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RAPID COMMUNICATION

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Inhibition of MAO-B protects against MDMA-induced neurotoxicity in the striatum

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Abstract The effects of the MAO-B inhibitors, L-deprenyl and MDL-72974 on MDMA-induced serotonergic neurotoxicity in rats were examined. MDMA alone produced a significant decrease in the number of 5-HT uptake sites, measured as a decrease in the B_{max} for binding of [^{3}H]paroxetine, and in 5-HT and 5-HIAA levels in the striatum. L-Deprenyl and MDL-72974 attenuated this MDMA-induced decrease in serotonergic markers. The data suggest a key role for MAO-B in the expression of the neurotoxicity produced by MDMA in the striatum.

Key words MDMA · Neurotoxicity · L-Deprenyl · MDL-72974

Introduction

Administration of high doses of 3,4-methylenedioxymethamphetamine (MDMA) to rats and nonhuman primates is known to induce degeneration of serotonergic neuron terminals in the central nervous system (McKenna and Peroutka 1990). However, the exact mechanism by which MDMA-induces this serotonergic toxicity remains unknown. Several studies have suggested a direct role for 5-HT (e.g. Berger et al. 1992) and for DA (e.g. Stone et al. 1988; Nash 1990; Schmidt et al. 1991). However, we have recently shown that the 5-HT precursors tryptophan and 5-hydroxytryptophan afford protection against MDMA-induced neurotoxicity (Sprague et al. 1994) suggesting that toxicity is not due to high extracellular 5-HT levels, or 5-HT metabolites. Support for the importance of DA comes from studies showing that MDMA-induced neurotoxicity can also be blocked by the DA synthesis inhibitor alpha-methyl-*p*-tyrosine, the DA uptake blocker GBR 12909 (Stone et al. 1988), and is potentiated by the DA synthesis precursor L-dopa (Schmidt et al. 1991).

Because MAO-B preferentially deaminates DA (refer to Westland et al. 1985), the present study was designed to determine whether the MDMA-induced serotonergic neurotoxicity could be the result of the action of MAO-B on the high levels of DA produced by MDMA.

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Materials and methods

Animals and experimental design

In the first series of experiments, 18 adult, male Sprague-Dawley rats were individually housed with ad lib access to food and water. The animals were given a single dose of saline, MDMA (40 mg/kg, SC), L-deprenyl (2 mg/kg, IP) or a combination of L-deprenyl 30 min before MDMA. Seven days after treatment the animals were killed by decapitation and the striatum was removed.

Similarly, in the second series of experiments, 18 animals were randomly allocated into three groups treated with saline, MDMA (40 mg/kg, SC), the selective MAO-B inhibitor MDL-72974 (1.25 mg/kg, IP) (Zreika et al. 1987), or a combination of MDL-72974 30 min before MDMA. Seven days after treatment the animals were killed by decapitation and the striatum was removed.

5-HT uptake site determination

An estimate of serotonergic neuron loss in the striatum was made using the [^{3}H]paroxetine binding method to label 5-HT uptake sites (Marcusson et al. 1988). Battaglia et al. (1987) have shown that only the B_{max} and not the K_d values are altered after MDMA treatment. Therefore, it is possible to estimate the number of 5-HT uptake sites with a single saturating (1 nM) concentration of [^{3}H]paroxetine. Nonspecific binding was determined with 1 μm fluoxetine.

5-HT and 5-HIAA determination

The striatum was sonicated for 15 s in 50 µl of a solution containing 0.1 M $HClO_4$, 0.05% Na_2EDTA, and 0.1% $Na_2S_2O_5$. The samples were then centifuged at 14000 *g* for 4 min. The supernatant was collected and 50 µl was injected onto the HPLC column (Brownlee C18, Anspec, Ann Arbor, Mich). A model 400 EG & G Princeton electrochemical detector (Princeton, N.J.) with series dual electrodes was utilized. The levels of 5-HT and 5-HIAA levels were determined from standard curves using the Dynamax Method Manager software (Rainin; Woburn, Mass).

Statistics

Data were analyzed by ANOVA with a Student Newman-Kuels post-hoc test. Significance was set at $P \leq 0.05$

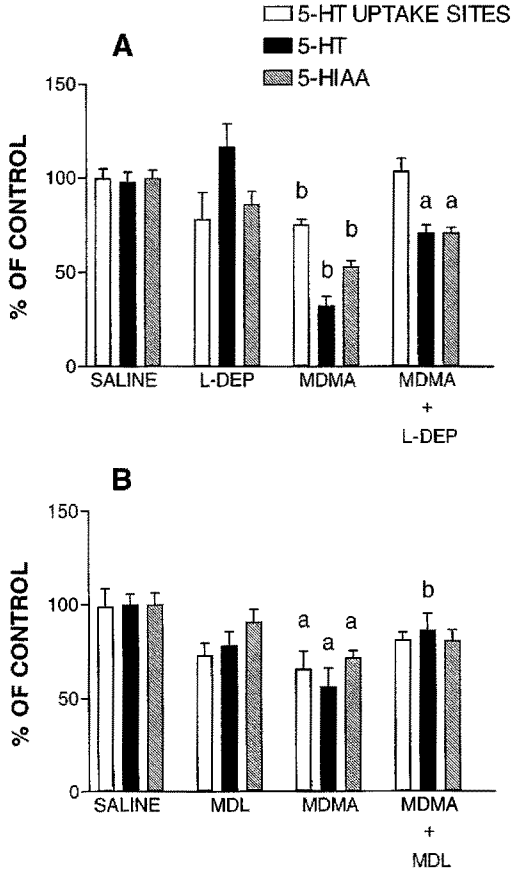

Fig. 1 **A** Effects of MDMA (40 mg/kg) and L-deprenyl (2 mg/kg) on serotonergic markers in the striatum 7 days after treatment. 5-HT, 5-HIAA, and the density of 5-HT uptake sites ([^{3}H]paroxetine B_{max}) for the striatum are reported as a percent of control values. Saline control values were: 5-HT 2157 ± 109, 5-HIAA 1308 ± 53 pg/mg wt; uptake sites 31.5 ± 1.5 fmol/g. Each value is the mean ± SEM for six animals. [a]Indicates significantly different from saline; [b]indicates significantly different from all other groups. **B** Effects of MDMA (40 mg/kg) and MDL-72974 (1.25 mg/kg) on serotonergic markers in the striatum 7 days after treatment. Saline control values in this experiment were: 5-HT 1719 ± 97, 5-HIAA 2561 ± 156 pg/mg; uptake sites 24.8 ± 2.3 fmol/g. Each value is the mean ± SEM for six animals. [a]Indicates significantly different from Saline; [b] indicates significantly different from MDMA alone

Results

MDMA produced a significant decrease in the number of 5-HT uptake sites in the striatum and in 5-HT and 5-HIAA levels (Fig. 1) in both studies. L-Deprenyl alone (Fig. 1A) and MDL-72974 alone (Fig. 1B) had no significant effects on these 5-HT neurotoxicity markers. L-Deprenyl and MDL-72974 both protected against the reduction in the number of 5-HT uptake sites and 5-HT and 5-HIAA levels.

Discussion

DA has clearly been implicated as a possible candidate in the neurotoxicity of MDMA. Nash and Nichols (1991) have shown that the acute increase in extracellular DA following a single dose of MDMA (20 mg/kg) was negatively correlated with 5-HT and 5-HIAA levels 7 days later. We speculate that the excessive DA may then compete with the lowered concentration of 5-HT for the 5-HT uptake carrier. Evidence for the 5-HT uptake protein recognizing DA as a substrate comes from data by Faraj et al. (1994) showing that the 5-HT transporter is not specific for 5-HT, but has relatively high affinity for DA. In fact, [^{3}H]DA uptake into human lymphocytes was 300–5000 times more potently inhibited by 5-HT uptake blockers than by DA norepinephrine uptake inhibitors. Fluoxetine, a 5-HT uptake blocker, has also been shown to block the toxicity of MDMA for up to 6 h after treatment (Schmidt and Taylor 1990).

The attenuation of MMA-induced serotonergic neurotoxicity by both L-deprenyl and MDL-72974 strongly suggests that deamination of DA by MAO-B may be an important component of the neurotoxic process. Because Westlund et al. (1985) have shown that serotonergic terminals immunocytochemically selectively stained for MAO-B, and *not* MAO-A, it seems possible that excessive deamination of DA *within* the serotonergic terminal could result in elevated intracellular levels of hydrozen peroxide. If the reductive capacity of the neuron was overwhelmed, extensive lipid peroxidation in the 5-HT terminal membrane could account for the selective neuronal degeneration.

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