Animals. Replacing thyroid hormones with levothyroxine (100 µg/kg s.c. × 5 days) resulted in a significant hyperthermic response compared with control (p < 0.01) albeit still significantly less than MDMA alone (p < 0.01). TX animals treated with MDMA only responded with a significant (p < 0.001) hypothermic response (Fig. 5B).

Fig. 3. Effects of MDMA (40 mg/kg s.c.) on 5-HT levels in the striatum (A) and hippocampus (B) of hypophysectomized rats 7 days after treatment. Each value is the mean ± S.E.M. (n = 6). *, significantly different from sham only (p < 0.03). **, significantly different from all treatment groups (p < 0.01). Sham striatal 5-HT levels were 280.6 ± 8.2 pg/mg tissue weight and sham hippocampal 5-HT levels were 100.6 ± 11.7 pg/mg tissue weight. Sham and HYPO treatment groups received saline (s.c.). MDMA only treatment groups also received sham surgeries.

Effects of Levothyroxine Supplementation on MDMA-Induced Thermogenic Response in TX Animals. Replacing thyroid hormones with levothyroxine (100 µg/kg s.c. × 5 days) resulted in a significant hyperthermic response compared with control (p < 0.01) albeit still significantly less than MDMA alone (p < 0.01). TX animals treated with MDMA only responded with a significant (p < 0.001) hypothermic response (Fig. 5B).

Effects of Thyroparathyroidectomy on MDMA-Induced Serotonergic Neurotoxicity. Striatal 5-HT concentrations were significantly (p < 0.01) decreased in the MDMA treatment group compared with sham only (Fig. 6A). Hippocampal 5-HT concentrations were also significantly (p < 0.01) decreased in the MDMA treatment group compared with all other treatment groups (Fig. 6B). Thyroparathyroidectomy attenuated this decrease in 5-HT levels seen in both regions.

Effects of Prazosin on MDMA-Induced Hyperthermia. The α1-receptor antagonist prazosin significantly (p < 0.01) attenuated this rise in rectal temperature but did not completely eliminate the hyperthermic response (Fig. 7). In the prazosin only treatment group, rectal temperatures remained constant throughout the monitoring period (data not shown). MDMA resulted in a significant (p < 0.001) hypothermia in the TX animals that was significantly (p < 0.05) potentiated by prazosin at the 3-h time point.
MDMA-induced a significant rise (p < 0.001) in skeletal muscle temperature that was not altered by prazosin pre-treatment at the 1- and 2-h time points (Fig. 8). At the 3-h time point, prazosin attenuated (p < 0.01) this rise in skeletal muscle temperature. Basal skeletal muscle temperature was significantly (p < 0.001) reduced after saline and prazosin treatment and remained constant throughout the remaining duration of the monitoring period (Fig. 8).

Effects of Cyanopindolol on MDMA-Induced Hyperthermia. The β2-receptor antagonist cyanopindolol had no effect on the MDMA-mediated increase in rectal temperature but significantly (p < 0.001) attenuated the increase in skeletal muscle temperature (Fig. 9B). Combining cyanopindolol and prazosin eliminated the hyperthermic response in both the rectum and skeletal muscle (Fig. 10).

Fig. 6. Effects of MDMA (40 mg/kg s.c.) on 5-HT levels in the rat striatum (A) and hippocampus (B) in TX animals 4 days after treatment. Each value is the mean percentage of control ± S.E.M. (n = 6–7). *, significantly different from all other groups (p < 0.05). Sham striatal 5-HT levels were 307.35 pg/mg and sham hippocampal 5-HT levels were 164.45 pg/mg. Sham and TX treatment groups received saline (s.c.). MDMA only treatment groups also received Sham surgeries.

Fig. 7. Effects of MDMA (40 mg/kg s.c.) on rat rectal temperature in TX and prazosin (0.2 mg/kg i.p. 30 min before MDMA)-treated animals. Each value is the mean ± S.E.M. (n = 6–7) for rectal temperatures. *, significantly different from all other groups (p < 0.001). **, significantly different from all other groups except TX + MDMA + prazosin (p < 0.001). $\Psi$, significantly different from all other groups except TX + MDMA (p < 0.001).

Fig. 8. Effects of MDMA (40 mg/kg s.c.) and prazosin (0.2 mg/kg i.p. 30 min before MDMA) on rat skeletal muscle temperature. Each value is the mean ± S.E.M. (n = 6) for skeletal muscle temperatures. *, significantly different from control and prazosin (p < 0.001). **, significantly different from all other groups (p < 0.01).

Fig. 9. Effects of MDMA (40 mg/kg s.c.) and cyanopindolol (4 mg/kg s.c. 30 min before MDMA) administration on rat rectal (A) and skeletal muscle (B) temperature. Each value is the mean ± S.E.M. (n = 6) for rectal temperatures. *, significantly different from control and cyanopindolol (p < 0.0001). **, significantly different from all other treatment groups (p < 0.001). $\Psi$, significantly different from MDMA only (p < 0.05).
observed that devices to record rectal and skeletal muscle temperature, we felt the possibility that hyperthermia may arise from heat induction in different tissue sites. By using thermocouple methods, we were able to look at the effects of prazosin on MDMA-mediated thermogenesis. Our results showing only a partial attenuation of the core hyperthermia by prazosin parallel those of Pedersen and Blessing (2001), who saw only a partial antagonism with surgical sympathectomy. The ganglionic blocker chlorisondamine has also been shown to reduce the amplitude of the hyperthermia induced by MDMA (Fernandez et al., 2002). Based upon these studies, we hypothesized that MDMA-induced core hyperthermia would be attenuated with prazosin pretreatment. Our results with prazosin’s effects on MDMA-mediated core temperature changes confirmed these previous observations. Skeletal muscle hyperthermia was, however, unaffected by α1-blockade.

Skeletal muscle thermogenesis occurs by three primary mechanisms: 1) contraction, or shivering, 2) thermogenic calcium cycling mediated by the dantrolene-sensitive ryanodine receptor (Paul-Pletzer et al., 2002), and 3) activation of mitochondrial proton leak by UCP-3, which is highly expressed in skeletal muscle and has recently been associated with skeletal muscle thermogenesis in transgenic mice overexpressing UCP-3 (Curtin et al., 2002) and yeast (Hinz et al., 1999). Dantrolene is the primary pharmacological line of defense in hospitalizations for MDMA-induced hyperthermia (Dar and McBrien, 1996). Despite its widespread use, dantrolene fails to adequately control the hyperthermic response seen after MDMA ingestion (Dar and McBrien, 1996). In animals, the skeletal muscle relaxant xylazine also fails to reduce the hyperthermic response (our unpublished observations). These data suggest that other mechanisms may contribute more readily to hyperthermia.

UCP-3 is both induced and activated by increased intracellular cAMP downstream of β3-adrenergic receptors and thyroid hormone (Gong et al., 1997). β3-Adrenergic agonists and thyroid hormone are thought to produce a synergistic activation of thermogenesis in animals (Silva, 1995). In the present study, the β3-antagonist, cyanopindolol, attenuated the rise in skeletal muscle temperature after MDMA treatment but had no effect on core temperature. Together, the data suggests that mitochondrial UCP-3 may contribute to increased skeletal muscle temperatures after MDMA.

Three other lines of evidence suggest that UCPs may be involved in the thermogenic response to MDMA. First, clinical presentations of severe hyperthermia induced by MDMA can include rhabdomyolysis, wherein skeletal muscle cells lose viability, lyse, and release myoglobin, which can lead to renal failure (Walubo and Seger, 1999). We recently demonstrated that overexpression of UCP-2, a homolog of UCP-3, induces ATP depletion and oncosis in transiently transfected and retrovirally infected cultured cells (Mills et al., 2002).
Second, we recently reported that MDMA regulates the levels of UCP-3 mRNA in rat skeletal muscle (Sprague et al., 2002). Third, MDMA has also been shown to increase proton leak in rat striatum, a specific functional correlate of UCP activity (Burrows et al., 2000).

MDMA-mediated dopamine release and subsequent activation of hypothalamic D_2 receptors has been shown to play an essential role in this hyperthermic response (Mechan et al., 2002). Activation of the hypothalamic axis following MDMA treatment is also confirmed by increased c-fos expression in the supraoptic and median preoptic nucleus of the hypothalamus following MDMA treatment (Stephenson et al., 1999). Curiously, our hypophysectomized and thyroparathyroidectomized animals showed a hyperthermic response to MDMA. Hypothyroidism has been shown to stimulate the SNS by increasing the amounts of norepinephrine in the plasma (Coulombe and Dussault, 1977). Despite increased amounts of norepinephrine, the normal thermogenic response to norepinephrine and cold is blunted in hypothyroid animals (Triandafillou et al., 1982; Sundin et al., 1984), supporting the notion that thyroid hormone plays a permissive role in SNS-mediated thermogenesis. According to Bianco et al. (1988), this blunted response is probably due to a reduced synergistic action between T_3 and norepinephrine at the gene level. In the absence of T_3, as would presumably be the case with our thyroparathyroidectomized animals, the norepinephrine response would be attenuated (Bianco et al., 1988). This defect in the norepinephrine signaling pathway may alter the stimulatory action of cAMP on UCP-3 activity and/or expression (Gripois and Valens, 1982; Young et al., 1982). Hypothyroidism has also been associated with an up-regulation of α_1- (Dicker et al., 1992) and β_2-receptors (Rubio et al., 1995b) and a down-regulation of β_1- and β_3-receptors (Rubio et al., 1995a). We cannot rule out the possibility that changes in sympathetic receptor levels in our TX animals may well play a role in the hyperthermic response seen in this study. Our data showing that levotyroxine supplementation restores the hyperthermic response to MDMA further supports our hypothesis that thyroid hormone is required for MDMA-mediated thermogenesis.

Much controversy surrounds the role of hyperthermia in the neurotoxic effects of MDMA in experimental animals. The 5-HT_2A/2C receptor antagonists ketanserin (Nash, 1990) and MDL 11,939 (Schmidt, 1987) not only prevent the neurotoxicity but also block the hyperthermia induced by a low dose (10 mg/kg) of MDMA. In both of these reports, higher doses of MDMA still produced hyperthermia, but serotoninergic neurotoxicity was nevertheless blocked. Malberg et al. (1996) showed that the ability of ketanserin to block the neurotoxicity of MDMA is lost by increasing the body temperature of the animal. Those authors further reported that pretreatment of the animals with α-methyl-para-tyroisine, a tyrosine hydroxylase inhibitor, induced hyperthermia and prevented the neurotoxicity of subsequently administered MDMA, but that warming the animals negated these protective effects. Although these data clearly suggest a link between hyperthermia and subsequent neurotoxicity, not all agents that prevent MDMA-induced neurotoxicity necessarily do so by blocking the hyperthermic response. Fluoxetine fails to prevent MDMA-induced hyperthermia, yet still affords protection against the neurotoxic process (Malberg et al., 1996). Antisense oligonucleotides targeting monoamine oxidase-B (Falk et al., 2002) or the dopamine transporter (Kanthasamy et al., 2002) also attenuate the serotoninergic neurotoxicity induced by MDMA without altering the hyperthermic response.

Lowering body temperature protects against brain damage induced by a variety of insults, most likely by simply slowing neurochemical processes. Bowyer et al. (1993) showed that if animals were placed in a cold environment, both dopamine release and neurotoxicity induced by methylphenidate treatment were decreased. Thus, agents that reduce body temperature may decrease the neurochemical effects of MDMA and provide protection against its acute peripheral effects and chronic neurotoxicity. Our results using surgically modified animals are consistent with previous suggestions that blocking hyperthermia protects against subsequent neurotoxicity. As our data also suggest, however, hyperthermia may be required for the magnitude of neurologic protection observed in our studies.

The results of the present study and supporting evidence that is extant in the literature would argue strongly for an interactive role between the SNS and the HPT axis in the hyperthermic response to MDMA. We propose that MDMA acutely activates the HPT axis and the SNS, and stimulates thyroid-, α_1-adrenergic-, and β_3-adrenergic-dependent core and skeletal muscle hyperthermia by the activation of uncoupling proteins. Furthermore, we propose that clinical intervention consistent with this mechanism of MDMA-induced hyperthermia may prove superior in protecting individuals from some of the acute peripheral and delayed neurological toxicities that may be seen after MDMA misuse.

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References
Farfel GM and Seiden LS (1995) Role of hyperthermia in the mechanism of protection...
against serotonergic toxicity, I. Experiments using 3,4-methylenedioxyamphetamine, discipline, CGS 19755 and NBQX. J Pharmacol Exp Ther 272:860–867.


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