Effect of ascorbate and cysteine on the 3,4-methylenedioxymethamphetamine-induced depletion of brain serotonin

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Summary. The extent of long-term depletion of serotonin (5-HT) produced by 3,4-methylenedioxymethamphetamine (MDMA) was assessed in rats treated with the antioxidants sodium ascorbate or L-cysteine. There was a 30–35% reduction in the striatal concentration of 5-HT 7 days following a single injection of MDMA (20 mg/kg, sc). MDMA had no significant effect on striatal concentrations of 5-HT in rats that had been treated with ascorbate (250 mg/kg, ip) or cysteine (500 mg/kg, ip) 30min prior to and 5hrs following the administration of MDMA. Treatment with ascorbate or cysteine did not alter the accumulation of MDMA in brain as determined by in vivo microdialysis. Moreover, neither ascorbate nor cysteine altered the stimulation of dopamine release elicited by MDMA. These data are supportive of the view that MDMA-induced toxicity of 5-HT neurons may be related to the production of free radicals and subsequent oxidative damage.

Keywords: 3,4-methylenedioxymethamphetamine (MDMA), dopamine, ascorbic acid, cysteine, serotonin.

Introduction

The single or repeated administration of 3,4-methylenedioxymethamphetamine (MDMA) produces a long-lasting, neurotoxicity of 5-HT neurons, as evidenced by a reduction in the density of 5-HT axon terminals, the number of 5-HT reuptake sites labelled by [3H]paroxetine, tryptophan hydroxylase activity and 5-HT concentrations (Commins et al., 1987; Schmidt, 1987; Stone et al., 1987; Battaglia et al., 1988; O’Hearn et al., 1988). The neurotoxicity resulting from high dose MDMA administration is selective for 5-HT, localized to the axon terminals, and reversible (Battaglia et al., 1988; Scanzello et al., 1993).
Considerable evidence exists in support of the hypothesis that the MDMA-induced depletion of brain 5-HT is due, in part, to the acute and sustained release of dopamine. Thus, disruption of dopaminergic function attenuates the MDMA-induced toxicity to 5-HT terminals (Stone et al., 1988; Brodkin et al., 1993). Although the nature of the dopamine-dependent processes involved in the MDMA-induced depletion of brain 5-HT remains to be determined, dopamine itself has been shown to produce neurotoxicity under a variety of experimental conditions (Filloux and Townsend, 1993; Michel and Hefi, 1990). It has been suggested that dopamine-mediated cellular toxicity is due to oxidative stress that results from the enzymatic or autoxidation of dopamine and the subsequent formation of reactive quinones and hydroxyl free radicals (Chiuheh et al., 1993; Hastings and Zigmound, 1994).

Gibb et al. (1990) have suggested that MDMA promotes oxidative stress within 5-HT terminals on the basis of the observation that the MDMA-induced inactivation of tryptophan hydroxylase is reversed by sulfhydryl reducing conditions (Stone et al., 1989). In addition, depletion of brain 5-HT by other amphetamine analogues (e.g., methamphetamine, parachloroamphetamine) has been shown to be attenuated by antioxidants and or free radical scavengers, such as cysteine, ascorbate or mannitol (Wagner et al., 1985; DeVito and Wagner, 1989; Steranka and Rhind, 1987).

In the present study, the effects of treatment with sodium ascorbate or L-cysteine on the MDMA-induced depletion of striatal 5-HT were determined. The effects of these antioxidants on the extracellular concentrations of MDMA in the striatum and on the MDMA-induced release of dopamine also were determined.

**Materials and methods**

Male rats of the Sprague-Dawley strain (200–275 g, Zivic Miller Labs, Allison Park, PA) were used in these studies. The animals were housed three per cage in a temperature and light-controlled room until the day of surgery for microdialysis. The animals had free access to food and water.

Rats were implanted with a stainless steel guide cannula under ketamine/xylazine (70/7 mg/kg, i.m.)-induced anesthesia 48–72 hrs prior to the insertion of the dialysis probe. On the morning of the dialysis experiment, a concentric style dialysis probe (4.5 mm of exposed membrane) was inserted through the guide cannula into the striatum: A, 1.2 mm; L, 3.1 mm; V, −6.5 mm from bregma, according to the stereotaxic atlas of Paxinos and Watson (1986). A modified Krebs-Ringer buffer (mM: NaCl, 145; NaHPO4, 6; CaCl2, 1.2; KH2PO4, 1.0; KCl, 2.0; pH 7.4) was delivered through the probe at a rate of 1.8 μl/min. After a 2-hr equilibration period, dialysis samples were collected every 30 min. At least four baseline samples were obtained prior to drug treatment.

The extracellular concentration of dopamine in the striatum was quantified with high performance liquid chromatography with electrochemical detection using methods similar to those described elsewhere (Gudelsky et al., 1994). Briefly, dialysis samples were injected onto a C18-column (Phenomenex, Torrance, CA) connected to a LC-4B amperometric detector (Bionalytical Systems, West Lafayette, IN). The mobile phase consisted of 35 mM citric acid, 54 mM sodium acetate, 50 mg/l of disodium ethylenediamine tetraacetate, 70 mg/l of octansulfonic acid sodium salt, 3% methanol, pH 4.2 pumped at a flow rate of 0.3 ml/min.

For post mortem analysis of brain 5-HT the rats were killed by decapitation 7 days after MDMA administration. Striatal samples were dissected from 1 mm slices, and the
tissue was kept frozen (−40°C) until analyzed for 5-HT. At the time of assay, the samples were sonicated for 5 s in 0.2 N perchloric acid containing 0.1% cysteine. The samples were subjected to centrifugation, and an aliquot of the resulting supernatant fluid was analyzed for 5-HT by high performance liquid chromatography with electrochemical detection using the conditions described for the analysis of extracellular dopamine content.

MDMA concentrations in dialysis samples were determined by high performance liquid chromatography with UV detection. MDMA was separated on a C18 reverse phase column (150mm × 3.9mm, 3 μm particle size) with a mobile phase (pH 4.0) consisting of 50mM sodium phosphate and 15% acetonitrile pumped at a flow rate of 1.0ml/min. The UV detector was set at 214nm and the sensitivity was set at 0.05 AUF. The limit of detection was approximately 1 ng/sample.

Analysis of the tissue concentrations of 5-HT was performed using a two-way analysis of variance. Differences between treatment groups were determined with the use of the Student Newman-Keuls test. Data from dialysis experiments were analyzed using a two-way repeated measures analysis of variance (Sigmastat, Jandel Scientific). Multiple pairwise comparisons were performed using the Student Newman-Keuls test. Treatment differences were considered statistically significant at P < 0.05.

Results

A single injection of MDMA (20mg/kg, sc) significantly reduced striatal concentrations of 5-HT by 30–35% 7 days following its administration (Fig. 1). However, there was no significant reduction of striatal concentrations of 5-HT in MDMA-treated rats that had received sodium ascorbate (250mg/kg, ip) 30min prior to and 5hrs after treatment with MDMA (Fig. 1). Treatment of rats with cysteine (500mg/kg, ip) 30min prior to and 5hrs following the administration of MDMA also significantly attenuated the MDMA-induced depletion of 5-HT in the striatum (Fig. 1).

In order to address the possibility that the neuroprotective effects of cysteine and ascorbate were due to alterations in the pharmacokinetics of MDMA, the extracellular concentrations of MDMA in the striatum were determined using in vivo microdialysis. There were no significant differences in the extracellular concentrations of MDMA in rats treated with MDMA alone compared with those for rats treated with ascorbate (250mg/kg, ip) or cysteine (500mg/kg, ip) and MDMA (Fig. 2).

Inasmuch as alterations in the extent of MDMA-induced dopamine release are associated with concomitant changes in the extent of MDMA-induced 5-HT depletion (Brodkin et al., 1993; Gudelsky et al., 1994; Schmidt et al., 1991), it also was decided to assess the magnitude of MDMA-induced dopamine release in rats of the treatment groups. Treatment of rats with either ascorbate or cysteine alone did not alter striatal dopamine release (data not shown). Moreover, there were no significant differences in the extent of stimulated dopamine release in the striatum of rats given MDMA alone compared to that in rats treated with ascorbate and MDMA or cysteine and MDMA (Fig. 3).

Discussion

The present findings of attenuation by ascorbate and cysteine of MDMA-induced 5-HT depletion are consistent with the view that the MDMA-induced
depletion of brain 5-HT involves oxidative damage that results from the generation of electrophilic substances (e.g., free radicals). The results are consistent with previous reports in which it was demonstrated that these antioxidants attenuated the depletion of brain dopamine or 5-HT elicited by other psychomotor stimulants, such as methamphetamine or p-chloroamphetamine (Wagner et al., 1985; DeVito and Wagner, 1989; Steranka and Rhind, 1987; Invernzizzi et al., 1989). In addition, Colado and Green (1995) have demonstrated that the MDMA-induced depletion of 5-HT is attenuated in rats treated with a spin trap agent, phenyl-butyl nitrone, which
Fig. 2. Extracellular concentrations of MDMA in the striatum after its systemic administration. Rats received vehicle, sodium ascorbate (250 mg/kg, ip) or cysteine (500 mg/kg, ip) 30 min prior to the administration of MDMA (20 mg/kg, sc). Dialysis samples were obtained every 30 min after the administration of MDMA. Each symbol represents the mean and SE of 5 rats.

Fig. 3. Effect of ascorbate or cysteine on MDMA-stimulated dopamine release. Ascorbate (250 mg/kg, ip), cysteine (500 mg/kg, ip) or the vehicle was injected 30 min prior to the administration of MDMA (20 mg/kg, sc), as indicated by the arrows. Each symbol represents the mean and SE of 6–9 rats.

acts presumably as a scavenger of free radicals. In further support of the view that MDMA induces a state of oxidative damage are the findings that the MDMA-induced inactivation of tryptophan hydroxylase is reversible by reducing agents (Stone et al., 1989) and that MDMA increases the formation of thiobarbituric acid reactive material (Sprague and Nichols, 1995), which often is viewed as an index of enhanced lipid peroxidation (Gutteridge and Halliwell, 1990).

The mechanism whereby MDMA might generate oxidative damage is unknown. MDMA produces a sustained increase in the extracellular concentration of dopamine (Nash, 1990; Gudelsky et al., 1994; Schmidt et al., 1994), and dopamine itself has been shown to be neurotoxic under different experi-
mental conditions (Filloux and Townsend, 1993; Michel and Hefti, 1990; Rosenberg, 1988). It can be envisioned that the sustained increase in the extracellular concentration of dopamine following MDMA results in the enzymatic or autoxidation of dopamine to free radicals. In support of this contention are the findings that methamphetamine and MDMA increase the formation of 2,3-dihydroxybenzoic acid (Giovanni et al., 1985; Gudelsky and Yamamoto, 1994), an index of the generation of hydroxyl free radicals (Floyd et al., 1984). Additionally, dopamine may be oxidized to form reactive quinones that bind to sulfhydryl groups on protein cysteinyl groups (Hastings and Zigmond, 1994). Finally, Cadet and coworkers (Cadet et al., 1994a,b; Hirata et al., 1995) have proposed that superoxide radicals mediate the neurotoxicity and lethality to MDMA and methamphetamine.

An alternative explanation is that MDMA induces oxidative damage as a result of the generation of catechols and reactive quinones formed from the oxidation of MDMA itself and/or its metabolite, 3,4-methylenedioxyamphetamine (Hiramatsu et al., 1990).

Consideration also was given to the possibility that the apparent neuroprotective effect of ascorbate and cysteine was due to alterations in the pharmacokinetic disposition of brain MDMA. Extracellular concentrations of MDMA were determined and neither ascorbate or cysteine altered the accumulation of MDMA in brain. Although Invernizzi et al. (1989) proposed that the antagonism of fenfluramine or p-chloroamphetamine induced 5-HT depletions by cysteine was due, in part, to a cysteine-induced reduction in brain concentrations of the drug, pharmacokinetic considerations do not appear to account for protection by the antioxidants of MDMA-induced depletion of striatal 5-HT.

The pharmacological manipulation of the extent to which MDMA increases dopamine release has been shown to be accompanied by alterations in the extent of the MDMA-induced depletion of 5-HT. Thus, suppression or enhancement of MDMA-induced dopamine release is accompanied by attenuation or exacerbation, respectively, of the MDMA-induced depletion of 5-HT (Brodkin et al., 1993; Gudelsky et al., 1994; Schmidt et al., 1991). For this reason extracellular concentrations of dopamine were determined following the administration of MDMA to rats treated with ascorbate, cysteine or the vehicle. Inasmuch as ascorbate or cysteine did not significantly alter the MDMA-induced release of dopamine, it is unlikely that these antioxidants attenuated MDMA-induced 5-HT depletion through an attenuation of stimulated dopamine release.

In summary, evidence in support of the hypothesis that MDMA-induced 5-HT depletion in the brain is related to oxidative damage includes the findings that (1) MDMA-induced inactivation of tryptophan hydroxylase is reversed by reducing conditions, (2) MDMA produces evidence of increased formation of hydroxyl free radicals and lipid peroxidation and (3), as evidenced in part by the present findings, MDMA-induced depletion of 5-HT is prevented by antioxidants and/or free radical scavengers.
MDMA-Induced 5-HT depletion

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References


Cadet JL, Ladenheim B, Baum I, Carlson E, Epstein C (1994a) CuZn-superoxide dismutase (CuZnSOD) transgenic mice show resistance to the lethal effects of methylenedioxymethamphetamine (MDA) and of methylenedioxymethamphetamine (MDMA). Brain Res 655: 259–262


Gudelsky G, Yamamoto B (1994) MDMA increases the extracellular concentration of 2,3-dihydroxybenzoic acid in the striatum: evidence for increased hydroxyl radical formation. Soc Neurosci Abstr 20: 343


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