On the Mechanisms Underlying 3,4-Methylenedioxymethamphetamine Toxicity: The Dilemma of the Chicken and the Egg

E. Puerta I. Hervias N. Aguirre

Department of Pharmacology, School of Medicine, University of Navarra, Pamplona, Spain

Key Words

3,4-Methylenedioxymethamphetamine ·
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Abstract

Administration of 3,4-methylenedioxymethamphetamine (MDMA, ecstasy) to various experimental animals has been shown to induce a selective damage to serotonergic axon terminals. While a great consensus appears to exist regarding the causative role of reactive oxygen species (ROS) in the mechanisms underlying MDMA toxicity, the source of free radicals is still a matter of debate. While some authors support dopamine metabolism/oxidation inside 5-hydroxytryptamine (5-HT) terminals as the key factor responsible for ROS formation and final 5-HT terminal degeneration, others believe it is MDMA metabolism into pro-oxidant compounds. Although at first sight both hypotheses appear to contend with each other, it may not be the case. This mini-review was therefore undertaken to try to reconcile both hypotheses and to address the dilemma of the causality of MDMA neurotoxicity.

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3,4-Methylenedioxymethamphetamine (MDMA or ecstasy) is an illicit drug used by young adults in the USA, Europe and elsewhere. The appeal of MDMA is related to

its unique profile of psychotropic actions, which includes amphetamine-like stimulant effects, coupled with feelings of increased emotional sensitivity and closeness to others [1, 2]. However, it is worth noting that MDMA-related medical complications have risen more than 20-fold in recent years, consistent with the increasing popularity of the drug [3]. The serious adverse effects of MDMA intoxication include cardiac arrhythmias, hypertension, hyperthermia, serotonin (5-HT) syndrome, hyponatremia, liver complications, seizures, coma and, in rare cases, death [4]. Further, accumulating evidence also indicates that long-term MDMA abuse is associated with cognitive impairments and mood disturbances, which can last for months after the cessation of drug intake [5–7].

On the other hand, it is well known that single or repeated injections of MDMA can cause long-lasting changes in neurochemical and histological markers of serotonergic function in brains of rats [8–11], primates [12, 13] and, possibly, humans [14]. Such neurotoxicity is evidenced by the decline in the activity of tryptophan hydroxylase [15], a decrease in the content of 5-hydroxytryptamine (5-HT) [16], a lower density of [³H]paroxetine-labeled 5-HT transporters in several regions of the brain [17, 18] and impairments of central 5-HT function. This constellation of findings, coupled with neuroanatomic observations using different techniques such as silver impregnation methods [19], neuronal staining for neuronal death [20] and immunohistochemistry [21], strongly sug-

Table 1. Drugs known to prevent MDMA-induced 5-HT depletion by preventing the acute hyperthermic response after MDMA administration

Pharmacologic mechanism	Drug	Reference
Glutamate receptor antagonists	MK-801, CGS 19755, NBQX	Farfel and Seiden [122]
Free radical scavenger	N-tert-butyl- α -phenylnitron	Che et al. [123] but see [48]
5-HT _{2A} receptor antagonist	Ketanserin	Malberg et al. [124]
Dopamine synthesis inhibitor	α -Methyl- <i>p</i> -tyrosine	Malberg et al. [124]
Nitric oxide synthase inhibitors	N(G)-nitro-L-arginine methyl ester	Taraska and Finnegan [125]
GABA mimetics	Chlormethiazole	Colado et al. [126]
Anesthetic	Pentobarbitone	Colado et al. [127]
Inhibitor of glucose transport/phosphorylation	2-Deoxy-D-glucose	Hervias et al. [18]
Glutathione conjugation	Diethylmaleate	O'Shea et al. [128]
Cannabinoid receptor agonists	Δ 9-Tetrahydrocannabinol; CP 55940	Morley et al. [129]

gests that MDMA damages 5-HT terminals in rats [reviewed by 22].

According to these evidences, a number of investigators have also tested whether MDMA abuse results neurotoxic in the human brain [23–33]. Some of these studies concluded that MDMA may also be toxic to humans, since the 5-hydroxyindoleacetic acid levels in the CSF are reduced in MDMA abusers [23, 24, 26, 28, 29]. Recent advances in neuroimaging techniques have been applied to the study of the serotonin system in the brains of humans with a history of MDMA abuse. Positron emission tomography or single-photon emission computed tomography used in combination with a 5-HT transporter ligand found a lower density of brain 5-HT transporter sites (SERT) and a reduction in their function in MDMA users [14, 27, 34–36].

While the exact mechanisms underlying MDMA-induced neurotoxicity remain unclear, the close relationship between hyperthermia and neurotoxicity engendered by MDMA is well established [37–41]. Thus, a common feature of many drugs known to prevent MDMA toxicity relies on their ability to block the acute hyperthermia induced by MDMA, with such protection disappearing if the temperature of the rats is kept elevated (table 1). Similarly, other nonpharmacological manipulations capable of preventing the acute hyperthermic response caused by MDMA also provide substantial protection against the neurotoxic effects of the drug [42]. Furthermore, the converse also occurs; any manipulation known to potentiate MDMA-induced hyperthermia exacerbates long-term 5-HT deficits caused by the drug and small increases in ambient temperature lead to large changes in both core body temperature and MDMA-in-

duced 5-HT neurotoxicity in the rat [39–41]. This represents a clinically relevant aspect in MDMA abusers, since MDMA is often taken at 'rave parties,' where dancing takes place in crowded conditions with a high ambient temperature, physical activity and dehydration, which may all contribute to increase the hyperthermic response induced by MDMA and thereby promote acute or long-term complications [7]. However, some compounds have been shown to protect MDMA-induced serotonergic deficits without affecting hyperthermia [43–45], suggesting that hyperthermia is not a primary contributing factor to 5-HT neurotoxicity [46], despite its key modulator effects [47].

On the other hand, there is overwhelming evidence supporting the role of oxidative stress due to the formation of free radicals in MDMA-induced damage to 5-HT terminals. The findings that free radical scavengers and antioxidants attenuate the MDMA-induced 5-HT deficits [15, 48–50] provide indirect evidence for the involvement of free radicals in the mechanism of MDMA neurotoxicity. In addition, MDMA has been reported to produce cellular changes, e.g. lipid peroxidation or protein nitration, consistent with the formation of free radicals [51–53]. Finally, Colado et al. [52, 54, 55], as well as Shankaran et al. [56–58], have demonstrated that MDMA increases the formation of hydroxyl radicals, as evidenced by an increased extracellular concentration of 2,3-dihydroxybenzoic acid following salicylic acid administration.

Despite the general agreement on oxidative stress as one of the main causes underlying MDMA-induced 5-HT toxicity, the source of reactive oxygen species (ROS) is still a matter of debate. Several authors have suggested

that the depletion of 5-HT in the rat brain is dependent, in part, upon dopamine (DA) metabolism/oxidation inside 5-HT terminals [59–61]. Other authors, however, have proposed that some quinone thioether adducts resulting from the peripheral metabolism of MDMA might be the ultimate mediators of its neurotoxicity [39, 62–66].

Involvement of DA in the Mechanisms Underlying MDMA Toxicity

A decade ago, Sprague et al. wrote the review entitled ‘An integrated hypothesis for the serotonergic axonal loss induced by 3,4-methylenedioxymethamphetamine’ [61]. In this work the authors integrated the current knowledge, at that time, proposing the following hypothesis as a plausible sequence of events leading to long-term 5-HT deficits after MDMA. (A) MDMA induces an acute release of 5-HT and of DA. (B) 5-HT released by MDMA activates postsynaptic 5-HT_{2A/2C} receptors located on GABA interneurons resulting in a decrease in GABAergic transmission and an increase in DA release and synthesis. (C) Extracellular DA, present in abnormally high amounts, then enters the depleted 5-HT terminal by means of the SERT, and (D) once inside the 5-HT terminal, DA is metabolized by monoamine oxidase-B (MAO-B) resulting in ROS formation and selective degeneration of the serotonergic terminals. A thorough revision of all the evidences supporting this hypothesis can be found in the above-mentioned review, and so, we will briefly summarize the main findings.

MDMA administration to rats induces an acute and rapid release of 5-HT. This has been broadly demonstrated not only in vitro following addition of MDMA to brain slices or synaptosomal preparations but also using in vivo microdialysis or voltametry [reviewed by 22]. MDMA-induced 5-HT release is also reflected by the fact that the 5-HT concentration in brain tissue decreases markedly during the first few hours following drug administration [16, 67–72]. On the other hand, it is also well known that MDMA causes both transporter- and impulse-mediated DA release, an effect that is in part dependent upon 5-HT transmission [73–74]. MDMA-induced DA release is potentiated in 5-hydroxytryptophan-treated rats [75], which is consistent with the view that 5-HT release after MDMA contributes to the effect of MDMA on DA release [74]. Microdialysis studies have shown that blockade 5-HT₂ receptors significantly reduces the efflux of endogenous DA [76–78], whereas the activation of these receptors ap-

pears to be necessary for MDMA-induced DA release [79–81], free radical formation [56, 57, 75] and neurotoxicity [82, 83]. Consistent with these observations, 5-HT_{2A} antagonists attenuated the increased L-DOPA utilization and extracellular levels of DA seen after MDMA administration as well as MDMA-induced neurotoxicity [78, 84]. Further, Nash and Nichols [85], using in vivo microdialysis, showed that the acute increase in extracellular DA concentration following MDMA was negatively correlated with the concentrations of 5-HT and its major metabolite, 5-hydroxyindoleacetic acid measured 7 days following drug administration. In a similar fashion, Shankaran et al. [56] showed that mazindol, a DA uptake inhibitor, suppressed acute DA release and 2,3-dihydroxybenzoic acid formation and attenuated the long-term depletion of 5-HT caused by MDMA administration in rats. It is worth mentioning that 5-HT precursors, tryptophan or 5-hydroxytryptophan, attenuate rather than exacerbate MDMA-induced 5-HT depletions in rats [86]. Although apparently at odds with the above evidences supporting the role of the 5-HT-DA connection in the mechanisms underlying MDMA toxicity, it appears reasonable to think that these amino acids may compete with tyrosine for the large neutral amino acid transporter located at the blood-brain barrier to enter the brain [87] (see below and also fig. 1).

The GABAergic system is thought to serve as a modulator of dopaminergic activity. Colado et al. [88] demonstrated that chlomethiazole, a GABA agonist, attenuated the serotonergic toxicity induced by MDMA treatment in rats, although in part due to the temperature-lowering effect of chlomethiazole. In another investigation, Yamamoto et al. [77] conducted microdialysis studies and observed an MDMA-induced increase in extracellular DA coupled with a decrease in extracellular GABA levels in the rat striatum that was reversed by the 5-HT_{2A} receptor antagonist, ritanserin. It is possible that MDMA-mediated reduction in GABA might enhance its effects on DA synthesis and release [61, 89, 90], thus potentiating its neurotoxic effects in the long term.

It is known that DA can enter the 5-HT terminal by means of the 5-HT transporter, an effect that may be especially relevant when DA is present in abnormally high amounts [91–94]. DA uptake by SERT is effectively inhibited by 5-HT uptake blockers [91], providing a likely explanation of how selective serotonin reuptake inhibitors prevent both the acute increase in hydroxyl radical formation which follows MDMA [57] and the long-lasting neurotoxic effects [10, 40, 95, 96]. Moreover, inhibition of DA metabolism is protective, as evidenced by the block-

Fig. 1. The proposed sequence of events resulting in serotonergic neurotoxicity after MDMA. Formation of toxic MDMA metabolites and DA metabolism promotes an oxidative environment responsible for MDMA toxicity towards 5-HT terminals in the brain. BBB = Blood-brain barrier; GSHT = glutathione transporter; LNAA = large neutral amino acid transporter.

ade of MDMA toxicity. Sprague and Nichols [51] showed that MAO-B metabolizes DA inside the 5-HT terminal, producing hydrogen peroxide which could lead to lipid peroxidation and general oxidative stress. Consistent with these findings, MAO-B inhibitors such as L-deprenyl or MDL-72974 or knocking down MAO-B enzyme expression using antisense oligonucleotides attenuate the long-term MDMA-induced 5-HT loss [51, 97].

Treatment with α -methyl-*p*-tyrosine, a DA synthesis inhibitor, prior to the administration of MDMA protects against 5-HT deficits [98]. In a similar fashion, complete protection against MDMA neurotoxicity is afforded when DA terminals are destroyed by prior administration of 6-hydroxydopamine [99]. Conversely, an increase in MDMA-induced neurotoxicity is observed following pretreatment with the DA precursor L-DOPA [100]. More

recently, Simantov and Tauber [101] demonstrated that programmed cell death caused by MDMA could be potentiated by DA but not by 5-HT in a human serotonergic cell line.

Despite all above evidences on the role of DA in mediating MDMA toxicity, there are also caveats associated with how DA may mediate MDMA-induced 5-HT depletions in brain areas that are sparsely innervated by DA such as the frontal cortex or the hippocampus. Another important drawback of the 'dopamine hypothesis' relies on the fact that MDMA administered directly into the brain causes DA release but not long-term 5-HT depletions [102]. Further, Ricaurte's group demonstrated that rats depleted of vesicular and cytoplasmic DA, using reserpine in combination with α -methyl-*p*-tyrosine, were still susceptible to MDMA neurotoxicity when drug-in-

duced hypothermia was averted by raising ambient temperature, suggesting that endogenous DA is not essential for the expression of MDMA-induced toxicity [38].

These important issues have been expanded upon recently [59]. The novel findings from this study are twofold. First, these authors showed that MDMA increases the concentrations of tyrosine in DA-sparse brain regions, which is converted to DOPA via the nonenzymatic hydroxylation of tyrosine to DOPA and subsequently to DA via aromatic L-amino acid decarboxylase. Further, conversion of tyrosine into DA can directly mediate toxicity as blockade of L-amino acid decarboxylase attenuates MDMA-induced 5-HT depletions. These findings do not contradict Ricaurte's data (see above) as endogenous DA may not be essential for MDMA toxicity, but DA formed within 5-HT terminals from tyrosine may be a critical factor. Second, the tyrosine levels increased in the brain after systemic but not after centrally administered MDMA, which could explain why MDMA is not toxic when directly administered into the brain [40, 65, 102].

It has been consistently shown that the striatum is more resistant to MDMA-induced 5-HT loss than the hippocampus and cortex [39, 42, 43, 103, 104]. Similar findings were described by Breier et al. [59] despite reporting a higher tyrosine concentration rise after MDMA in the striatum as compared to the hippocampus. A plausible explanation given by the authors for this apparent discrepancy may be related to the differential presence of DA endogenous to each region. Thus, DA terminals of the striatum may buffer much of the increased tyrosine after MDMA, whereas absence of DA terminals in the hippocampus may render this brain region more vulnerable to lower tyrosine concentrations due to its lower capacity to enzymatically metabolize tyrosine to DA for neurotransmission and subsequent enzymatic degradation [59].

Noteworthy, Breier et al. [59] assume that the preexistence of hydroxyl free radicals is responsible for the oxidation of tyrosine into DOPA. This issue is of key importance, especially if we take into account that a systemic injection of L-tyrosine in combination with the intrastriatal perfusion of MDMA in hyperthermic rats does not result in long-lasting reductions in 5-HT [66]. It should be noted that the dose of L-tyrosine and the concentration of MDMA in the perfusate used in this latter study give rise to the range of extracellular MDMA and serum concentrations of L-tyrosine observed following peripheral administration of neurotoxic doses of MDMA [65, 66].

Involvement of MDMA Metabolism in the Mechanisms Underlying MDMA Toxicity

Since direct injection of MDMA into the brain fails to reproduce the serotonergic neurotoxicity seen following systemic administration [40, 65, 102], some authors have postulated that peripheral metabolism of MDMA into toxic compounds is responsible for neurotoxicity. In rats, MDMA is mainly N-demethylated by several cytochrome P450 isoenzymes to form 3,4-methylenedioxymphetamine (MDA). MDMA and MDA are further O-demethylated to 3,4-dihydroxymethamphetamine and 3,4-dihydroxyamphetamine, respectively (fig. 2). 3,4-Dihydroxymethamphetamine and dihydroxyamphetamine are highly redox-unstable catechols and can be rapidly oxidized to their corresponding orthoquinones forming adducts with GSH and other thiol-containing compounds [105–108].

Due to their structure, these catechols would not be expected to cross the blood-brain barrier. However, the rapidly formed quinones may conjugate with GSH to form adducts such as 5-(glutathion-S-yl)- α -methyl dopamine (5-GSyl- α -MeDA) or 5-(glutathion-S-yl)-N-methyl- α -MeDA [108, 109] that may cross the blood-brain barrier using a GSH transporter. Indeed, the brain uptake index for 5-GSyl- α -MeDA is reduced by GSH coadministration, indicating a competitive uptake mechanism [110]. Further support for the possible role of these compounds arose from the observation that treatment with acivicin, a γ -glutamyl transpeptidase inhibitor (an enzyme involved in the breakdown of GSH and GSH S-conjugates), not only increased the brain uptake index of 5-GSyl- α -MeDA but also the neurotoxicity of systemically administered MDA or MDMA, presumably due to increased concentrations of thioether conjugates in the brain [64]. Interestingly, intracerebroventricular administrations of GSH or N-acetylcysteine conjugates of MDMA metabolites resemble not only some of the acute neurobehavioral effects of the parent drug but also its neurotoxic pattern [62–64, 110, 111].

The *in vivo* detection of quinone thioether adducts in the brain of rats after systemic administration of MDMA and the evaluation of the neurotoxicity of some of these metabolic species further strengthens the hypothesis that MDMA metabolic disposition contributes significantly to the induction of neurotoxicity [112]. Moreover, the capability of some of these putative thioether adducts of MDMA metabolites to generate ROS in a 5-HT-transporter-dependent manner has been demonstrated [93]. Cytotoxicity of these metabolites has been shown not

Fig. 2. Postulated pathways of MDMA metabolism [adapted from 88, 98]. Only structures considered in this mini-review are shown.

only in neurons [113] but also in other tissues such as cardiomyocytes [114], hepatocytes [115, 116] or human renal proximal tubular cells [117]. Importantly, these cytotoxic effects are aggravated under hyperthermic conditions [118, 119] and can be formed by human liver microsomes in a CYP2D6-dependent manner [111, 120].

As stated above, a close relationship appears to exist between MDMA-induced changes in core body temperature and long-term 5-HT loss. Accordingly, Goñi-Allo et al. [39] investigated whether MDMA metabolic disposition is affected by changes in core body temperature. These authors showed that there is a direct relationship between plasma concentrations of MDMA metabolites and core body temperature. The administration of MDMA at 15°C blocked the hyperthermic response and long-term 5-HT depletion found in rats treated at an am-

bient temperature of 22°C. At 15°C, the plasma concentrations of MDMA were significantly increased, whereas those of MDA, 4-hydroxy-3-methoxymethamphetamine or 4-hydroxy-3-methoxyamphetamine (fig. 2) were reduced when compared to rats treated at 22°C. By contrast, hyperthermia and 5-HT deficits were exacerbated in rats treated at 30°C. Noteworthy, the plasma concentrations of MDMA metabolites were greatly enhanced in these animals. Furthermore, interfering in MDMA metabolism using the catechol-O-methyltransferase inhibitor entacapone potentiated the neurotoxicity of MDMA, indicating that metabolites that are substrates for this enzyme may contribute to neurotoxicity. This study became the first report showing a direct relationship between core body temperature and MDMA metabolism – a finding that has implications on both the temperature depen-

dence of the mechanism of MDMA neurotoxicity and human use, as hyperthermia is often associated with MDMA abuse in humans.

In agreement with other authors, the effects produced by MDMA are likely to be a result of its metabolism to not 1 neurotoxic thioether metabolite but a combination of several adducts already mentioned or adducts still to be discovered [121], and in light of the above-mentioned findings, we believe this metabolic pathway to be an area of particular relevance to human users of MDMA. It is important to note that enzymes that participate in the activation of MDMA (CYP2D6) and inactivation of MDMA metabolites (COMT) are highly polymorphic in the human, and so, the fraction converted to toxic metabolites may vary greatly in human abusers exposed to similar doses of MDMA [112].

According to the above-mentioned findings there appears to exist overwhelming data supporting each of the 2 hypotheses, making it difficult to opt for one of them. However, it may not be the case. The tyrosine/DA hypothesis states that the pre-existence of hydroxyl radical is necessary for the conversion of tyrosine into DOPA. In an elegant study by Jones et al. [93] it was shown that 5-(GSyl)- α -MeDA and 2,5-bis(GSyl)- α -MeDA, and to a lesser extent MDA and MDMA, induced a concentration- and time-dependent increase in ROS formation in human SERT-transfected cells. Moreover, both metabo-

lites stimulated DA uptake in these cells, an effect that contributed further to ROS formation and that was attenuated by fluoxetine. Moreover, rats treated with a nontoxic dose of MDMA showed significant reductions in brain 5-HT content when given under heating conditions or if combined with entacapone or acivicin, which interfere with MDMA metabolism or increase brain MDMA metabolite availability, respectively. By contrast, the brain 5-HT content remained unchanged after the administration of a nontoxic dose of MDMA in combination with a dose of L-tyrosine known to produce a similar increase in serum tyrosine levels to those found after a toxic dose of MDMA [66]. Altogether, these data indicate that while tyrosine may contribute to MDMA-induced toxicity, MDMA metabolism appears to be the key limiting step, which somehow unravels the dilemma of the chicken and the egg. As depicted in figure 1, we propose that both tyrosine and MDMA metabolism into pro-oxidant compounds act in concert inside the serotonergic terminals to promote oxidative stress and final terminal loss.

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