Pergamon

Life Sciences, Vol. 55, No. 15, pp. 1193-1198, 1994

0024-3205/94 $6.00 + .00

0024-3205(94)00252-5

ATTENUATION OF 3,4-METHYLENEDIOXYMETHAMPHETAMINE (MDMA) INDUCED NEUROTOXICITY WITH THE SEROTONIN PRECURSORS TRYPTOPHAN AND 5-HYDROXYTRYPTOPHAN

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(Received in final form July 29, 1994)

Summary

Treatment of rats with serotonin (5-HT) precursors tryptophan (TRP, 400 mg/kg) and 5-hydroxytryptophan (5-HTP, 50 mg/kg) was shown to attenuate MDMA (20 mg/kg) induced serotonergic neurotoxicity as measured by [^{3}H]-paroxetine binding in the striatum, hippocampus, and frontal cortex of the rat brain. Hippocampal 5-HT and 5-HIAA levels were also indicative of the protective effects of TRP and 5-HTP. These results suggest that depletion of 5-HT stores is important for MDMA-induced neurotoxicity. The possible significance of this 5-HT depletion in MDMA-induced serontonergic terminal degeneration is also discussed.

Key Words: 5-hydroxytryptamine, neurotoxicity, MDMA, tryptophan

3,4-methylenedioxymethamphetamine (MDMA or "Ecstasy") was first synthesized in the early 1900's as an anorexic agent. However, MDMA had not received much attention until the past decade. In 1985, the Drug Enforcement Administration invoked their emergency powers to place MDMA into Schedule 1, because of it's wide-spread use among young professionals and college students (1). This popularity is probably due to both its euphoric action and its unique effects on emotion, without the sensory distortion seen with strong psychedelics such as lysergic acid diethylamide (LSD). MDMA has been reported to increase interpersonal communication and empathy, leading several therapists to propose the use of MDMA as an adjunct to psychotherapy (2). However, no double-blind experiments have been undertaken to examine MDMA's therapeutic efficacy in this setting.

MDMA has also been shown to deplete selectively the concentrations of serotonin (5-HT) and its major metabolite, 5-hydroxyindoleacetic acid (5-HIAA), and to induce serotonergic neuron terminal degeneration in the brain of rodents and non-human primates following administration of a single high dose (3,4). This latter effect has further hindered clinical evaluation of MDMA. Studies of the mechanisms by which MDMA depletes these markers suggest a role for dopamine (DA) in the neurotoxicity of MDMA (5,6). *In vitro* (6) and *in vivo* (7) studies have shown that MDMA induces a dramatic increase in the extracellular concentration of DA in the striatum. Nash *et al.* (5) also demonstrated that MDMA stimulates DA biosynthesis *in vivo*, as measured by dihydroxyphenylalanine (DOPA) accumulation in the striatum. This effect was blocked by the 5-

$HT_{2A/2C}$ antagonist, ketanserin, which has been shown to attenuate the MDMA-induced increase in striatal DA *in vivo* and also to attenuate the neurotoxicity induced by MDMA (7). The selective 5-HT_{2A} receptor antagonist, MDL 28,133A has also recently been shown to block MDMA induced DA synthesis and release and to prevent MDMA-induced neurotoxicity (8). Thus, proposed components of the mechanism of MDMA-induced neurotoxicity may be as follows: 1) MDMA induces a release of 5-HT; 2) This 5-HT activates the 5-$HT_{2A/2C}$ receptor complex; 3) This activation, in conjunction with MDMA-induced DA release, leads to a profound increase in DA synthesis and release; 4) This increased DA then somehow plays a major role in the neurotoxicity process. The exact mechanisms of all the steps are currently under investigation.

In a recent report, Huang and Nichols (9) proposed that 5-HT depletion from neuron terminals may render them vulnerable to DA toxicity or the action of some other toxin. In order to investigate this hypothesis, we pretreated animals with 5-HT precursors, namely, tryptophan (TRP) and 5-hydroxytryptophan (5-HTP).

Materials and Methods

Thirty-two adult, male Sprague-Dawley rats (175-199g; Harlan, Indianapolis, IN) were used in this study. Upon arrival animals were transferred to individual cages with *ad lib* access to food and water.

The animals were then randomly assigned to four treatment groups (n = 8). Treatments were given twice daily for four days at 7 AM and 7 PM. This treatment regimen was selected since it is known to produce the most extensive sertonergic deficits. The experimental groups were as follows:

Group I = saline
Group II = MDMA (20 mg/kg)
Group III = MDMA + TRP (400 mg/kg)
Group IV = MDMA + RO 4-4602 (50 mg/kg) + 5-HTP (50 mg/kg)

All doses of MDMA were given subcutaneously; all other drugs were given intraperitoneally. RO 4-4602 was used in conjunction with 5-HTP to inhibit the peripheral decarboxylase. The drug pretreatments were given 30 minutes before MDMA. One week after the last treatment, the animals were sacrificed by decapitation; the brain regions were dissected out and stored at -70 °C until assay. An estimate of serotonergic neurotoxicity in the striatum, hippocampus, and frontal cortex was made using a [^{3}H]paroxetine binding method to label 5-HT uptake sites (11,12). 5-HT and 5-HIAA levels in the hippocampus were also estimated at this time. The hippocampus was selected since it was one of the regions shown to have long term reductions in 5-HT and 5-HIAA levels after MDMA treatment (3,4)

MDMA hydrochloride was synthesized in our laboratory (10). [^{3}H]Paroxetine was purchased from New England Nuclear (Boston, MA) at a specific activity of 20.5 Ci/mmol. Fluoxetine was generously provided by Eli Lilly and Co. (Indianapolis, IN). RO 4-4602 was kindly donated by Hoffman-LA Roche (Nutley, NJ). All other reagents were purchased from Sigma Chemical Co. (St. Louis, MO).

A modified procedure of Marcusson *et al.* (11) was employed to measure [^{3}H]paroxetine binding sites. Since it has been previously reported that only the B_{max} and not the K_D values are

altered after MDMA treatment (12), 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.

Hippocampal 5-HT and 5-HIAA levels were also determined. Tissue samples were sonicated for 15 seconds in 0.1N $HClO_4$ containing 0.05% Na_2EDTA, and 0.1% $Na_2S_2O_5$. The samples were then centrifuged at 14,000 x g for 4 minutes with a tabletop centrifuge. The supernatant was collected and 50 μl injected onto the HPLC column (Brownlee C18, Anspec; Ann Arbor, MI.). A model 400 EG & G Princeton electrochemical detector (Princeton, NJ) with series dual electrodes was utilized. The mobile phase consisted of 50 mM NaH_2PO_4, 30 mM citric acid, 0.1 mM Na_2EDTA, 0.034 % sodium octyl sulfate and 25% methanol. Peaks were integrated with the Dynamax Methods Manger software (Rainin; Woburn, MA.).

Statistical treatment of groups was performed with an ANOVA. Differences between treatment groups were determined with a Student Neuman-Keuls multiple range test with significance set at $p<0.05$.

Results

Fig. 1 presents the results of the treatments on the number of 5-HT uptake sites for all brain regions examined. MDMA produced a significant reduction of [^{3}H]paroxetine-labeled sites in all brain regions examined. MDMA produced the greatest reduction (72%) in uptake sites in the hippocampus. TRP and 5-HTP protected all three brain regions from MDMA-induced neurotoxicity. TRP and 5-HTP afforded the greatest protection in the frontal cortex and the least protection in the hippocampus (28% & 32% reduction, respectively). TRP alone had no effect on [^{3}H]paroxetine binding in any of the brain regions (data not shown).

Table 1 presents the effects of the treatments on 5-HT and 5-HIAA levels in the hippocampus. MDMA produced a significant reduction in both 5-HIAA and 5-HT levels. TRP and 5-HTP protected against this reduction in 5-HIAA and 5-HT.

Table 1

Treatment	5-HT (% of control)	5-HIAA (% of conrtol)
SAL	100.1 ± 6.8	100.0 ± 6.8
MDMA	43.3 ± 6.3*	69.3 ± 6.6*
MDMA + TRP	72.4 ± 7.5	103.9 ± 5.7
MDMA + 5-HTP	125.5 ± 21	88.9 ± 5.5

5-HT and 5-HIAA levels in the hippocampus one week after multiple doses of MDMA and MDMA drug combinations. Basal 5-HT and 5-HIAA levels were 648 ± 48 and 972 ± 65 pg/mg wet weight, respectively. *significantly different from all other treatment groups

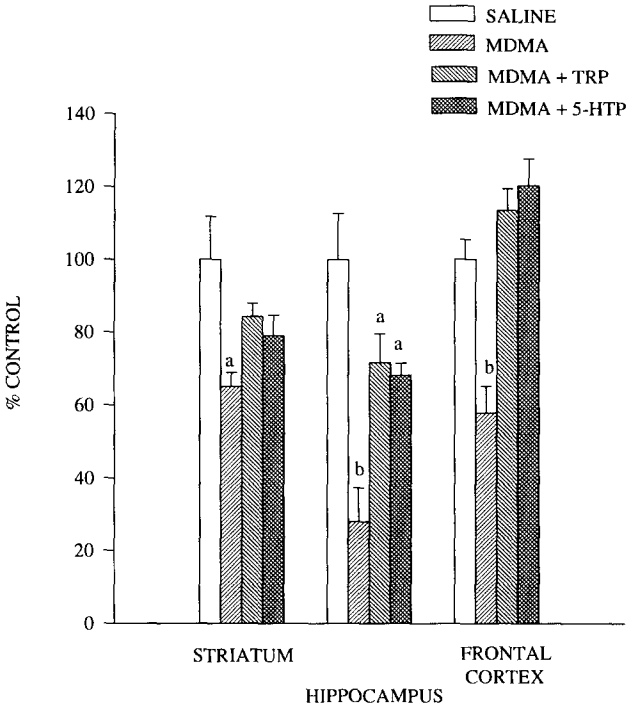

Fig. 1

[^{3}H]paroxetine binding one week after multiple doses of MDMA and MDMA drug combinations. SAL = saline treatment group; MDMA + TRP = MDMA + TRP pretreatment; MDMA + 5-HTP = MDMA + 5-HTP pretreatment. Basal [^{3}H]paroxetine binding site densities in the striatum, hippocampus, and frontal cortex were 27.9 ± 3.4, 16.6 ± 2.0, and 18.4 ± 1.0 fmol/g wet weight, respectively. a=significantly different from saline control. b=significantly different from all other treatment groups.

Discussion

The results of the present study suggest that in order for MDMA to induce serotonergic neurotoxicity, the neuron terminals must be depleted of serotonin. Studies in many laboratories have shown that MDMA and structural analogues (e.g. N-methyl-1-(1,3-benzodioxol-5-yl)-2-butanamine (MBDB), and N-ethyl-3,4-methylenenedioxyamphetamine (MDE)) which acutely deplete 5-HT, lead to long term decreases in concentrations of 5-HT and 5-HIAA in the brain of rodents and non-human primates (13,14,15,16,). These decreases in serotonergic parameters have also been linked to 5-HT axon terminal degeneration (17). Further support for a role of 5-HT depletion recently came from Bradberry *et al.* (18), who showed that neurotoxicity induced by para-chloroamphetamine (PCA) could be blocked by TRP pretreatment.

Acute MDMA treatment has also been shown to decrease tryptophan hydroxylase activity (19). This original report suggested that the decrease in tryptophan hydroxylase activity might

play an important role in MDMA-induced neurotoxicity. However, the results of the present study showing that 5-HTP was no more effective that TRP suggest that the decrease in tryptophan hydroxylase activity may not be important.

Berger *et al.* (20) have shown that if monoamine stores are depleted with reserpine prior to PCA treatment, serotonergic neurotoxicity can be blocked. These authors speculated that 5-HT itself or a toxic metabolite of 5-HT (i.e. 5,6- or 5,7-dihydroxytryptamine) might therefore be responsible for the neurotoxicity. The present results suggest that such toxins are probably not responsible for serotonergic neurotoxicity. If in fact this were the case, pretreatment with TRP or 5-HTP would be expected to enhance the concentrations of these substances and potentiate the neurotoxicity.

In summary, the results of this study suggest that depletion of 5-HT stores increases the vulnerability of serotonergic neurons to the toxic effects of MDMA. This is a necessary but not sufficient condition to produce neurotoxicity (21). This depletion may then allow the carrier-mediated uptake of the ultimate toxicant (DA?) into the 5-HT terminal. Such a scenario would explain why MDMA or PCA-induced neurotoxicity can still be inhibited by the 5-HT uptake blocker fluoxetine up to six hours after drug administration (22,23).

Acknowledgements

This work was supported by USPHS Grant DA04758 from the National Institute on Drug Abuse. The authors thank Stewart Frescas for the synthesis of MDMA.

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