Neuroscience Letters, 104 (1989) 178–182

NSL 06304

Reserpine does not prevent 3,4-methylenedioxymethamphetamine-induced neurotoxicity in the rat

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(Received 18 March 1989; Revised version received 5 May 1989; Accepted 8 May 1989)

Key words: 3,4-Methylenedioxymethamphetamine; Serotonin; Dopamine; Reserpine; Rat

3,4-Methylenedioxymethamphetamine (MDMA; 'Ecstasy') is a known neurotoxin to 5-hydroxytryptamine (5-HT) nerve terminals. Recent studies have suggested that endogenous dopamine (DA) and/or 5-HT may mediate the MDMA-induced neurotoxicity. The central monoamine stores of rats were significantly decreased with reserpine (5 mg/kg) prior to toxic injections of MDMA. Rats given MDMA (30 mg/kg) displayed significant decreases in the density of 5-HT nerve terminals labeled by [^{3}H]paroxetine both with ($51 \pm 8\%$) and without ($43 \pm 20\%$) reserpine pre-treatment. These data suggest that the degeneration of 5-HT nerve terminals following MDMA is independent of the presence of endogenous stores of DA or 5-HT.

3,4-Methylenedioxymethamphetamine (MDMA; 'Ecstasy') has recently received much attention due to its widespread recreational use [1, 11] coupled with several reports documenting its neurotoxic potential [2–5, 8–10, 12, 14, 16]. MDMA is a selective neurotoxin to 5-hydroxytryptamine (5-HT; serotonin) nerve terminals, since dopamine (DA) and norepinephrine terminals are damaged to a much lesser degree [5]. The mechanism by which MDMA induces degeneration of nerve terminals remains unknown. Stone et al. [15] have suggested that endogenous DA stores may be a partial mediator in the MDMA-induced neurotoxicity. They reported that prior depletion of DA with reserpine, α-methyl-*p*-tyrosine (AMPT) or 6-hydroxydopamine (6-OHDA) partially attenuates the typical drop in tryptophan hydroxylase (TPH) activity seen after MDMA injections. They suggested that high concentrations of extracellular DA brought about cytotoxic changes, possibly by causing the production of destructive oxidants.

A similar case could be made for endogenous 5-HT stores mediating the neurotoxicity of MDMA. In support of this hypothesis, those compounds related to MDMA

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which induce the most substantial release of 5-HT such as 3,4-methylenedioxyamphetamine, *p*-chloroamphetamine and fenfluramine [7] also cause marked degeneration of 5-HT terminals. Conversely, compounds such as 3,4-methylenedioxyethylamphetamine cause relatively less 5-HT release and comparatively less toxicity. One possible explanation for the possible correlation between 5-HT release and neurotoxicity is that the surplus of extracellular 5-HT is metabolized into a neurotoxic compound such as 5,7-dihydroxytryptamine.

The present study was undertaken in order to address the hypothesis that endogenous neurotransmitters such as DA and 5-HT mediate MDMA-induced toxicity. Reserpine was used to significantly reduce central monoamine stores prior to administration of neurotoxic doses of MDMA. We now report that depletion of central monoamines has no significant effect on MDMA-induced neurotoxicity as measured by the density of 5-HT uptake sites.

Male Sprague–Dawley rats (200–225 g; Simonsen Labs: Gilroy, CA) were housed in a 12-h light–dark cycle with unlimited access to food and water. Rats ($n=4$ per condition) were administered either reserpine (5 mg/kg in Serpasil, i.p.) or vehicle, followed 24 h later by either (±)-MDMA (30 mg/kg, i.p.) or saline. Rats from each of the 4 experimental groups were sacrificed 2 weeks following the MDMA injection. The brains were dissected on ice and the density of cerebral cortical 5-HT nerve terminals was quantified using [^{3}H]paroxetine.

Additionally, animals were also given reserpine or vehicle and were sacrificed 24 h after injection. Cerebral 5-HT and striatal DA content was measured using high-performance liquid chromatography according to the method of Sleight et al. [13] with the following modifications: separation took place on a column of length 300 mm and internal diameter of 5 mm, and was packed with 5 μm of NovaPak.

5-HT uptake sites were quantified according to the method of Habert et al. [6] with slight modifications. Briefly, cerebral cortices were homogenized in 50 volumes of buffer (50 mM Tris-HCl, pH 7.4, containing 120 mM NaCl and 5 mM KCl) using a Brinkmann polytron (setting of 9). The homogenate was centrifuged at 48,000 *g* for 10 min. The resuspended pellet was incubated at 37°C for 10 min and then centrifuged at 48,000 *g* for 10 more min. Aliquots of the resuspended pellet (10 mg of wet wt./ml final volume) were incubated with increasing concentrations of [^{3}H]paroxetine (0.025–3.2 nM) in the presence and absence of 0.1 mM fluoxetine. The assays were rapidly filtered through no. 32 glass filter papers pre-soaked with 0.5% polyethylenimine. The filters were rinsed with 10 ml buffer, dried and equilibrated in 3a70 Scintillation Cocktail (Research Products, IL). Radioactivity was measured in a Packard 1900CA Liquid Scintillation Analyzer at 61% efficiency. The means for density of 5-HT uptake sites (B_{max}) and uptake receptor affinity (K_d) were compared with a two-way analysis of variance (ANOVA) test.

Intraperitoneal administration of reserpine reduced rat cerebral cortical levels of 5-HT (56 ± 10 μg/g tissue) to 17% of control values (330 ± 2 μg/g tissue). Striatal levels of DA (410 ± 300 μg/g tissue) were reduced to 21% of control values (2000 ± 30 μg/g tissue) in the reserpinized rats. These measurements were made 24 h after reserpine injection, i.e., at the time that MDMA was given in the dose regimen. In the

absence of reserpine pre-treatment, a single 30 mg/kg dose of MDMA reduced the B_{max} of [^{3}H]paroxetine binding to 5-HT nerve terminals to 43 ± 20% of control values. As shown in Table I, in rats pre-treated with reserpine, MDMA also significantly reduced the B_{max} of [^{3}H]paroxetine binding (51 ± 8% of control). Reserpine treatment alone had no significant effect on the B_{max} value as compared to control animals.

A two-way ANOVA test showed that the reduction in B_{max} following MDMA was statistically significant ($F_{1,12}=37$, $P<0.01$). By contrast, the effect of reserpine ($F_{1,12}=0.58$, $P>>0.05$) and the interaction effect between MDMA and reserpine ($F_{1,12}=0.038$, $P>>0.05$) were not significant. None of the experimental conditions affected the K_d of [^{3}H]paroxetine binding (Table I). The two-way ANOVA demonstrated that neither the effect of MDMA ($F_{1,12}=0.42$, $P>>0.05$), the effect of reserpine ($F_{1,12}=0.42$, $P>>0.05$), nor the effect of the interaction between MDMA and reserpine ($F_{1,12}=0.21$, $P>>0.05$) on the K_ds were statistically significant.

The major finding of the present study is that the MDMA-induced destruction of 5-HT nerve terminals is not protected by the reserpine-induced reduction of endogenous monoamines. Depletion of monoamines using reserpine did not alter the reduction in the density of [^{3}H]paroxetine-labeled 5-HT uptake sites measured 2 weeks after injection of MDMA. These results appear to contrast with those of Stone et al. [15] who reported that depletion of endogenous dopamine with reserpine, AMPT or 6-OHDA did protect rats from developing 'long-term' MDMA-induced neurotoxicity. However, the methodology of Stone et al. [15] differs significantly from the present studies in a number of ways.

First of all, Stone et al. [15] studied animals at 3 days following MDMA whereas the present study measured 5-HT terminal density at 2 weeks following drug injection. Battaglia et al. [4] have shown that degeneration of 5-HT uptake sites in some regions of the cerebral cortex can be observed as early as 18 hours following MDMA. In other regions, however, significant depletions are not measurable until 14 days after treatment. In order to ensure the elapse of sufficient time for terminal degeneration to take place, we chose to examine [^{3}H]paroxetine binding at 14 days after injection.

TABLE I

EFFECT OF SALINE, RESERPINE, AND/OR MDMA ON THE DENSITY AND AFFINITY OF 5-HT UPTAKE SITES IN RAT BRAIN

The density (B_{max}) and affinity (K_d) of [^{3}H]paroxetine labeled 5-HT uptake sites for all experimental groups ($n=4$). The dosage for reserpine was 5 mg/kg and for MDMA was 30 mg/kg. Measurements were made 2 weeks following the MDMA injections.

Condition	B_{max} (fmol/mg)	% Control B_{max}	K_d (nM)
Control	29 ± 6	–	0.09 ± .04
MDMA	12 ± 6	43 ± 20	0.11 ± .02
MDMA + reserpine	15 ± 2	51 ± 8	0.12 ± .04
Reserpine	30 ± 5	105 ± 20	0.10 ± .04

The second methodological difference is that Stone et al. [15] injected rats with 5–20 mg/kg MDMA whereas rats in the present study received 30 mg/kg MDMA. Finally, Stone et al. [15] used TPH activity to measure MDMA effects while the present study examined decreases in [^{3}H]paroxetine-labeled 5-HT uptake sites.

Therefore, these significant methodological differences are likely to be the basis of the different conclusions between the two studies. The role of endogenous monoamines in MDMA-induced neurotoxicity is important since it may indicate the possible neurotoxic mechanism of action of MDMA. According to Stone et al. [15], their data support the hypothesis that endogenous dopamine is a key intermediary in MDMA-induced neurotoxicity. By contrast, our data suggest that monoamine depletion, at least under our experimental conditions, does not prevent MDMA-induced toxicity. This observation supports the hypothesis that other factors (e.g. the production of a toxic metabolite of MDMA) may be responsible for the actual destruction of 5-HT nerve terminals induced by MDMA.

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