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Neurotoxic Effects of the Alpha-Ethyl Homologue of MDMA Following Subacute Administration

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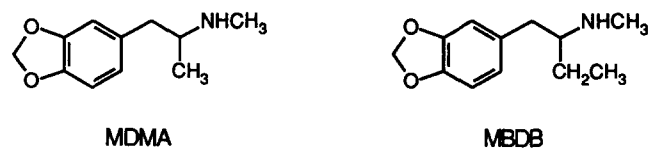
JOHNSON, M. P. AND D. E. NICHOLS. *Neurotoxic effects of the alpha-ethyl homologue of MDMA following subacute administration.* PHARMACOL BIOCHEM BEHAV **33**(1) 105–108, 1989.—The possible neurotoxic effects of the α-ethyl homologue of MDMA, N-methyl-1-(1,3-benzodioxol-5-yl)-2-butanamine (MBDB), were examined following a regimen of twice daily dosing for four days. The levels of norepinephrine, serotonin and its metabolite 5-HIAA were quantitated by standard HPLC-EC techniques. In addition, the number of 5-HT uptake sites was estimated by examining the binding of [^{3}H]-paroxetine to rat cortex homogenate. With 20 mg/kg (IP) subacute dosing of MDMA, a nearly 60% reduction in 5-HT, 5-HIAA, and 5-HT uptake sites was found, with no change in NE, two weeks posttreatment. A behaviorally equipotent dose of MBDB (25 mg/kg, IP) also produced a significant decrease in the serotonergic markers, 5-HT, 5-HIAA and [^{3}H]-paroxetine binding sites. However, a comparison of the relative toxic effects of MDMA and MBDB indicates that MBDB may be slightly less neurotoxic. It was also found that MDMA but not MBDB caused a significant increase in dopamine levels at 3 hours following a single IP injection. The results are discussed in relation to the therapeutic index of MBDB and the relative importance of dopamine release in the neurotoxicity of MDMA.

MBDB MDMA Neurotoxicity Serotonin Dopamine [^{3}H]-Paroxetine

IN the past few years 3,4-methylenedioxymethamphetamine (MDMA) or "Ecstasy" has received widespread coverage both in the popular media and the scientific journals. In fact, such concern arose over the abuse potential of this compound that the Drug Enforcement Administration has now permanently placed it into Schedule 1. One of the concerns that led to this action was the neurotoxicity associated with the N-desmethyl analog of MDMA (i.e., MDA) (13). Indeed, it has now been shown by several investigators that both MDA and MDMA cause similar long-term depletion of serotonin (5-HT) and its metabolite, 5-hydroxyindoleacetic acid (5-HIAA) (17,21). MDMA also causes a decrease in the number of 5-HT uptake sites, as measured by binding of the 5-HT uptake inhibitor, [^{3}H]-paroxetine (1), and the V_{max} for [^{3}H]-serotonin uptake (3,15). These deficits have since been attributed to a selective degeneration of the fine axon serotonergic projections ascending from the dorsal raphe nucleus (12).

MDMA has been found to possess unique psychopharmacological effects that are characterized as a state of enhanced emotional and sensory awareness (5). Several reports suggest that the α-ethyl homologue of MDMA, N-methyl-1-(1,3-benzodioxol-5-yl)-2-butanamine (MBDB), has a similar pharmacological action. For example, in the two-lever drug discrimination paradigm, MBDB fully substituted in rats trained to discriminate (±)-MDMA from saline (11). In addition, MDMA has been found to substitute in *S*-(+)-MBDB-trained rats (10), indicating that the discriminative cue properties of the two compounds are very similar.



MDMA MBDB

FIG. 1. The structures of MDMA and MBDB.

However, subtle differences in the action of these two compounds have been reported. While both compounds appear to affect serotonergic and noradrenergic pathways in vitro, MBDB does not significantly inhibit the uptake of dopamine into rat brain striatal synaptosomes (18) or induce the release of dopamine from rat caudate nucleus slices (7). By contrast, both MDA and MDMA have appreciable effects in these assays (7,18). Furthermore, while both (+)-amphetamine and cocaine fully substitute in rats trained to discriminate (±)-MDMA from saline (10,11), only

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TABLE 1

THE EFFECTS OF MBDB AND MDMA AT 3 HOURS POSTINJECTIONS

Treatment	Monoamine and Metabolite Levels (pg/mg wet wt.) NE	5-HT	5-HIAA	DA	DOPAC	HVA
Saline	473 ± 61	312 ± 15	163 ± 10	70 ± 10	26 ± 3	58 ± 7
MDMA	458 ± 23	90 ± 6*	116 ± 6†	220 ± 36§	25 ± 4	67 ± 7
MBDB	425 ± 37	86 ± 11*	130 ± 12‡	74 ± 9	17 ± 2	77 ± 6

*Significantly decreased from control ($p<0.0001$, Student's t-test).
†Significantly decreased from control ($p<0.005$, Student's t-test).
‡Significantly decreased from control ($p<0.05$, Student's t-test).
§Significantly increased over control ($p<0.0005$, Student's t-test).
Saline, (±)-MDMA·HCl (20 mg/kg), or (±)-MBDB·HCl (25 mg/kg) was injected IP and animals were sacrificed 3 hours later. The frontal cortex brain region was removed and stored at −70°C until assayed. Monoamines and their metabolite levels were determined using HPLC-EC techniques. Values are presented as the mean ± S.E. for an n=6.

partial substitution occurs in (+)-MBDB-trained rats (10). These assays indicate that MBDB has little effect on dopaminergic systems in the brain, while MDA and MDMA have a component to their action that is "amphetamine-like," and presumably related to an effect on dopamine pathways. This suggestion is consistent with the one report on the human psychopharmacology of MBDB, where this compound is described as producing less euphoria than MDMA (9).

Some investigators have suggested that, like the structurally related methamphetamine, the mechanism of MDMA serotonin neurotoxicity may involve its ability to interact with dopaminergic pathways (14, 16, 19, 20). This would suggest vide supra that the neurotoxic effects of MBDB might be attenuated relative to MDMA. Therefore, the potential neurotoxic effects of MBDB were examined following a multiple dosing regimen, utilizing the levels of monoamines and their metabolites, and the density of serotonin uptake sites as indicators of neuronal toxicity (1).

METHOD

[^{3}H]-Paroxetine (23.1 Ci/mmol) was obtained from New England Nuclear (Boston, MA). MDMA and MBDB were synthesized in this laboratory. Fluoxetine was kindly provided by Eli Lilly (Indianapolis, IN). All other compounds were purchased from Sigma Chemical Co. (St. Louis, MO).

Male Sprague-Dawley rats (Harlan Industries, Indianapolis, IN), weighing 175 to 200 g, were individually housed and given access to food and water ad lib. All drugs were dissolved in saline vehicle. Eight animals were used for each treatment. Rats were injected IP every 12 hours for 4 days with behaviorally equipotent (10) doses of either (±)-MDMA·HCl (20 mg/kg) or (±)-MBDB·HCl (25 mg/kg) and were sacrificed 2 weeks posttreatment. To assess the more immediate effects of these drugs, a second group of animals was sacrificed 3 hr following a single injection (IP) of either saline, (±)-MDMA·HCl (20 mg/kg), or (±)-MBDB·HCl (25 mg/kg). After sacrifice, the hemispheres were separated and the frontal cortex and hippocampus were dissected over ice (4) and immediately frozen in liquid nitrogen for storage at −70°C until assays were performed.

The brain regions from one hemisphere were weighed and homogenized in 1.0 ml of 0.4 N $HClO_4$ containing 0.05% Na_2EDTA, 0.1% $Na_2S_2O_5$ and 50 ng/ml of dihydroxybenzylamine (DHBA), using a motor-driven teflon mortar and pestle. The samples were then centrifuged at 11,000 × g for 20 min and the supernatant was assayed for catecholamines, 5-HT, and their metabolites using HPLC-EC techniques. A reverse-phase C18 analytical cartridge column (Brownlee Laboratories, Ann Arbor, MI) and a mobile phase, containing 0.05 M NaH_2PO_4, 0.03 M citric acid, 0.1 mM Na_2EDTA, 0.25% sodium octyl sulfate and 25% methanol (pH = 2.85) were used to separate the monoamines and their metabolites. An Hitachi (D-2000) integrator was used to quantitate the concentrations. A flow rate of 0.7 ml/min gave the following retention times: NE, 5.10 min; DOPAC, 5.95 min; DHBA, 6.50 min; 5-HIAA, 6.95 min; DA, 7.85 min; HVA, 10.86 min; and 5-HT, 13.20 min, respectively.

[^{3}H]-Paroxetine Binding Studies

The procedures of Habert *et al.* (6) were employed, with minor modifications. The brain regions from the other hemisphere were thawed and weighed before being homogenized in 15 ml of 50 mM Tris with 120 mM NaCl and 5 mM KCl (pH = 7.4) with a Brinkman polytron (setting 6, 2 × 20 sec). The homogenates were centrifuged twice at 30,000 × g for 10 min, with an intermittent wash, and were then resuspended in the same buffer.

Increasing concentrations of [^{3}H]-paroxetine (0.1 to 2.5 nM) in the presence or absence of 10 μM fluoxetine were incubated in a total volume of 2 ml containing 200–400 μg of protein. The tubes were allowed to equilibrate for 1 hr at 24°C before being filtered through GF/C filters (presoaked in 0.05% PEI) using a Brandel Cell Harvester. The tubes were then washed twice with 5 ml of buffer and the filters were allowed to air dry. Filters were placed into scintillation vials, ACS (Amersham) was added, and the vials were allowed to sit overnight at room temperature before counting at 46% efficiency in a Packard 4000 Scintillation counter. The concentration of radioligand was determined by counting the DPM's added for each concentration of [^{3}H]-paroxetine. Data analysis utilized the least squares curve fitting procedures embodied in the computer programs EBDA and LIGAND as adopted for the IBM PC by McPherson (8). The concentration of protein per tube was determined using the method of Bradford (2).

RESULTS AND DISCUSSION

As seen in Table 2, the results clearly indicate a persistent decrease in 5-HT and 5-HIAA, and in 5-HT uptake sites in rat frontal cortex and hippocampus with MDMA treatment. These results, and the levels of neurotransmitters reported for vehicle administration, are in reasonable agreement with other studies (1,3). Based on loss of 5-HT uptake sites, the multiple dose regimen employed in this study apparently destroyed 55 to 60% of

TABLE 2

THE NEUROTOXIC EFFECTS OF MBDB AND MDMA

Treatment	Monoamine and Metabolite Levels (pg/mg wet wt.) NE	5-HT (% remaining)	5-HIAA	5-HT Uptake Sites B_{max} (fmol/mg protein) (% remaining)
Cortex:				
Saline	554 ± 18	543 ± 21 (100%)	308 ± 18 (100%)	543 ± 26 (100%)
MDMA	579 ± 22	215 ± 22* (40%)	129 ± 19* (42%)	248 ± 27* (46%)
MBDB	611 ± 18	304 ± 24* (56%)	179 ± 17* (58%)	324 ± 16*† (60%)
Hippocampus:				
Saline	608 ± 56	573 ± 34 (100%)	653 ± 45 (100%)	N.D.
MDMA	627 ± 42	188 ± 25* (33%)	262 ± 35* (40%)	N.D.
MBDB	623 ± 65	248 ± 40* (43%)	354 ± 59* (54%)	N.D.

*Significantly decreased from control ($p<0.0001$, Student's t-test).

†Significantly greater than MDMA ($p<0.05$, Student's t-test).

N.D. indicates not determined.

Saline, (±)-MDMA·HCl (20 mg/kg), or (±)-MBDB·HCl (25 mg/kg) was injected IP every 12 hr for 4 days. Animals were sacrificed two weeks later and frontal cortex and hippocampus regions were removed and stored at −70°C until assayed. Monoamines and their metabolite levels were determined using HPLC-EC techniques. The number of 5-HT uptake sites was estimated using [^{3}H]-paroxetine receptor binding techniques (see the Method section). DA, HVA and DOPAC levels did not significantly change with either treatment (data not shown). Values are presented as the mean ± S.E. for an n=8. The K_D values for [^{3}H]-paroxetine binding following the three treatments were not significantly different in the cortex: saline, 0.11 ± 0.01 nM; MDMA, 0.18 ± 0.03 nM; MBDB, 0.14 ± 0.02 nM.

the serotonergic terminals in the cortex and hippocampus, without significantly altering the catecholamines or their metabolites at 2 weeks posttreatment.

It is evident from the data in Table 2 that MBDB is also toxic to serotonin neurons. There was a significant decrease in 5-HT and 5-HIAA, and in 5-HT uptake sites, indicating a 40 to 50% reduction in serotonergic neuron terminals in the cortex and hippocampus. Similar to MDMA, MBDB did not significantly alter the long-term (2 weeks posttreatment) levels of NE or DA or their metabolites (data not shown). It is also apparent, from comparisons of the relative toxicity at behaviorally equipotent doses, that MBDB is somewhat less toxic than MDMA, although the only statistically significant difference between the two was the number of 5-HT uptake sites remaining after treatment. This would imply that MBDB may have a higher therapeutic index than MDMA, but the risk of neurotoxicity is still present.

It has been previously shown by others (17) that MDMA induces a significant increase in brain dopamine levels, 3 hours following drug treatment. Several studies have focused on this aspect of the action of MDMA, and have presented some evidence to suggest that this dopaminergic interaction may be involved in the neurotoxic effects of MDMA (16, 19, 20). However, while MDMA does induce the release of dopamine from striatal slices, and inhibits the reuptake of dopamine into striatal synaptosomes, MBDB does not possess similar effects (7,18). Table 1 presents data showing that, while MDMA also acutely increases brain dopamine levels, in agreement with an earlier report (17), MBDB fails to produce such an effect in rat frontal cortex. In view of the similar neurotoxicity of MBDB and MDMA, this casts some doubt on the importance of dopamine pathways in the expression of the neurotoxic effects.

The results in Table 1 provide further support for the view that MBDB has little impact on dopaminergic pathways. While the dopaminergic properties of MDMA may well mediate its amphetamine-like effects in drug discrimination or self-administration studies, the similar psychopharmacology of MBDB (9), but apparent lack of significant dopaminergic properties, indicates that the unique pharmacology of MDMA, and other entactogens, is distinct from that of amphetamine-like central stimulants.

In summary, the present results clearly show that after multiple dosing with MBDB, a decrease in indices associated with serotonergic function has occurred. This neurotoxic effect was somewhat less than that seen with behaviorally equipotent doses of MDMA. MDMA acutely increased brain dopamine levels, while MBDB did not. This seems to argue against an essential role for dopamine in the expression of serotonin neurotoxicity, although other explanations such as differences between the rates of metabolism, distribution or elimination cannot be excluded.

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