

The heat is on: Molecular mechanisms of drug-induced hyperthermia

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Abbreviations: α_1 AR, α_1 -adrenoceptor; β_3 AR, β_3 -adrenoceptor; BAT, brown adipose tissue; Ca^{2+} , calcium ion; FADH_2 , flavin adenine dinucleotide; MAOIs, monoamine oxidase inhibitors; LSD, lysergic acid diethylamide; MDMA, ecstasy, methylenedioxyamphetamine; NADH, nicotinamide adenine dinucleotide; NST, non-shivering thermogenesis; NE, norepinephrine; RYR1, ryanodine receptor; SSRIs, selective serotonin reuptake inhibitors; SERCA, sarcoplasmic/endoplasmic reticulum-ATPase pump; SKM, skeletal muscle; SNS, sympathetic nervous system; UCP1, uncoupling protein 1; UCP3, uncoupling protein 3; TCA, tricyclic antidepressant.

Thermoregulation is an essential homeostatic process in which critical mechanisms of heat production and dissipation are controlled centrally in large part by the hypothalamus and peripherally by activation of the sympathetic nervous system. Drugs that disrupt the components of this highly orchestrated multi-organ process can lead to life-threatening hyperthermia. In most cases, hyperthermic agents raise body temperature by increasing the central and peripheral release of thermoregulatory neurotransmitters that ultimately lead to heat production in thermogenic effector organs skeletal muscle (SKM) and brown adipose tissue (BAT). In many cases hyperthermic drugs also decrease heat dissipation through peripheral changes in blood flow. Drug-induced heat production is driven by the stimulation of mechanisms that normally regulate the adaptive thermogenic responses including both shivering and non-shivering thermogenesis (NST) mechanisms. Modulation of the mitochondrial electrochemical proton/pH gradient by uncoupling protein 1 (UCP1) in BAT is the most well characterized mechanism of NST in response to cold, and may contribute to thermogenesis induced by sympathomimetic agents, but this is far from established. However, the UCP1 homologue, UCP3, and the ryanodine receptor (RYR1) are established mediators of toxicant-induced hyperthermia in SKM. Defining the molecular mechanisms that orchestrate drug-induced hyperthermia will be essential in developing treatment modalities for thermogenic illnesses. This review will briefly summarize mechanisms of thermoregulation and provide a survey of pharmacologic agents that can lead to hyperthermia. We will also provide an overview of the established and candidate molecular mechanisms that regulate the actual thermogenic processes in heat effector organs BAT and SKM.

Introduction to Toxicant-Induced Hyperthermia

The 2 broad categories of medical conditions that result in increased body temperature are hyperthermia and fever. Hyperthermia refers to a catastrophic rise in body temperature resulting from an imbalance between the accumulation of body heat (either from environmental temperature extremes and/or body heat generation) and the capacity to dissipate heat. Hyperthermia typically involves a failure of central hypothalamic autoregulatory body temperature control. In contrast, fever is a regulated process and presents as an adaptive, physiologic response to infection involving a rise in the hypothalamic body temperature “set

point.” Although fever can be treated with non-steroidal anti-inflammatory agents (e.g. aspirin, acetaminophen), these drugs are ineffective in the therapeutic use for hyperthermia.¹

A variety of pharmacologic and toxicologic agents can trigger hyperthermia, and in most cases, they do so in large part by altering hypothalamic and sympathetic nervous system (SNS) regulation of body temperature. As depicted in **Table 1**, the most well characterized drug-induced hyperthermic syndromes include neuroleptic malignant syndrome, serotonin syndrome, sympathomimetic syndrome, and malignant hyperthermia. Hyperthermia typically coincides with devastating medical complications, including hyperkalemia, renal failure, metabolic acidosis, liver

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Table 1. Overview of drug-induced hyperthermic conditions

Drug-Induced Hyperthermia	Causative Agents	Implicated Molecular Mechanisms
Sympathomimetic Syndrome	Sympathomimetics: <i>Amphetamine, Methamphetamine, Cocaine</i>	Uncoupling proteins in BAT and SKM
Serotonin Syndrome	Antidepressants: <i>MAOIs</i> Antidepressants: ↑ Serotonin synthesis: <i>L-Tryptophan</i> ↓ Serotonin reuptake: <i>SSRIs, TCAs</i> ↓ Serotonin breakdown: <i>MAOIs</i> Sympathomimetics: ↑ Serotonin release: <i>MDMA, Methamphetamine, Cocaine</i> Serotonin agonists: <i>Lithium, LSD, Sumatriptan, Buspirone</i>	Uncoupling proteins in BAT and SKM
Malignant Hyperthermia	Inhalational anesthetics: <i>Halothane, Sevoflurane, Desflurane</i> Depolarizing neuromuscular agents: <i>Succinylcholine, Decamethonium, Gallamine Triethiodide</i>	Mutations in ryanodine receptor (RYR1) in SKM
Neuroleptic Malignant Syndrome	Atypical antipsychotics: <i>Olanzapine, Risperidone, Clozapine</i> Dopamine antagonists: <i>Haloperidol, Metoclopramide, Prochlorperazine, Promethazine</i>	Unknown

Abbreviations: Monoamine oxidase inhibitors (MAOIs), Lysergic acid diethylamide (LSD), Methylenedioxyamphetamine (MDMA, Ecstasy), Selective serotonin reuptake inhibitors (SSRIs), Tricyclic antidepressant (TCAs).

failure, intravascular coagulation, rhabdomyolysis and death.^{2,3} Thus, it is essential to understand the molecular changes that are involved in order to effectively treat hyperthermic crises.⁴ In most cases, the precise mechanisms of drug-induced heat generation are either unknown or only beginning to be understood. Treatment options that target thermogenic responses are currently limited to only small fraction of drug-induced hyperthermic illnesses, and our existing approaches for the vast majority of cases focus mainly on providing supportive care, which involves, external cooling, anxiolytic drugs, and rehydration.^{2,3,5}

Mechanisms of Thermoregulation: The Thermogenic Effector Systems in BAT and SKM

At thermoneutrality, the temperature at which animals need not make “extra” body heat to conserve normal body temperature (~37°C), basal temperature is maintained by the combined inefficiency of all exergonic cellular reactions.⁶ This is commonly referred to as obligatory thermogenesis. By contrast, in response to chronic cold exposure, feeding, and infection, endotherms can also rapidly increase thermogenesis to defend core body temperature or raise it through physiological heat-generating processes collectively referred to as facultative thermogenesis (see Table 2). The hypothalamus is the predominant, master controller of obligatory and facultative thermogenesis and also coordinates cooling mechanisms that dissipate heat, including sweating (in humans) and cutaneous vasodilation.⁷⁻⁹ A considerable body of work has defined many of the efferent and afferent neuroanatomical networks that signal within, to and from the hypothalamus, spinal cord, and periphery to control thermogenesis and heat dissipation. Although, the central and peripheral regulators of the neurochemical mechanisms that coordinate body temperature and thermogenesis are not the main focus of this manuscript, these pathways have been well-reviewed elsewhere.^{10,11}

When considering the direct thermogenic effector mechanisms of body heat production, only a dramatic increase in cellular “work” (e.g., muscle contraction) or other exergonic biochemical reactions in organs of sufficient metabolic capacity (e.g., BAT, SKM) can increase body temperature. Rapid muscle contraction / shivering is a highly thermogenic mode of SKM facultative thermogenesis that mediates an early and temporary component of the adaptive response to cold and infection. However, shivering is energetically costly and impractical to sustain for extended periods of time. Therefore, endotherms have evolved alternative mechanisms of heat generation that are recruited to withstand prolonged periods of cold exposure without shivering, i.e. non-shivering thermogenesis (NST).¹²

The 2 predominant thermogenic organs are BAT and SKM. SNS stimulation of BAT mitochondrial uncoupling protein 1 (UCP1) is the prototypical mechanism of NST. The role of UCP1 (originally identified as thermogenin) in heat production was initially characterized in the 1980s.¹³ UCP1 is part of a highly conserved family of mitochondrial solute carriers that have the ability to dramatically increase mitochondrial respiration and uncouple oxidative phosphorylation from ATP production by dissipating the proton gradient.¹⁴ By allowing protons to leak across the mitochondrial inner membrane and circumvent the F₁/F₀-ATPase complex of the electron transport chain, UCP1 releases the energy stored in the electrochemical gradient in the form of heat. Mitochondrial proton leak sets up what is commonly referred to as a biochemical futile cycle where 2 metabolic pathways (proton extrusion and proton leak) run simultaneously in opposite directions. The thermogenic futile cycle induced by mitochondrial proton leak is simulated by the metabolic toxicant dinitrophenol, a weak acid that works by localizing to the mitochondrial inner membrane and inducing dose-dependent proton leak. Persons exposed to the drug, either unintentionally in munitions factories or during its brief stint as

an anti-obesity medicine routinely developed hyperthermia and many died.^{15,16}

Sarcoplasmic reticulum calcium extrusion and ATP dependent calcium uptake in SKM is another futile cycle that is implicated in adaptive NST as well as drug-induced hyperthermia (discussed below). Other examples of futile cycles that may be involved in thermoregulation include the simultaneous occurrence of protein synthesis and breakdown (especially in muscle), and leakage of the sodium-potassium ATPase pump.¹⁷ However, in general, UCP1-dependent mitochondrial proton leak is the most well characterized physiological mechanism of NST in mammals. The extent to which other futile cycles contribute to whole body thermogenesis is unclear.

In the canonical response to cold, the hypothalamus activates the SNS, triggering the release of norepinephrine (NE) from sympathetic neurons that densely innervate BAT tissue depots.¹⁸ NE binds to β_3 -adrenoreceptors (β_3 AR) on brown adipocytes, initiating a signaling cascade that leads to the generation of free fatty acids from cytoplasmic multilocular lipid droplets via the cyclic AMP-protein kinase A-hormone sensitive lipase signaling pathway.^{19,20} Free fatty acids are then transported into BAT mitochondria via the carnitine shuttle system, where they are used as substrates for β -oxidation, and activate UCP1-mediated thermogenesis.^{20,21} Studies in genetic mutant mouse models with defects in fatty acid transport and metabolism that exhibit cold-sensitivity phenotypes²² reinforce the physiological importance of fatty acids in thermogenesis. On a side note, NE can also interact with α_1 -adrenoreceptors (α_1 AR) to stimulate vessel constriction and therefore block heat dissipation.²³ Support for the classic pathway of β_3 AR-stimulated thermogenesis has been demonstrated in studies where lipolysis and BAT thermogenesis are blocked by administration of the PKA inhibitor, H-89,²⁴ and by adrenergic blocking agents.²⁵ Likewise, experiments in brown adipocytes show that treatment with fatty acids alone can simulate NE-induced thermogenesis, but only in the presence of UCP1.²⁶ Recent elegant studies using mitochondrial patch clamping demonstrated that UCP1 requires long chain fatty acids to associate with and activate UCP1-driven thermogenic proton leak.²⁷ Thus, it is the presence and activation of UCP1 by fatty acids in brown adipocytes that endows these cells the specialized ability to generate heat. Although it is unclear the extent to which UCP1 participates in toxicant- and drug-induced hyperthermia, unpublished work in our laboratory shows that UCP1-null mice harbor dramatically blunted thermogenic responses to methamphetamine. These findings are consistent with recent studies demonstrating that mice with surgically ablated or denervated BAT exhibited lower peak temperatures and shorter durations of MDMA-induced hyperthermia.²⁸ Together, these findings validate the contribution of sympathetic regulation of BAT-UCP1 thermogenesis in drug-induced hyperthermia.

UCP1 is thought to be primarily, if not exclusively, expressed in BAT. Compared to rodents and newborns, only a fraction of adult humans have significant amounts of BAT.^{29,30} However, BAT tissue is not only activated by SNS stimulation, it can also increase in size in response to SNS activation.³¹⁻³³ Nonetheless, it remains uncertain the extent to which BAT contributes to NST in adults.³⁴ It is likely that alternative thermogenic systems in addition to BAT have evolved for the regulation of NST. This

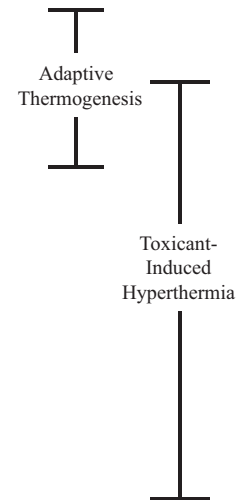
notion is supported by studies in animals that completely lack BAT (amphibians, reptiles, and birds) yet still exhibit SNS-dependent thermogenic responses.³⁵⁻³⁷ Furthermore, mice lacking UCP1 still exhibit some, albeit blunted NE-induced thermogenesis.³⁸

Skeletal muscle (SKM) is an attractive site of NST because it is the largest and most highly metabolic organ in the body. However, the mechanisms involved in muscle NST are far less well characterized compared to BAT. Several studies have implied that thermogenic systems similar to the UCP1-regulated pathways may also exist in SKM.³⁹⁻⁴¹ The idea that these SKM specific systems are regulated similarly to BAT thermogenesis through adrenergic stimulation is demonstrated in studies showing that NE administration in isolated muscle increases metabolism/thermogenesis.⁴² Interestingly, most if not all types of drug-induced hyperthermic conditions are associated with SKM damage.⁴³ For example, malignant hyperthermia (MH) involves a well-defined pharmacogenetic mechanism of drug-induced hyperthermia that arises from muscle in individuals harboring mutations in the SKM ryanodine receptor calcium channel (RYR1). In these patients, in response to volatile anesthetic administration, a highly exergonic futile cycle is created by the excessive release of Ca^{2+} in to the cytoplasm, thus uncoupling the sarcoplasmic / endoplasmic reticulum-ATPase pump (SERCA) from ATP hydrolysis.⁴⁴ Although it is not established whether RYR1 is a direct regulator of NST under normal, physiological circumstances, it is very likely that intramyocyte calcium handling, in general, plays an important role in normal thermoregulation. This idea is corroborated by recent studies showing that mice lacking sarcolipin, a negative regulator of SERCA were unable to defend core body temperature in cold environments.⁴¹

The skeletal muscle-enriched UCP1 homolog, UCP3, has also been implicated in drug-induced hyperthermia⁴⁵ and physiological NST responses to thyroid hormone.^{46,47} UCP3, shares more than 50% amino acid homology with UCP1 and has been shown to regulate proton flux in reconstituted systems *in vitro*.^{48,49} Compared to UCP1, UCP3 is more broadly expressed in heart, skeletal muscle, BAT,⁵⁰ and as we recently demonstrated, skin.⁵¹ UCP3 knock-out SKM mitochondria also show decreased proton leak (i.e., increased "coupling") in some studies^{52,53} and transgenic mice that overexpress UCP3 exhibit enhanced SKM thermogenesis.⁵⁴ Furthermore, UCP1-null mice exhibit skeletal muscle-specific, cold-induced adaptive thermogenesis that correlates with increased expression of UCP3.⁵⁵ In addition, thyroid hormone, arguably the most important positive endocrine regulator of the majority of obligatory and facultative thermogenic processes, also modulates SKM metabolic efficiency and proton leak.^{46,56} Interestingly, thyrotoxicosis, a dangerous feature of hyperthyroid conditions, is often accompanied by hyperthermia and rhabdomyolysis, but is associated with decreased activity of BAT UCP1.⁵⁶ Thus, the hyperthyroid hyperthermic crisis of thyrotoxicosis may preferentially involve SKM over BAT. The tenet that UCP1 homologues are mediators of thyroid thermogenesis is also supported with the finding that UCP3 is significantly increased at the protein level by thyroid hormone in rodents.^{57,58} Together, the data support the argument that

Table 2. Mechanisms of Obligatory and Facultative Thermogenesis

Category	Component	Site	Main Mediators	Description of Thermogenic Systems
Obligatory Thermogenesis	Standard Metabolic Rate	All Tissues	Thyroid Hormone, Leptin, Insulin, Glucagon <i>Drugs: Levothyroxine</i>	Heat produced from all metabolic reactions necessary to maintain basic organ function
Facultative Thermogenesis	Shivering Thermogenesis	SKM	CNS	Heat produced from muscle movement in response to cold
	Non-Shivering Thermogenesis	BAT	SNS, Thyroid Hormone	Heat produced in response to cold and diet. UCP1 and UCP3(?)
		SKM	SNS, Thyroid Hormone, Catecholamines <i>Drugs: Sympathomimetics, Serotonergic Agents</i>	Heat produced in response to cold and diet. UCP3 and Ca ²⁺ cycling
	Movement-Related Heat Production	SKM	CNS	Heat produced from exercise (voluntary muscle movement).
	Malignant Hyperthermia	SKM	Genetic mutation in RYR1 in SKM <i>Drugs: Inhalational Anesthetic agents</i>	Heat produced from unregulated Ca ²⁺ cycling when exposed to inhalation anesthetics



thermogenic mechanisms in SKM, involving the sarcoplasmic reticulum RYR1 and mitochondrial UCP3, are strong candidate mediators of muscle thermogenesis.

It should be mentioned that controversy exists dealing with UCP3 as a physiological thermogenic regulator. Soon after its identification in 1997, UCP3-null mice were generated and shown to protect body temperature normally as compared to wild type mice when exposed to cold.⁵⁷ As made evident in numerous subsequent reviews, this singular observation led the UCP field to conclude that UCP3 may not, unlike UCP1, mediate NST at least in response to cold exposure. However, the mere lack of a cold-phenotype does not rule out the involvement of UCP3 in thermogenesis by different mechanisms, or the possibility that a thermogenic compensatory process has fulfilled the heat requirements in the UCP3-null background. As mentioned above, thyroid hormone is a potent inducer of SKM UCP3 protein along with its upstream regulator β_3 AR.^{46,56} In a study using a severely cold intolerant, $\beta_{1,2,3}$ AR adrenergic receptor-deficient “ β -less” mouse model, T3 administration upregulated skeletal muscle UCP3, and drove oxygen consumption in muscle in the β -less background, but not in the β -less UCP3-null background.⁵⁹ Although UCP3 is not an established regulator of BAT NST, these results provide evidence that it is a key thermogenic effector in SKM, at least in response to certain types of drug- or hormonally-regulated stimuli.

Sympathomimetic-Induced Hyperthermia

Amphetamine-type drugs and their derivatives have long been associated with increased body heat production,

hypermetabolism, catastrophic and lethal hyperthermia and skeletal muscle rhabdomyolysis.⁶⁰ It is worth noting that amphetamine-type drugs and other sympathomimetics have different affinities for protein targets that affect the release and uptake of monoamines.^{61,62} For example, while cocaine and related analogs are selective, competitive inhibitors for dopamine monoamine transporters, MDMA acts as a competitive substrate for monoamine transporters and is relatively specific for serotonin.⁶³ Therefore, these sympathomimetics have distinct neuropharmacological profiles that contribute to the central and peripheral effects of these agents, depending on their direct and indirect sympathomimetic actions. However, the final common pathway of SNS-hyperthermia through adrenergic mechanisms appears similar for sympathomimetics as a class.

3,4-methylenedioxymethamphetamine (MDMA, Ecstasy) is a prototypical sympathomimetic amine agent and a worldwide major drug of abuse in large part because of its euphoric and stimulant properties.⁶⁴ MDMA increases body heat production via multiple mechanisms of thermoregulation. Unsurprisingly, even though catastrophic hyperthermia is a relatively rare event in MDMA use, one of the most commonly reported subjective effects of MDMA in humans is a feeling of “being hot”.⁶⁵ The idea that MDMA disrupts central regulation of body temperature is supported by observations that environmental ambient temperature and physical activity can profoundly affect the variability of MDMA-hyperthermic responses.^{66,67} In rodent models, hyperthermia occurs when MDMA is administered at ambient temperatures of 24°C or greater,⁶⁸ and the degree of hyperthermia positively correlates with increasing ambient temperature.^{69,70} In contrast, low environmental temperatures can block the hyperthermic effects of MDMA,⁷¹ and even result in hypothermic responses.⁶⁸ These observations

have important implications for MDMA users, where the drug is typically used in the setting of dance parties referred to as “raves” that increase the susceptibility to hyperthermia.

The challenge that arises from treating MDMA-induced hyperthermia stems from the complex and peripheral effects this agent can have on normal pathways of thermoregulation. MDMA administration is thought to cause a central deregulation of thermogenesis by altering the release of key monoamine neurotransmitters including serotonin, dopamine, and NE⁷²⁻⁷⁵ and by affecting reuptake and transport of these neurotransmitters. Neuronal activation in response to neurotransmitter release of specific hypothalamic pathways in the supraoptic and median preoptic nuclei has been demonstrated with *c-fos* expression staining.⁷⁶ MDMA and amphetamine derivatives have been shown to increase NE release,⁷⁷ and also potentiate the effects of NE by blocking reuptake through the NE transporter.^{61,78-80} Furthermore, MDMA can act as substrate for the monoamine transporter that can be taken up into nerve terminals to cause the redistribution of cytoplasmic monoamine vesicles and lead to the reverse transport of neurotransmitters.⁶³ These complex changes in neurotransmitter release and accumulation lead to alternations noreadrenergic signaling that contribute to the peripheral effects of MDMA by affecting cutaneous vasoconstriction of blood flow and stimulation of heat production in thermogenic effector organs SKM and BAT.^{62,81,82}

UCP3 and Thyroid Hormone in MDMA-Induced Hyperthermia

Several lines of evidence support the hypothesis that SKM UCP3 is a major molecular mediator of sympathomimetic-induced hyperthermia. Foremost, the strongest evidence is that UCP3 knockout mice have severely blunted (80–100%) thermogenic responses to MDMA *in vivo*.⁴⁵ Resistance to MDMA thermogenesis in UCP3 knockout mice also corresponds with complete protection from the lethal effects of the drug. Moreover, recent work showed that MDMA treatment in rodents led to a change in UCP3 protein phosphorylation and increased proton leak in the MDMA exposed animal SKM compared to controls.⁸³ Consistent with the aforementioned study in β -less mice administered thyroid hormone, hyperthyroid and hypothyroid rats show dramatic increases or complete blunting, respectively, of MDMA hyperthermic responses, with body temperatures correlating in a near linear fashion with levels of UCP3 protein in isolated SKM mitochondria.⁸⁴ Interestingly, a published case study showed that a woman that allegedly ingested 1–1.5 MDMA tablets (usually 150 mg MDMA) and died from hyperthermia had, on autopsy, subclinical hyperthyroidism.⁸⁵ This suggests the likelihood that some of the idiosyncratic hyperthermic reactions to sympathomimetics could be thyroid hormone related.

The identification of UCP3 (and now also UCP1) as a thermogenic effector of sympathomimetic agents provides the opportunity to target these thermogenic proteins or their upstream mediators to mitigate in human cases of MDMA toxicity. After MDMA administration, the plasma levels of NE and sympathetic

activity are elevated in human subjects.⁸⁶ In rodents, NE increases by 35-fold in the bloodstream prior to the development of peak hyperthermia,²⁵ as do levels of plasma free fatty acids.⁸⁷ The absence of a concomitant rise in the NE metabolite dihydroxyphenylglycol following MDMA administration suggests that peripheral NE reuptake is also blocked.²⁵ In response to increased plasma NE, triglyceride lipolysis leads to fatty acid release from white adipose tissue depots into the bloodstream. It is postulated that they serve as endocrine “thermokinins” to activate UCP3 for SKM thermogenesis.⁸⁷ Expression levels of α_1 - and β_3 AR are relatively low in skeletal muscle.⁸⁸⁻⁹⁰ However, adrenergic actions may also be mediated directly in SKM, as made evident by observations from the Astrup lab that infusion of various adrenergic agonists and sympathomimetics can directly stimulate oxygen consumption in perfused muscle in rodents and humans.^{91,92} As depicted in **Figure 1**, the fatty acid activation model of SKM UCP3 contrasts with that of BAT, where the storage, lipolysis, liberation of fatty acid, and fatty acid activation of UCP1 each occurs within the brown adipocyte and its mitochondria.

Owing to the central role of the SNS, NE, and β_3 AR, along with the α_1 AR induced vasoconstriction in sympathomimetic hyperthermia, treatment of rodents with experimental adrenergic blocking drugs prevents MDMA hyperthermia in rodents, and dramatically decreases SKM rhabdomyolysis.²⁵ Similarly, in clinically relevant work, treatment of rats with the pan α AR / β AR antagonist carvedilol completely blocks MDMA hyperthermia, and importantly, fully and rapidly resolves peak hyperthermia back to pre-treatment baseline temperatures.²⁵ Importantly, carvedilol is the only FDA approved drug that antagonizes β_3 AR. It is quite likely that this activity endows its treatment benefit in MDMA hyperthermia, because studies have shown in rodents that non-selective antagonists of β_{1-2} AR fail to block MDMA hyperthermia in the least.²⁵ Results from the carvedilol study using rodents were recently repeated in MDMA-exposed humans, where the MDMA-induced rise in body temperature was fully blocked, but not the euphoric effects.⁹³ As a bonus, carvedilol is generally regarded as a safe and well tolerated cardiovascular medicine and may be easy to employ in those cases when other supportive cooling measures and benzodiazepines fail to cool the hyperthermic patient. However, it is worth noting that the carvedilol is only available as an oral drug and exhibits variability in bioavailability due to poor gut perfusion. Furthermore, due to ethical considerations in the aforementioned clinical studies, subjects were given relatively low doses of MDMA and experienced only moderate hyperthermic responses. Therefore, additional studies are crucial in characterizing the drug’s clinical effectiveness in severe cases associated with MDMA toxicity.

Uncoupling Proteins and Molecular Mechanisms in Other Thermogenic Conditions

As mentioned above, many drug- and toxicant-induced hyperthermic conditions present with similar clinical symptoms when compared to sympathomimetic hyperthermia, and in most every

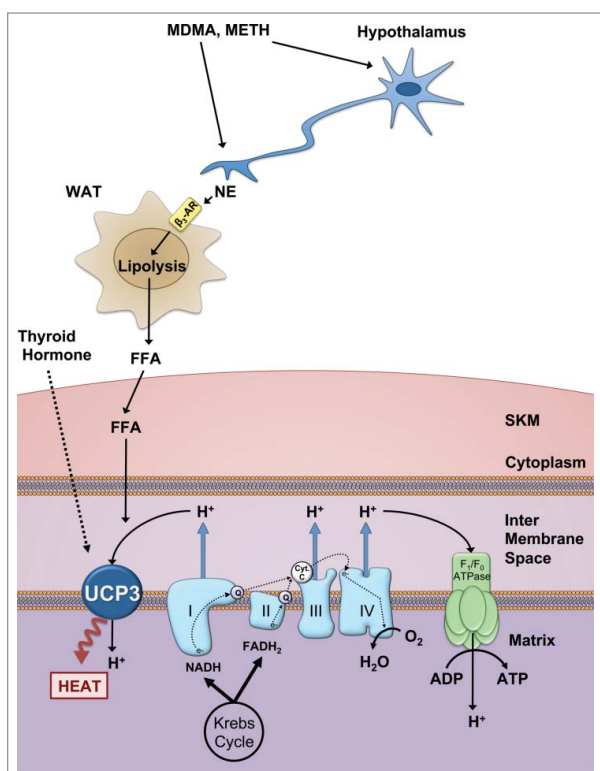


Figure 1. Hypothetical mechanism of MDMA-induced hyperthermia in response to treatment with amphetamine-like agents, hypothalamic neurons signal to the sympathetic nervous system causing the peripheral release of norepinephrine (NE). MDMA and other amphetamines also inhibit reuptake of NE at SNS synapses. NE binds to β_3 adrenergic receptors on target tissues, notably white adipocytes, where it triggers lipolysis, resulting in an increase in circulating free fatty acids (FFA). FFA are taken up by skeletal muscle (SKM), where they are used as substrates for β -oxidation, and also directly activate uncoupling protein 3 (UCP3). UCP3 stimulates proton (H^+) leak back into the mitochondrial matrix, circumventing ATP production and releasing the free energy from proton flux as heat. Thyroid hormone plays a contributory role in this model, through the regulation of UCP3 gene and protein expression in SKM. Abbreviations: Nicotinamide adenine dinucleotide (NADH), Flavin adenine dinucleotide (FADH₂)

case is thought to be associated with altered hypothalamic regulation of sympathetic outflow. As with MDMA, and as we have also shown with methamphetamine⁹⁴ it is highly likely that activation of the uncoupling mechanism by UCP3 in SKM (and perhaps UCP1 & UCP3 in BAT) accounts for a significant portion of thermogenesis induced by most sympathomimetics. The exemplars in this class are amphetamines, cocaine, ephedrine, and a new series of drugs on the worldwide market referred to as “bath salts.”⁹⁵ Serotonin syndrome is characterized by a variety of symptoms including cognitive, autonomic, neuromuscular dysfunction, and hyperthermia and can be induced by a variety of agents that have different actions involving serotonin.⁹⁶⁻⁹⁸ Along with sympathomimetics that increase serotonin release, other agents associated with serotonin syndrome that either act as agonists to serotonin or affect serotonin synthesis, reuptake, and breakdown respectively including LSD, L-tryptophan, tricyclic

antidepressants, and MAOIs⁹⁹ (Table 1). The tenet that the toxic thermogenic mechanisms associated with serotonin syndrome largely overlap with sympathomimetic syndrome is supported with experiments in animal models of serotonin syndrome showing enhanced levels of serotonin and norepinephrine in the anterior hypothalamus.^{100,101} It is likely that elevated serotonin levels increase central catecholamine release to alter hypothalamic regulation of the thyroid axis and peripheral release of NE.^{102,103} Unlike the hyperthermia observed with sympathomimetics, these central serotonin releasers are thought to have indirect effects on α_1 - and β_3 -adrenergic receptors, and that the potential uncoupling activity and heat generation in these conditions in BAT and SKM arises predominantly from NE release rather than a combined blockade of reuptake. Future work will be necessary to test the involvement of uncoupling proteins in response to non-sympathomimetic thermoregulation agents.

UCP Hyperthermia Versus RYR1 “Malignant Hyperthermia”

It has classically been assumed that MDMA hyperthermia must share a common mechanism with RYR1-mediated malignant hyperthermia, especially because the clinical and metabolic changes in both conditions are very similar. However, the literature indicates that the final heat-generating mechanisms of each syndrome are distinct, despite some potential upstream common regulatory features.¹⁰⁴ As mentioned, MH is an autosomal dominant disorder caused by a mutation in RYR1, a major regulator of Ca^{2+} transport in SKM.¹⁰⁵ It is triggered in susceptible patients by exposure to general anesthetic gases, and is clinically characterized by metabolic acidosis, hyperthermia, muscle rigidity, and rhabdomyolysis. The postulate that the mechanisms of MDMA induced hyperthermia are analogous to MH is reasonable based on observations that both conditions produce SKM damage, and alterations in NE and serotonin signaling.¹⁰⁶⁻¹⁰⁸ However, unlike sympathomimetic hyperthermia, where activation of the SNS is the primary culprit behind massive heat generation, whether or not SNS and serotonergic modulation is causal or merely correlative in MH is not clear. Similar to what has been observed with MDMA toxicity in rodent models, MH susceptible adrenalectomized pigs were also more resistant to MH toxicity,¹⁰⁹ and treatment with thyroid hormone led to an earlier onset of MH in pigs,¹¹⁰ implicating activation of the hypothalamic-pituitary-adrenal and -thyroid axes in MH. Despite the similarities, a recent study done by Schutte et al.¹¹¹ to investigate whether MDMA could trigger MH in susceptible pigs found that there were no metabolic differences between MH-susceptible pigs and MH-normal pigs following MDMA exposure. This study, along with the finding that MDMA increases intracellular Ca^{2+} through a mechanism independent of the ryanodine receptor pathway in SKM,¹¹² strongly suggests that MDMA is not a classic trigger of MH and works through a distinct biochemical mechanism to produce heat. This is also supported by studies using the RYR1 inhibitor dantrolene, the established and effective MH antagonist.¹⁰⁴ Because of the erroneous assumption that

MDMA hyperthermia and MH arise from the same mechanism, dantrolene has been indicated for treatment of MDMA hyperthermia.¹¹³ However, clinicians should be cognizant of the fact that the molecular mechanisms underlying these 2 pathologies are significantly divergent and should consider the possibility that other strategies may provide greater therapeutic benefit compared to dantrolene (i.e. adrenergic blockade) in extreme cases of MDMA hyperthermia.

Concluding Remarks

As shown in studies with MDMA and methamphetamine, it is likely that the family of uncoupling proteins, chiefly UCP1 and UCP3 play the dominant roles in the generation of the hyperthermic and SKM toxic responses to the vast majority of drugs that increase SNS activity via hypothalamic, serotonergic and direct peripheral adrenergic mechanisms. If true, this would predict that the use of the drug carvedilol may not only be useful for MDMA poisoning, but also in response to overdose with the cadre of sympathomimetics, and perhaps the newest members of this class – the bath salts. Testing the involvement of UCPs in a variety of thermogenic responses to other drugs and toxins will be of obvious importance.

It should also be noted that very interesting recent work highlights the fact that it would be erroneous to assume all sympathetic activity is “the same” when considering thermogenesis and hyperthermia. Indeed, SNS activation by different stimuli can lead to diverse thermogenic responses. This was recently demonstrated in a clinical report showing that cold exposure activated human BAT, but systemic treatment with a thermogenic dose of the sympathomimetic drug ephedrine failed to do so.¹¹⁴ This highlights the fact that much is to be learned regarding the neuro-anatomic distinctions that govern body temperature regulation to diverse SNS regulatory stimuli. Because so very few treatments exist, investigators will need to use strong mechanistic tools, tissue-targeted knockout mice, and neuro-tracing methodologies to provide a comparative assessment of the detailed mechanisms that distinguish the varied drug induced thermogenic syndromes. Recent clinical work identifying carvedilol as a potential sympathomimetic hyperthermia treatment is a step in the right direction.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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