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GUEST EDITOR REVIEW

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The role of the sympathetic nervous system and uncoupling proteins in the thermogenesis induced by 3,4-methylenedioxymethamphetamine

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Abstract Body temperature regulation involves a homeostatic balance between heat production and dissipation. Sympathetic agents such as 3,4-methylenedioxymethamphetamine (MDMA, ecstasy) can disrupt this balance and as a result produce an often life-threatening hyperthermia. The hyperthermia induced by MDMA appears to result from the activation of the sympathetic nervous system (SNS) and the hypothalamic-pituitary-thyroid/adrenal axis. Norepinephrine release mediated by MDMA creates a double-edged sword of heat generation through activation of uncoupling protein (UCP3) along with α_1- and β_3-adrenoreceptors and loss of heat dissipation through SNS-mediated vasoconstriction. This review examines cellular mechanisms involved in MDMA-induced thermogenesis from UCP activation to vasoconstriction and how these mechanisms are related to other thermogenic conditions and potential treatment modalities.

Keywords 3,4-Methylenedioxymethamphetamine · Rhabdomyolysis · Hyperthermia · Uncoupling proteins · UCP-3

Abbreviations *AR:* α_1-Adrenoreceptor · *BAT:* Brown adipose tissue · *DNP:* Dinitrophenol · *HPA:* Hypothalamic-pituitary-adrenal · *HPT:* Hypothalamic-pituitary-thyroid · *MDMA:* 3,4-Methylenedioxymethamphetamine · *MH:* Malignant hyperthermia · *NMS:* Neuroleptic malignant syndrome · *ROS:* Reactive oxidant species · *SNS:* Sympathetic nervous system · *UCP:* Uncoupling protein

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Introduction

Every weekend around the world thousands of teenagers and young adults gather at dance parties called raves. What originated in the late 1980s as clandestine gatherings of British youth interested in all-night marathon dancing has developed into a new culture. Similar to the

hippie culture of the 1960s and 1970s, raves are signified by their own dress, lingo, artwork, music, and drugs [1]. At the center of the rave movement is the drug ecstasy (3,4-methylenedioxymethamphetamine, MDMA). This was originally patented by Merck in 1914 as a precursor compound for the production of other therapeutically active agents [2, 3]. MDMA enjoyed a brief stint as an adjunct to psychotherapy in the late 1970s and early 1980s but did not become popular as a drug of abuse until the emergence of the rave party scene. Despite early beliefs that MDMA was safe, there have been increasing numbers of reported fatalities and severe adverse effects associated with its use [4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17]. Of these adverse effects, hyperthermia is one of its most acute, life-threatening toxicities [18].

In general, body temperature regulation is very complex and requires an orchestrated balance between heat production and dissipation. Increased body temperature occurs when metabolic heat production exceeds heat dissipation. The preoptic nucleus of the anterior hypothalamus responds to core temperature changes and activates the autonomic nervous system to induce cutaneous vasodilation in order to dissipate heat by convection when body temperature is elevated, or induces vasoconstriction to conserve heat during adaptation to cold (for a review see [19]). Thus, MDMA-induced hyperthermia may be the result of pharmacological interactions involving heat production and/or dissipation of heat.

Recent studies have begun to shed new light on the molecular mediators of MDMA-mediated thermogenesis and have revealed a complex interaction between the hypothalamic-pituitary-thyroid (HPT) axis, sympathetic nervous system (SNS), and the activity of uncoupling proteins (UCP; namely UCP3). This review summarizes new findings in this field and focuses on MDMA as the prototype thermogenic sympathomimetic agent.

Clinical effects of MDMA

Hyperthermia induced by MDMA is the most serious toxicity associated with its use and has been linked to severe complications, including rhabdomyolysis [20], renal failure [21], and ultimately death [22]. Mortality

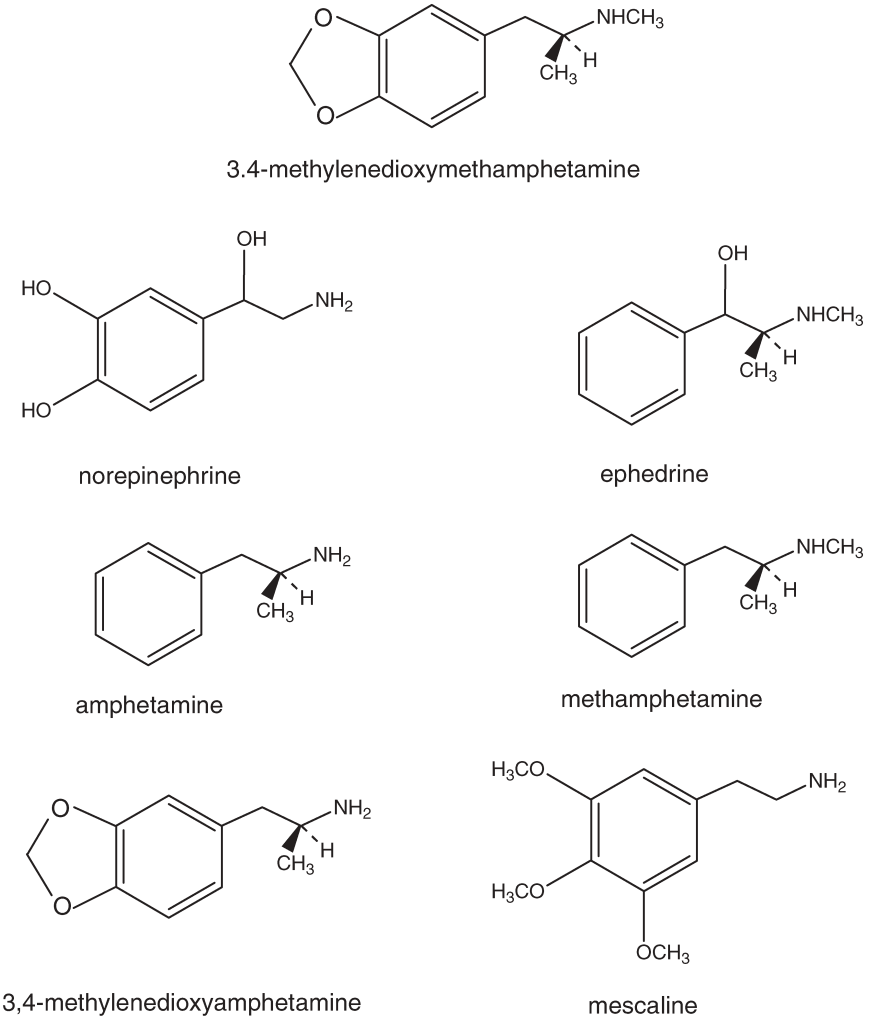

Fig. 1 Sample structures of phenethylamine type sympathomimetic agents which mimic the pharmacological/toxicological effects MDMA

rates in reported MDMA cases are directly correlated with peak core body temperature: two-thirds of cases in which body temperature exceeded 41.5°C were fatal [18]. Structurally, MDMA is related to other sympathomimetic agents such as methamphetamine and the hallucinogen mescaline (Fig. 1). Mild symptoms of MDMA intoxication include tachycardia, diaphoresis, and sensory amplification (described as an increased intensity in light, sound, smell, taste, touch, and emotional sensitivity). These pharmacological effects have led to its popularity as a rave party drug [23]. Severe intoxications usually produce a clinical picture characterized by marked hyperpyrexia, diaphoresis, tachycardia, muscle rigidity, rhabdomyolysis, metabolic acidosis, seizures, hyperkalemia, coagulopathy, and death. Temperatures greater than 41.7°C [6, 7, 9, 12, 13, 20, 24] and as high as 43.9°C [24] have been reported in cases of MDMA use. Along with hyperthermia, muscle rigidity and hyperreflexia [9, 13, 25, 26], rhabdomyolysis [12] and renal failure [21] have been reported in cases of MDMA intoxication. Deaths have been reported following overdose [13] and after just a single dose [12].

In the 1930s, a group of physicians published a paper describing the beneficial weight loss properties of a compound called dinitrophenol (DNP) [27]. Despite their recommendation that the compound be used only in controlled research settings the popularity of DNP rose with the lay population, and soon case reports of deaths associated with DNP appeared in the literature [28, 29, 30]. Patients dying after ingestion of DNP were reported to have severe hyperpyrexia, 46.1°C in one case [30], and muscle rigor. Cases of poisoning with DNP and similar compounds such as, dinitro-orthocresol and pentachlorophenol, have a strikingly similar clinical presentation to MDMA toxicity, with the most common reported symptoms being marked hyperpyrexia, muscle rigidity, metabolic acidosis, tachycardia, and diaphoresis [31, 32, 33, 34, 35]. In contrast to MDMA, the molecular mechanisms of DNP toxicity are well established [36, 37]. The clinical syndrome of DNP poisoning results from uncoupling of oxidative phosphorylation, whereas MDMA toxicity has been attributed to heat stroke [38], serotonin syndrome [39], neuroleptic malignant syndrome [14], and malignant hyperthermia [40]. Only recently have scientific studies of the mechanisms of MDMA-mediated thermogenesis begun to shed light on the role of mitochondrial uncoupling in this hyperthermic response.

CNS effects of MDMA

The effects of MDMA on body temperature appear to be directly correlated with the ambient temperature. Several laboratories have shown that when MDMA is given to rats in a 24°C or greater environment, hyperthermia results [41, 42]. However, if the ambient temperature is lowered to 10°C, a hypothermic response occurs [41]. These latter results led Gordon et al. [41] to conclude that MDMA induces a central deregulation of thermogenesis.

Activation of the hypothalamic axis following MDMA treatment has been confirmed by increased c-fos expression in the supraoptic and median preoptic nucleus of the hypothalamus following MDMA treatment [43]. Thermoregulation within the hypothalamus has been suggested to be controlled by serotonin [44], dopamine [45], and norepinephrine [46] all of which are altered by MDMA administration.

Most in vivo studies on the acute neurochemical effects of MDMA have focused on specific brain regions but have unfortunately not examined the hypothalamus. In general, pharmacological alterations in brain biogenic amine pathways following treatment with MDMA occur in a biphasic fashion. The first phase is an initial release of neuronal serotonin, followed by recovery within 24 h. The second phase, the long-term decrease in serotonin and serotonin neuronal markers, occurs within approximately 3 days and persists for up to a year [47]. The initial release of serotonin also upregulates dopamine biosynthesis and release by activation of serotonin 2A postsynaptic receptors [42, 48]. MDMA-mediated dopamine release and subsequent activation of D_1 receptors has been shown to play an essential role in this hyperthermic response [49]. In vitro studies with hypothalamic synaptosomes have demonstrated that MDMA can increase norepinephrine release [50] and inhibit norepinephrine reuptake [51]. Thus, MDMA may modify central regulation of body temperature through alterations of the biogenic amines involved in temperature regulation.

Uncoupling proteins and their role in thermogenesis

In endothermic or warm-blooded animals heat production is divided into two categories: obligatory and facultative. *Obligatory thermogenesis* refers primarily to the basal metabolic thermogenic processes that maintain a constant body temperature necessary to sustain the life of cells and tissues. On the other hand, *facultative thermogenesis* refers to rapidly inducible heat production driven by the hypothalamus and sympathetic nervous system in response primarily to cold or excess food intake [52]. The two primary sites of facultative thermogenesis in mammals are skeletal muscle and brown adipose tissue (BAT), a specialized thermogenic organ found in rodents and hibernating mammals and in newborn infants. BAT thermogenesis is also referred to as nonshivering because when activated it significantly increases body temperatures in the absence of shivering. Skeletal muscle is thought to participate in facultative thermogenesis primarily by rapid contraction, or shivering. Under normal conditions adult humans have little if any BAT, suggesting that the primary site of inducible thermogenesis in man is skeletal muscle. Interestingly, not all types of hyperthermia in man, including that produced in response to MDMA, are associated with significant amounts or

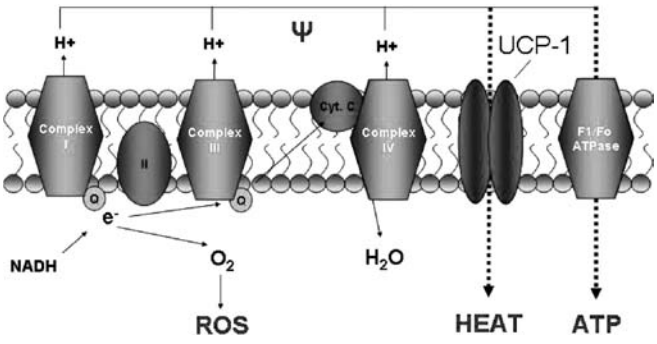

Fig. 2 Role of UCP1 in thermogenesis. As electrons are passed down the mitochondrial electron transport chain, protons are extruded from the inside (matrix) to the outside of the inner membrane, thus forming an electrochemical proton gradient, also referred to quantitatively as the mitochondrial membrane potential (Ψ). Activation of UCP1 induces a leak of protons through the membrane and "uncouples" the free energy stored in the electrochemical gradient from ATP synthesis

duration of shivering, suggesting that some BAT-independent mechanism may exist for the induction of nonshivering thermogenesis in humans [53, 54].

In 1980, the molecular mechanism of BAT thermogenesis was attributed to a cold- and diet-inducible mitochondrial protein highly expressed in brown adipocytes known as thermogenin [53]. Evidence accumulated over the next few years to show that thermogenin is the physiological mediator of heat production in BAT by virtue of its ability to drastically increase the respiration rate of brown adipocyte mitochondria through the "uncoupling" of mitochondrial respiration from ATP synthesis, and it has been since referred to as UCP1 [55].

Brown fat possesses a very rich innervation by the sympathetic nervous system (SNS) and is highly vascularized. When a BAT-bearing animal (e.g., grizzly bear, rodent, newborn human) is exposed to cold, an almost immediate hypothalamic activation of the relevant sympathetic outflow occurs, which releases an abundant supply of norepinephrine in brown fat depots [56]. Upon binding to β_3-adrenoreceptors (AR) on brown adipocytes norepinephrine stimulates the cAMP-dependent liberation of intracellular free fatty acids, which act as second messengers and perhaps direct activators in the activation of UCP1-induced proton leak and thermogenesis [57]. Both α_1AR and β_3AR selective agonists stimulate brown fat thermogenesis in rodents, with the latter being a far more potent stimulus [58]. Activation of α_1AR by norepinephrine strongly potentiates the thermogenic action of β_3AR [59]. However, the mechanism for this interaction is not known.

An appreciation of how respiratory uncoupling and thermogenesis are related requires a brief summary of mitochondrial respiration and "respiratory control" [60], see Fig. 2. The mitochondrial inner membrane electrochemical gradient or mitochondrial membrane potential is formed by the reduction/oxidation (redox) reactions of electrons with the respiratory complex proteins and the resultant extrusion of protons from the inner matrix side to the cytosolic side of the inner mitochondrial membrane. The free energy stored in this electrochemical gradient is consumed in two ways: (a) during ATP synthesis (i.e., oxidative phosphorylation), a process in which protons flow back into the mitochondrial matrix through the F_0 portion of the F_1/F_0-ATPase, where the free energy released is coupled to the phosphorylation of ADP to ATP, and (b) by "proton leak" across the inner membrane, a nonproductive process in which the free energy liberated from proton flux is not coupled to a biochemical reaction or "work" and thus, based upon the first law of thermodynamics, is released as heat. In order to maintain ATP at a relatively constant level in cells, mitochondrial respiration is stoichiometrically coupled to, and rate-limited by, ATP synthesis or steady state levels of ADP and ATP, and this coupling simplistically is referred to as *respiratory control*. At high ratios of ATP:ADP, respiration slows and vice versa. By effectively short circuiting the electrochemical gradient across the inner membrane, mitochondrial uncoupling bypasses respiratory control mechanisms and can dramatically increase mitochondrial respiration rates (e.g., increase electron flow and oxygen consumption) without concomitantly increasing levels of ATP. By analogy, a copper wire placed across the positive and negative terminals of an automotive battery short-circuits (i.e., provides a path of much lower resistance for) the highly resistant electron flow through the wiring that connects and drives the electrical components of an automobile, and the free energy is released as explosive amounts of heat. Mitochondrial uncoupling provides the same type of low resistance path for proton flux through the mitochondrial membrane and bypasses normal proton flux through the high resistance pore, the F_1/F_0-ATPase, thereby unleashing the massive free energy stored in the mitochondrial electrochemical gradient as heat.

Much excitement was shared by biochemists and physiologists surrounding the discovery and characterization of UCP1 from brown fat, but many questions remained regarding its relevance to man. First, adult humans have little or no BAT and likewise lack UCP1, which is expressed exclusively in brown adipocytes [61]. Second, under basal conditions other tissues, such as liver and skeletal muscle, exhibit levels of respiratory uncoupling as high as 25% and 52%, respectively, of total respiration, suggesting that mechanisms similar to uncoupling protein in brown fat must also exist in these and perhaps other tissues [62, 63]. After nearly two decades of intense searching, in 1997, two distinct proteins with greater than 50% homology to UCP1 were cloned and thus named UCP2 [64] and UCP3 [65, 66], both of which appear to regulate a fatty-acid dependent proton leak or uncoupling in vivo [67]. UCP2 is expressed primarily in spleen, pancreatic beta cells, macrophages, certain brain regions (e.g., hypothalamus) and in rapidly dividing cell types such as gut epithelium [68, 69]. UCP3 is expressed primarily in skeletal muscle, and is also found in BAT and heart [65]. Two more recent UCP1 homologues have been

cloned and are expressed in brain, UCP4 [70], and UCP5 [71], but their characterizations as bona fide uncoupling proteins has yet to be established. Since 1997, much interest has focused upon the determination of the functional similarities between the prototypic uncoupling protein, UCP1, and the novel UCP isoforms as well as their mechanisms of activation and transcriptional regulation.

Generally speaking, levels of UCP1, UCP2, and UCP3 in various tissues are regulated transcriptionally by thyroid hormone and by dietary fatty acids [72], in part through the activation of transcription factors, among others, belonging to the peroxisome-proliferator activated receptor family [73]. UCPs are acutely activated by fatty acids, superoxide, and lipid peroxides and are rapidly inhibited by nucleotides such as GDP [74, 75, 76].

Because it seems on the surface that organisms would evolve strategies to maximize the efficiency of energy production, it appears rather paradoxical that in most cold or warm blooded animals, under normal conditions there is an overall 15–25% respiratory inefficiency that is thought to be largely attributed to mitochondrial proton leak [77]. What then are the possible functions of such an apparently wasteful process?

(1) Thermogenesis: norepinephrine, through its activation of BAT UCP1 can double the respiration rate of BAT mitochondria without changing ATP concentrations, a process that is highly thermogenic and is required for cold adaptation in rodents and newborn infants [78]. UCP1-deficient mice do not survive prolonged exposure to cold [79]. UCP2 [80] and UCP3 [81] knockout mice, however, adapt normally to cold, a finding which has led to the conclusion that these novel isoforms are not involved in thermogenesis. The importance of UCP2 and UCP3 in facultative thermogenesis in man may be greater than for rodents because rodents rely upon BAT thermogenesis for cold adaptation yet adult humans have no BAT. Because shivering can be incapacitating, it seems plausible that some adaptive mechanism other than shivering and analogous to brown fat uncoupling exists in man to account for thermogenesis during at least mild cold adaptation or the response to overfeeding and during hyperthermic conditions. However, this mechanism has yet to be described.

(2) Reduction in mitochondrial oxidant production: As electrons flow along the mitochondrial respiratory chain, at certain points they can interact with molecular oxygen to form reactive oxidant species (ROS), such as superoxide, which can then form deleterious ROS such as hydrogen peroxide or the highly damaging hydroxyl radical. At high mitochondrial membrane potentials, which based upon respiratory control mechanisms occur when mitochondria have an excess supply of energy substrates (e.g., from overfeeding), ROS production is exponentially increased [82]. By mildly dissipating the mitochondrial proton gradient, UCP1 dramatically, or exponentially, lowers ROS production. This function is supported by several lines of evidence. (a) UCP1, UCP2, and UCP3 are activated by intramitochondrial superoxide and lipid peroxides and in response lower mitochondrial superoxide production in vitro and thus appear to participate in a negative feedback loop controlling ROS production [76]. (b) Transgenic overexpression of UCP2 in vivo greatly protects tissues such as heart and brain from ROS-associated injury following ischemia, stroke, and trauma [83]. (c) Knockout animals deficient in either UCP1, UCP2, or UCP3 show increased mitochondrial ROS production, respectively, in BAT, macrophages, and skeletal muscle [84].

(3) Regulation of ATP-independent cell death (oncosis): One caveat to mitochondrial uncoupling is that with strong uncoupling, the electrochemical gradient may not be adequate to drive sufficient ATP synthesis necessary for the basic functions of the cell. Some evidence suggests that despite their ability to lower ROS production and to be protective when mildly activated, excessive uncoupling in cells or tissues can be deleterious, perhaps by compromising cellular energetics: (a) When transiently overexpressed in certain cell types in vitro, UCP2 induces a form of cell death known as oncosis, where cells rapidly lose ATP, swell, and lyse. Inhibition of UCP2 in this system using a dominant-negative mutant led to a reduction in hypoxia-induced oncosis [85]. (b) In rodents excessive uncoupling and thermogenesis in skeletal muscle induced by MDMA is associated with skeletal muscle cell lysis (oncosis) and the liberation of creatine kinase into the bloodstream [88]. In humans, MDMA can induce rhabdomyolysis subsequent to such skeletal muscle breakdown [87]. In rodents, drugs which block skeletal muscle thermogenesis (uncoupling) induced by MDMA significantly protect muscle from lytic breakdown [88]. (c) In vivo, excess UCP activity in rodent pancreatic beta cells is associated with ATP depletion, loss of insulin secretion, and beta cell death, whereas UCP-deficient beta cells have higher ATP levels and enhanced insulin secretion, suggesting that UCP2 plays a potentially important role in the development of diabetes [89, 90, 91]. (d) UCP2 induction in fatty liver predicts a slower course of healing and an exacerbated pathological response to subsequent fatty acid or alcohol stimulation [90].

"Coupling" of the pharmacological effects of MDMA and UCP activity

The peripheral pharmacological effects of MDMA parallel UCP activation in many ways. As outlined above, UCP activation results in thermogenesis, as does MDMA [92]. UCP3 is highly expressed in skeletal muscle and has recently been associated with skeletal muscle thermogenesis in transgenic mice overexpressing UCP3 [93]. Uncoupling proteins are also associated with muscle oncosis [85]. Moreover, $UCP3^{-/-}$ mice are also less responsive to the hyperthermic and lethal effects of MDMA [86]. The latter findings suggest that MDMA mediated

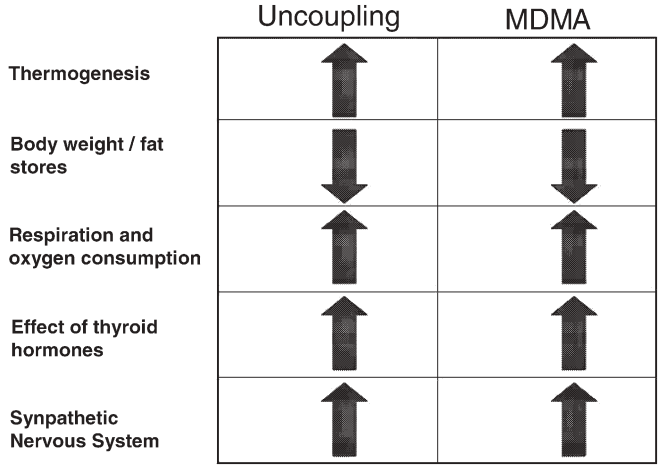

Fig. 3 "Coupling" of the pharmacological effects of MDMA and UCP activity. Direct uncoupling agents such as DNP or sympathomimetic agents such as MDMA have been shown to increase mitochondrial respiration and oxygen consumption resulting in thermogenesis. Both groups of agents have also been shown to reduce body weight and fat stores. Thyroid hormones have been demonstrated to potentiate uncoupling activity and the thermogenic effects of sympathomimetic agents. Furthermore, MDMA activates the relevant sympathetic outflow; a response associated with the activation of uncoupling. See text for details

thermogenesis involves the activation of uncoupling proteins in a pattern which parallels direct uncoupling agents such as DNP (Fig. 3). Figure 3 summarizes the similarities between the activation of uncoupling and the pharmacological effects of MDMA.

Although amphetamines and MDMA are known to reduce brain [94] and skeletal muscle [95] ATP levels, the ability of MDMA to uncouple oxidative phosphorylation does not appear to be direct. In isolated mitochondrial studies, MDMA was devoid of any direct ability to increase oxygen consumption as was seen with the known uncoupling agent, carbonyl cyanide chlorophenylhydrazone [96]. In a coculture model study with liver microsomes and L6 skeletal muscle cells, MDMA and/or its potential metabolites also did not cause toxicity [95, 96]. These findings lend support to the notion that MDMA-induced activation of the relevant sympathetic outflow is the penultimate step in the activation of uncoupling through α_1AR and β_3AR.

Role of the sympathetic nervous system in MDMA-mediated thermogenesis

Recently, the hyperthermia induced by MDMA was shown to involve a complex interaction between the HPT axis and SNS [92]. In studies with isolated skeletal muscle mitochondria, oxygen consumption (i.e., thermogenesis) was dependent on a coordinated activity between thyroid function and an acute increase in norepinephrine, an effect which could be reversed by sympathetic blockade [97]. MDMA increases central norepinephrine release [98], and we have recently demonstrated a greater than 35-fold increase in plasma norepinephrine levels 30 min after MDMA administration (unpublished findings). Norepinephrine is an agonist at both α_1AR and β_3AR receptors and has also been shown to be elevated in human subjects following exposure to MDMA [99]. A 20-min intravenous infusion of norepinephrine has been demonstrated in rats to generate a rapid elevation in both brown fat and core body temperatures that quickly reverses after the discontinuation of the infusion [100]. Similarly, norepinephrine infusions into the skeletal muscle of rats increase oxygen consumption through β_3AR activation [101]. Treatment of rats with the α_1AR antagonist prazosin 30 min before MDMA partially attenuates the rise seen in rectal temperature, and the use of the nonselective, β_3AR antagonist, cyanopindolol, partially attenuates the rise in skeletal muscle temperature. However, when these agents were coadministered, the thermogenic effects of MDMA were blocked [92]. Furthermore, when animals were treated 30 min before MDMA with prazosin plus the selective β_3AR antagonist SR59230A, hyperthermia was attenuated, as was the rise in markers of rhabdomyolysis [88]. The rise in core temperature was initially attributed to MDMA-induced peripheral release of norepinephrine [92] and subsequent α_1-mediated vasoconstriction [102, 103]. The rise in skeletal muscle temperature was attributed to β_3AR activation of UCP3 [86]. β_3AR appear to regulate UCP3 [104, 105]. However, the finding that α_1AR potentiate the thermogenic effects of β_3AR in BAT [59] suggests that both β_3AR and α_1AR mediate MDMA-induced increases in mitochondrial proton leak and uncoupling.

α_1AR [106] and β_3AR [107, 108, 109] are present at relatively low concentrations in skeletal muscle. De Matteis et al. [110] found a lack of β_3AR in skeletal muscle and suggested that this is the result of their low or different expression patterns in certain regions of skeletal muscle but also concluded that functionally they must be present. Although β_3AR are in low concentration on skeletal muscle, their functional regulation of UCP activity is well documented. β_3AR appear to regulate UCP3 gene and protein expression in skeletal muscle [105, 111, 112]. Additionally, there is evidence for the presence of functional β_3AR in norepinephrine-mediated thermogenesis in the rat hindlimb skeletal muscle [108].

Role of thyroid hormones in MDMA-mediated thermogenesis

Although MDMA [92] and other amphetamines [113] have been demonstrated to induce a marginally significant increase in serum thyroxine levels in rodents and humans, thyroid hormones appear to play a modulating role through the regulation of UCP3 expression [114, 115, 116] and/or adrenergic receptor synthesis [117]. Hypothyroid animals respond to typical thermogenic doses of MDMA [92] and other chemically related phenethy-

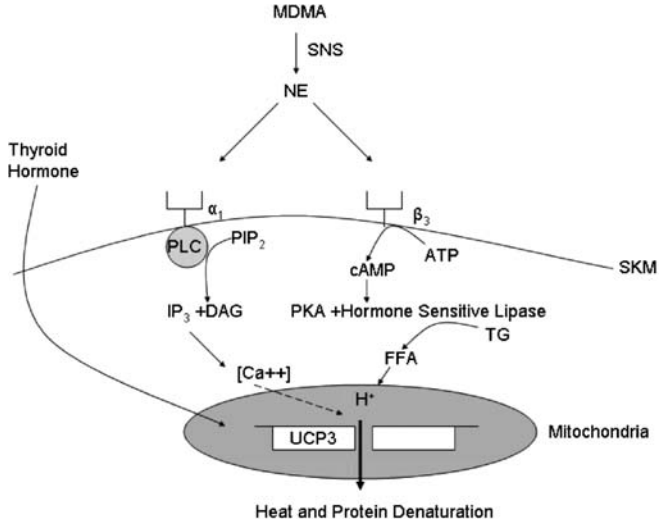

Fig. 4 Integrated hypothesis for the thermogenic and myonecrotic effects of MDMA. In this model, the robust increase in plasma norepinephrine activates the α_1AR and β_3AR on the skeletal muscle tissue. β_3AR activation increases cellular cAMP levels which stimulates hormone-sensitive lipase activity increasing the conversion of triglycerides (*TG*) to free-fatty acids (*FFA*). The FFAs supply the protons for uncoupling to occur. As the skeletal muscle temperature increases, protein denatures and rhabdomyolysis may develop as a result of lost skeletal muscle integrity. The α_1AR activation increases intracellular calcium levels through a phospholipase C dependent fashion. The role of intracellular calcium in UCP activation is not currently well understood. Thyroid hormones play a permissive role through the regulation of UCP3 gene and protein expression

lamines, such as amphetamine [118] with a hypothermic response. Hyperthyroidism has also been shown to enhance the hyperthermia and mortality rate of amphetamines [118, 119]. Similar to MDMA, methamphetamine-induced thermogenesis is dependent upon the HPT axis and UCP3 [120]. However, methamphetamine does not induce an acute change in thyroxine or triiodothyronine levels, supporting rather a permissive role for thyroid hormones in the thermogenesis mediated by sympathomimetic agents through the regulation of UCP3 expression.

In Fig. 4, we propose an integrated hypothesis for the thermogenic and myonecroctic effects of MDMA. In this model, the robust increase in plasma norepinephrine induced by MDMA activates the α_1AR and β_3AR on the skeletal muscle tissue. β_3AR activation increases cellular cAMP levels which in turn stimulates hormone sensitive lipase activity, thereby increasing the conversion of triglycerides to free fatty acids which may supply the protons for uncoupling to occur [121]. As the skeletal muscle temperature increases, proteins can denature and rhabdomyolysis may develop as a result of lost skeletal muscle integrity. α_1AR activation increases intracellular calcium levels through a phospholipase C dependent mechanism. However, the role of intracellular calcium in UCP activation is not currently well understood. In this model, thyroid hormones play a permissive role through the regulation of UCP3 gene and protein expression.

Hemodynamic changes associated with MDMA-induced hyperthermia

Cutaneous vascular control contributes to normal temperature regulation. Hyperthermia activates sympathetic vasodilator activity increasing cutaneous blood flow to the skin and subsequently increasing convective heat transfer from the core to the periphery [19, 122]. The converse is also true; hypothermia activates sympathetically mediated vasoconstriction to maintain temperature. Cutaneous vasoconstriction also appears to contribute to the thermogenesis induced by MDMA [103, 123]. In these studies, MDMA increased ear pinna sympathetic nerve activity and reduced blood flow in rabbits and tail blood flow in rats. The atypical antipsychotic agent clozapine was shown to reverse MDMA-mediated activation of sympathetic nerve activity and subsequently hyperthermia [123]. However, clozapine has a broad spectrum of action and antagonizes many receptor systems, namely dopamine D_1, D_4 receptors, serotonin 2A receptors, and α_1AR [124].

Antagonism of each of these receptor subtypes attenuates the thermogenic effects of MDMA. D_1 receptor antagonism appears to inhibit activation of the hypothalamus by MDMA [125]. Serotonin 2A receptor antagonism may directly alter regulation of sympathetic nerve activity or may reduce MDMA-mediated dopamine release [126] and subsequently may indirectly attenuate hypothalamic activation [127]. The α_1AR appears to possess a dual role in regulating this thermogenic process influencing UCP3 activity and vasoconstriction [92]. Thus, clozapine may reverse MDMA-mediated hyperthermia as a result of a combination of a host of pharmacological effects. The role of vasoconstriction in the thermogenic effects of MDMA cannot be understated [103] and may play a major role in the inability to dissipate the heat produced as a result of indirect UCP3 activation. This creates a potent dual mechanism for MDMA-mediated thermogenesis with UCP3 activation generating heat and vasoconstriction preventing the dissipation of this heat.

Clinical applications to other thermogenic conditions

Serotonin syndrome, neuroleptic malignant syndrome, and malignant hyperthermia are toxicological conditions that may present with clinical manifestations that parallel those of MDMA. Similar to MDMA-induced thermogenesis, the hyperthermia induced by theses conditions is associated with the activation of the relevant sympathetic outflow and the hypothalamic-pituitary-adrenal (HPA) axis.

Other sympathomimetic agents in the phenethylamine class such as amphetamine [128] and methamphetamine [129] as well as structurally dissimilar stimulants such as cocaine [130] also activate the SNS and HPA axis generating heat and muscle rigidity [113, 118, 120, 131, 132, 133, 134, 135, 136]. Similarly, administered catecholamine agents (exogenous epinephrine and dobutamine) that do not cross the blood-brain barrier, and that do not activate β_3AR are generally devoid of the thermogenic effects of MDMA.

Because MDMA releases serotonin, the acute toxicities associated with MDMA are often referred to as a serotonin syndrome [137]. A true serotonin syndrome is an idiopathic disorder characterized by the development of cognitive, autonomic, and neuromuscular dysfunction which occurs typically in patients taking one or more serotonergic agents [138]. Indistinguishable from MDMA-induced toxicities, severe cases of serotonin syndrome can present with intense hyperthermia, muscle rigidity and clonus [139]. Numerous agents with pharmacologically indistinct structures have been associated with the serotonin syndrome, including monoamine oxidase inhibitors, tricyclic antidepressants, serotonin reuptake inhibitors, and lithium to name but a few [140]. As with MDMA, serotonergic agents that can induce a serotonin syndrome also activate the SNS and HPA axis. Rats treated with the serotonin precursor 5-hydroxytryptophan and the monoamine oxidase A inhibitor clorgyline not only develop a serotonin syndrome but also elevations in both serotonin and norepinephrine concentrations in the anterior hypothalamus [141, 142]. Increases in cerebral spinal fluid norepinephrine levels during the acute phase of serotonin syndrome have also been demonstrated in humans [143, 144]. Along with increasing central adrenergic neurotransmission, serotonin receptor agonists also stimulate the HPA axis, an effect that may be mediated by central noradrenergic stimulation [145].

Malignant hyperthermia (MH) is a clinical syndrome characterized by metabolic acidosis, hyperthermia, muscle rigidity, and rhabdomyolysis [146]. This syndrome occurs in persons with genetic defects in the ryanodine receptor which controls intracellular calcium and is typically seen with general anesthetic agents. While initiation of MH is thought to be from sudden increases in intracellular calcium, the SNS plays an important contributing role. In susceptible pigs, induction of MH has been shown to cause significant elevations in circulating norepinephrine [147, 148], with therapeutic α-blockade increasing animal survival [149]. Along with norepinephrine, elevated levels of serotonin have also been demonstrated in MH [150]. Likewise, MH-susceptible pigs have been shown to develop exaggerated responses to drugs with serotonergic activity, including MDMA [151, 152, 153]. Serotonin antagonists, however, have not been shown to be effective in preventing MH in susceptible swine [154]. Activation of the HPA axis as well is thought to occur in MH as pigs undergoing adrenalectomy appear more resistant the lethal effects of a halothane challenge [155]. Together these results suggest that activation of the relevant sympathetic outflow with subsequent release of norepinephrine likely plays a contributing role in the etiology of MH [147, 156].

Neuroleptic malignant syndrome (NMS) is a rare idiosyncratic reaction typically occurring in persons taking neuroleptics or after sudden withdrawal of dopamine agonists. NMS patients typical presents with hyperthermia, encephalopathy, skeletal muscle rigidity, and autonomic dysfunction [157]. NMS in general is thought to be due to central dopamine antagonism, particularly within the hypothalamus [158]. Although central dopamine function is likely important for the initiation of NMS, the SNS appears to play a critical role in its development [159]. In patients with NMS, peripheral and cerebral spinal fluid catecholamines have been shown to be elevated [159, 160, 161]. In particular, cerebral spinal fluid levels of norepinephrine during the acute phase of illness were two times greater than those of matched controls or during convalescence [162]. Other aspects of the HPA axis to date have not been studied with NMS.

Although the initiation of each of the above discussed syndromes is different, a common factor of activation of the SNS, particularly norepinephrine, and perhaps the HPA axis occurs in each of them. As we have previously discussed, activation of the SNS and HPA axis are central to the etiology of MDMA-mediated thermogenesis and toxicity. We believe that further studies need to be carried out to establish the role of skeletal muscle uncoupling proteins in these and other disorders a thermoregulation. If uncoupling proteins are shown to have a role, futures studies should target α_1AR and β_3AR antagonists as potential treatment modalities.

Conclusions/future directions

Here, we summarize recent progress in our understanding of the role of the HPT axis, SNS, and UCP activity in the thermogenesis induced by MDMA (Fig. 4). Pharmacological and genetic knockout mouse studies have clearly demonstrated a critical role for α_1AR, β_3AR and UCP3 in MDMA-mediated hyperthermia. As a result of these findings, pharmacological interventions targeting these receptors may be useful tools in the therapeutic armamentarium used in the treatment of sympathomimetic-induced thermogenesis.

Thermogenesis is complex and requires well-orchestrated interactions between the central nervous system, peripheral nervous system, and cell signaling. As a result of this complexity, several questions remain to be answered. What role do UCP1 and BAT play in the thermogenesis induced by MDMA? What effects do acute and chronic changes in thyroid and adrenal function have on MDMA-induced hyperthermia and UCP3 expression? What effects would the combination of pharmacological agents capably of targeting central activation on the HPT axis or SNS have with peripherally acting agents in

treating MDMA-induced hyperthermia and other thermogenic conditions? As our understanding of the cellular mechanisms involved in generating centrally mediated peripheral thermogenesis become clearer, the findings seen with MDMA-induced hyperthermia may have applications to other thermogenic diseases and conditions.

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