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α-MDA Not Neurotoxic via I.C.V.

Major metabolites of (±)3,4-methylenedioxyamphetamine (MDA) do not mediate its toxic effects on brain serotonin neurons

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The two major metabolites of (±)3,4-methylenedioxyamphetamine (MDA), alpha-methyldopamine (α-MeDA) and 3-*O*-methyl-α-methyldopamine (3-*O*-Me-α-MeDA), were administered to rats intracerebroventricularly and into brain parenchyma. In addition, their precursors, (α-MeDOPA and 3-*O*-Me-α-MeDOPA, respectively) were administered systemically, individually and in combination. None of these treatments produced a lasting depletion of brain serotonin (5-HT). These findings suggest that neither of MDA's major metabolites mediate its toxic effects on 5-HT neurons and that either a minor metabolite is responsible or that alternate mechanisms are involved.

(±)3,4-Methylenedioxyamphetamine (MDA) is one of several ring-substituted amphetamine derivatives [3,4-methylenedioxymethamphetamine (MDMA, 'Adam'), 3, 4-methylenedioxyamphetamine (MDEA, 'Eve')] used recreationally by humans. It is also a major metabolite of MDMA, both in vivo and in vitro[3,30]. Previous studies have shown that all of these amphetamine analogues (MDA, MDMA and MDEA) possess serotonin (5-HT) neurotoxic activity. Neurotoxicity of MDA and related compounds has been demonstrated in the rat[2,4,16–18,23,27], guinea pig[4], squirrel monkey[19,20,29], and rhesus monkey[10,12]. The neurotoxic potential of these drugs in man is currently under evaluation[22].

MDA's serotonergic neurotoxic effects have been characterized both chemically and anatomically. Neurochemically, MDA-treated animals exhibit lasting reductions in the concentration of brain serotonin (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA)[2,16,27] as well as in the number of 5-HT uptake sites[2,17]. Morphologically, evidence of axonal damage has been obtained using silver degeneration methods[4,17], as well as immunocytochemical techniques[15,16]. While the occurrence of MDA neurotoxicity is widely accepted, the mechanism by which it takes place has yet to be elucidated.

Since there is reason to suspect that MDA itself is not neurotoxic[15], the present study sought to test the hypothesis that a major metabolite of MDA might be responsible for its adverse effects on 5-HT neurons. In particular, this investigation evaluated the 5-HT neurotoxic potential of alpha-methyl-dopamine (α-MeDA) and 3-*O*-methyl-α-methyl-dopamine (3-*O*-Me-α-MeDA), which together comprise a major fraction of MDA's metabolic products in the rat[8,13], dog and monkey[14].

Male albino Sprague–Dawley rats (Harlan Co., Indianapolis, IN) weighing 180–220 g were used. Animals were housed in clear plastic cages (6 per cage) with free access to food (Purina Rodent Chow) and water. They were kept in a temperature-controlled colony room (22 ± 1 °C) which had fluorescent lighting automatically turned on at 06.00 h and off at 18.00 h. Rats were treated according to one of 3 drug regimens. In treatment regimen No. 1, rats first received carbidopa (25 mg/kg; i.p.), then α-methyl-dihydroxyphenylalanine (α-Me-DOPA) or 3-*O*-methyl-α-methyl-dihydroxyphenylalanine (3-*O*-α-Me-DOPA) subcutaneously at a dose of 200 mg/kg. Both α-MeDOPA and 3-*O*-Me-α-MeDOPA were dissolved in acidified dimethylsulfoxide (DMSO, pH = 3–4) and were given twice daily (08.00 and 17.00 h) for 4 consecutive days. Each drug was tested in a group of 6 rats, with a third group receiving a combination of the two drugs (200 mg/kg of each). In treatment regimen No. 2, α-MeDA and 3-*O*-Me-α-MeDA were given i.c.v. as previously described[21]. Both compounds were given at doses ranging from 100 to 400 μg/10 μl. Each dose was tested in 6 rats. Sham controls (n = 6) received only 10 μl of the vehicle (physiological saline). Animals for this

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TABLE I

Effect of α-MeDOPA, 3-O-Me-α-MeDOPA and MDA on regional 5-HT levels in rat brain

	Striatum[3]	*Hippocampus*	*Cortex*
(A) α-MeDOPA[1]			
Control	0.27 ± 0.02	0.27 ± 0.03	0.17 ± 0.02
α-MeDOPA	0.21 ± 0.02	0.25 ± 0.03	0.17 ± 0.02
(B) 3-O-Me-α-MeDOPA[1]			
Control	0.21 ± 0.02	0.18 ± 0.02	0.27 ± 0.02
3-O-Me-α-MeDOPA	0.16 ± 0.03	0.20 ± 0.02	0.26 ± 0.02
(C) Methylenedioxyamphetamine (MDA)[2]			
Control	0.24 ± 0.02	0.28 ± 0.02	0.19 ± 0.02
MDA	0.06 ± 0.01*	0.07 ± 0.02*	0.06 ± 0.01*

[1] α-MeDOPA and 3-O-Me-α-MeDOPA were given at a dose of 200 mg/kg s.c. twice daily for 4 days.
[2] MDA was given at a dose of 20 mg/kg twice daily for 4 days.
[3] Values represent the mean ± S.E.M. and are expressed in μg/g tissue.
*$P < 0.05$, two-tailed Student's *t*-test.

experiment were pretreated with pargyline (50 mg/kg; i.p.) 30–45 min prior to i.c.v. administration of either compound. In treatment regimen No. 3, α-MeDA was administered intrastriatally to 6 rats at a dose of 400 μg/μl. The compound was dissolved in physiological saline. A total of 10 μl were injected over a 90-s interval. The injection needle was kept in place for an additional 30 s. Again, sham controls received 10 μl of the vehicle in an identical manner. A final treatment group (positive control, $n = 6$) received MDA hydrochloride s.c. twice daily for 4 days. Ten to 14 days after drug treatment, rats were killed for determination of regional brain serotonin content as detailed elsewhere[18]. α-MeDOPA, 3-O-Me-α-MeDOPA and serotonin creatinine sulfate were purchased from the Sigma Chemical CO., St. Louis, MO; carbidopa monohydrate and 3-O-Me-α-MeDA were generous gifts from Merck, Sharp and Dohme, West Point, PA; α-MeDA was kindly provided by the Sterling-Winthrop Research Institute, Rensellaer, NY and (±)3, 4-methylenedioxyamphetamine was obtained from the National Institute of Drug Abuse, Rockville, MD.

TABLE II

Effect of α-MeDA, 3-O-Me-α-MeDA and 5,7-DHT on regional 5-HT levels in rat brain

	Striatum[3]	*Hippocampus*
(A) α-MeDA[1]		
Control	0.39 ± 0.03	0.36 ± 0.02
α-MeDA	0.37 ± 0.03	0.37 ± 0.03
(B) 3-O-Me-α-MeDA[1]		
Control	0.37 ± 0.03	0.33 ± 0.02
3-O-Me-α-MeDA	0.38 ± 0.03	0.30 ± 0.03
(C) 5,7-DHT[2]		
Control	0.25 ± 0.03	0.35 ± 0.03
5,7-DHT	0.02 ± 0.03*	0.05 ± 0.02*

[1] α-MeDA and 3-O-Me-α-MeDA were given at a dose of 400 μg/10 μl.
[2] 5,7-DHT was given at a dose of 200 μg/10 μl.
[3] Values shown are the mean ± S.E.M. and are expressed in μg/g tissue.
*$P < 0.05$, two-tailed Student's *t*-test.

TABLE III

Effect of intrastriatal α-MeDA[1] on 5-HT level in the striatum of the rat

Ipsilateral striatum	0.36 ± 0.04[2]
Contralateral striatum	0.33 ± 0.02

[1] α-MeDA was given at a dose of 400 μg/10 μl.
[2] Values represent the mean ± S.E.M. μg of 5-HT.

Neither α-MeDOPA nor 3-O-Me-α-MeDOPA caused a lasting depletion of regional brain 5-HT when administered subcutaneously 10–14 days earlier (Table IA–B). Combined treatment with the two drugs was also without effect (data not shown). By contrast, subcutaneous administration of MDA produced a profound depletion of 5-HT in the rat brain (Table IC). Rats given intraventricular α-MeDA or 3-O-Me-α-MeDA did not show a depletion of regional brain 5-HT two weeks later (Table IIA–B). In contrast to this, rats given 5,7-dihydroxytryptamine (5,7-DHT) by the same route showed a marked depletion of 5-HT (Table IIC). Intrastriatally administered α-MeDA did not deplete striatal 5-HT on a long-term basis (Table III).

The results of the present study indicate that neither of MDA's major metabolites, α-MeDA or 3-O-Me-α-MeDA, produces a long-term depletion of rat brain 5-HT. Since a lasting depletion of brain 5-HT is generally a reliable indicator of 5-HT neurotoxicity[2,17,29], the present findings argue against a role of either of those metabolites in the 5-HT neurotoxic action of MDA. While this conclusion seems warranted, several alternate explanations merit consideration. First, while neither metabolite alone may be capable of inducing 5-HT neural damage, it may be that if given in combination with the other, either metabolite might be capable of producing 5-HT neural damage. Mitigating against this possibility is the fact that combined administration of α-MeDOPA and 3-O-Me-α-MeDOPA did not induce a long-term depletion of rat brain 5-HT. A second possibility is that the presence of MDA itself, in conjunction with one or both of its major metabolites, is essential for expression of 5-HT neurotoxicity. Unfortunately, this possibility is difficult to evaluate because MDA, by itself, depletes rat brain 5-HT[17,27]. Yet another possibility is that a minor, as yet unidentified, reactive metabolite of MDA is responsible for its 5-HT neurotoxic effect. If so, the present study, by eliminating two of the major metabolites,

should aid in its identification. Finally, it could be that the 'toxic metabolite' hypothesis is untenable, and that alternative mechanisms need to be explored.

To date, 5 other mechanisms have been postulated to play a role in the 5-HT neurotoxic effect of MDA and related compounds. These include: (1) endogenous formation of 5,6-dihydroxytryptamine (5,6-DHT)[5], (2) a dopamine-mediated event leading to damage of 5-HT neurons[24,28], (3) generation of neurotoxic electrophilic intermediates[26], or (4) an excitotoxin-mediated process leading to stimulation of *N*-methyl-D-aspartate (NMDA) receptors[25] with subsequent 5-HT neural damage and (5) calcium (Ca^{2+})-dependent toxicity, possibly linked to 5-HT release[1]. While each of these postulated mechanisms has attractive features, none is without problems. For example, while 5,6-DHT has been shown to be formed endogenously in rats given a single high dose of methamphetamine[5], it is not found in all rats showing a lasting depletion of 5-HT. With regard to the view that dopamine may somehow mediate the 5-HT neurotoxic action of ring-substituted amphetamines[24,28], this notion is limited by the fact that it does not account for the occurrence of 5-HT neurotoxicity in areas lacking dopamine innervation (e.g. somatosensory cortex). As to the possible involvement of neurotoxic electrophilic intermediates (hydrogen peroxide and/or other toxic byproducts of monoamine metabolism)[26], the major shortcoming of this proposal is that while such byproducts have been well characterized in catecholaminergic systems[9], their formation within 5-HT neurons is less well established. With respect to the possible involvement of NMDA receptors[25], the weakness of this proposal is that, to date, there is only one report that an NMDA receptor blocking agent protects against MDMA-induced 5-HT neurotoxicity[7], and in this study, complete protection was not seen in all brain regions. Finally, as to the possible involvement of calcium[1], data to support this hypothesis have only been obtained in cell culture. Extension of this interesting observation to the intact animal will be important. Thus, while various hypotheses have been advanced to explain the 5-HT neurotoxicity of MDA and related compounds, for the moment, all of them await validation.

Although it is difficult to completely exclude the possibility that MDA's major metabolites did not induce lasting 5-HT deficits because of insufficient dosage or inappropriate route or frequency of drug administration, this seems unlikely for several reasons. First, with regard to dosage, both α-MeDOPA and 3-*O*-Me-α-MeDOPA were given at high dosage (200 mg/kg), a dosage that is ten-fold higher than the dose of MDA that produced severe 5-HT depletion (Table I). Since 24 h following MDA administration, over 65% of MDA in rat brain tissue has been metabolized to α-MeDA and another 30% has been converted to 3-*O*-Me-α-MeDA[13], insufficient dosage of the metabolites is unlikely to be the cause of their inactivity as 5-HT neurotoxic agents. As to the possibility that the s.c. route of drug administration may not have been adequate, it should be noted that MDA was given by the same route and it depleted 5-HT quite effectively. In addition, both metabolites were also tested i.c.v. and intrastriatally, and still found to be without effect. Finally, with respect to frequency of drug administration, the precursors of both metabolites were given twice daily for 4 days. When MDA is given according to this schedule of drug administration, it causes a profound depletion of brain 5-HT (Table I). It therefore seems improbable that drug delivery factors account for the failure of the MDA metabolites to deplete 5-HT. More likely, the lack of 5-HT-depleting activity of α-MeDA and 3-*O*-Me-α-MeDA is related to their lack of 5-HT neurotoxic activity.

In conclusion, the results of the present study suggest that the major metabolites of MDA, α-MeDA and 3-*O*-Me-α-MeDA, do not mediate its adverse effects on rat brain 5-HT neurons. As such, it may be that a minor metabolite (acting alone or in concert with other metabolites) is involved. Alternatively, it could be that the neurotoxicity of MDA is not mediated by a metabolite, but rather is related to one (or a combination) of the other mechanisms discussed above. Clearly, further studies are needed to elucidate the mechanisms by which MDA and related drugs damage central 5-HT neurons. Insight into these mechanisms should prove useful in the assessment of the public health risks of novel synthetic amphetamine analogs. In addition, such knowledge could yield helpful clues regarding the etiology of idiopathic forms of neurodegenerative diseases involving brain monoaminergic neurons (Alzheimer's disease and Parkinson's disease).

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