Microdialysis studies on 3,4-methylenedioxymethamphetamine and structurally related analogues

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The acute effect of 3,4-methylenedioxymethamphetamine (MDA) and three structural analogues on the extracellular concentrations of dopamine (DA) and its major metabolites, dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), in the striatum was studied using in vivo microdialysis in awake, freely moving rats. MDA significantly (P < 0.001) increased and decreased the extracellular concentrations of DA and its metabolites, respectively, following i.p. administration. Similarly, acute administration of the N-methyl (MDMA) and N-ethyl (MDE) derivatives of MDA significantly (P < 0.05) increased the concentration of DA in the striatum. The α-ethyl homologue of MDA, MBDB, increased the extracellular concentration of DA but significantly (P < 0.05) less than MDA, MDMA or MDE. The rank order of potency for these amphetamine derivatives to increase the extracellular concentration of DA was: MDA > MDMA > MDE > MBDB > vehicle. The increase in extracellular DA concentration following a single administration of these compounds was negatively correlated with the level of serotonin (5-HT) and its major metabolite, 5-hydroxyindoleacetic acid (5-HIAA) in the contralateral striatum measured 7 days following drug administration. Thus, extending the alkyl group on the nitrogen or α-carbon of MDA reduces the ability of these compounds both to increase acutely the extracellular concentration of DA and to produce long-lasting depletions of 5-HT in the brain.

Dopamine; 5-HT (5-hydroxytryptamine, serotonin); Microdialysis (in vivo); MDA (methylenedioxymethamphetamine); MDMA (3,4-methylenedioxymethamphetamine); Neurotoxicity

1. Introduction

The report by Ricaurte et al. (1985) offered the first evidence that repeated administration of high doses of 3,4-methylenedioxymethamphetamine (MDA) produced a long-lasting decrease in the concentrations of serotonin (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) in select forebrain regions in the rat. That initial study was followed by numerous reports which have unequivocally established that single or repeated administration of MDA and structural analogues (i.e. 3,4-methylenedioxymethamphetamine HCl (MDMA), N-methyl-1-(3-benzodioxol-5-y1)-2-butanamine HCl (MBDB), N-ethyl-3,4-methylenedioxymethamphetamine HCl (MDE)) decrease tryptophan hydroxylase activity as well as the concentrations of 5-HT and 5-HIAA in the brain of rodents and non-human primates (Stone et al., 1986; Ricaurte et al., 1988; Johnson and Nichols, 1989; Gibb et al., 1990). Moreover, the decreases in these serotonegic parameters have been linked to 5-HT axon terminal degeneration (O’Hearn et al., 1988).

It has been suggested that excessive release of dopamine (DA) may play a role in the MDMA-induced 5-HT neurotoxicity (Stone et al., 1988; Nash et al., 1990). In this regard, MDA and its N-methyl homologue, MDMA, have been reported to release DA from brain slice preparations (Johnson et al., 1986) as well as to inhibit the uptake of DA into striatal synaptosomes (Steele et al., 1987). Lengthening the methyl substitution on the nitrogen to ethyl (MDE), decreases its potency with respect to DA release from striatal brain slices (Schmidt, 1987). Similarly, increasing the length of the alkyl substitution on the α-carbon of these 3,4-methylenedioxymethylamines diminishes their effect on DA release and uptake (Johnson et al., 1986; Steele et al., 1987). Thus, alkyl substituents longer than methyl on either the nitrogen or α-carbon decrease the ability of these compounds to release neuronal stores of DA as measured in vitro.

MDMA has been reported to increase the extracel-
ular concentration of DA in the striatum, as measured by in vivo voltammetry (Yamamoto and Spanos, 1988) and microdialysis (Hiramatsu and Cho, 1990; Nash, 1990). Moreover, Hiramatsu and Cho (1990) found that the S(+) isomer of MDMA increased the concentration of DA to a greater extent than the R(-) isomer. These in vivo studies are in agreement with the aforementioned in vitro studies and suggest that the active isomer of MDMA resembles amphetamine in its ability to increase DA concentrations.

Extension of the nitrogen or α-carbon alkyl reduces the ability of these 3,4-methylenedioxy-phenethylamines to produce long-lasting decreases in the concentrations of 5-HT and 5-HIAA in the brain. For example, a single administration of MDE had no long-term effect on the concentration of 5-HT in the cortex (Schmidt, 1987). In contrast, a single administration of MDA or MDMA has been reported to decrease significantly 5-HT and 5-HIAA content, the number of 5-HT uptake sites and tryptophan hydroxylase activity in several forebrain regions (Stone et al., 1986; Gibb et al., 1990; Nash et al., 1991). Thus, increasing the length of the alkyl substitution on the nitrogen decreases the long-term 5-HT depleting effect of MDA.

The objective of the present study was to determine the effect of nitrogen or α-carbon alkyl substitution in MDA on the drug-induced changes in extracellular concentrations of DA and its major metabolites in the ventralateral striatum, as measured by in vivo microdialysis. In addition, the ability of a single dose of MDA, MDMA, MDE and MBDB to decrease the concentration of 5-HT and 5-HIAA in the striatum 7 days following administration was determined.

2. Materials and methods

2.1. Materials

Racemic 3,4-methylenedioxyamphetamine HCl (MDA), 3,4-methylenedioxyamphetamine HCl (MDMA) and N-ethyl-3,4-methylenedioxyamphetamine HCl (MDE) were generously provided by the National Institute on Drug Abuse. The racemic mixture of N-methyl-1(1,3-benzodioxol-5-yl)-2-butanamine HCl (MBDB) was synthesized by procedures established in one of our laboratories (Nichols et al., 1986). The structure of these amphetamine derivatives is illustrated in fig. 1. Chloral hydrate and the HPLC standards were purchased from Sigma Chemical Co. (St. Louis, MO). All drugs were dissolved in saline and administered i.p. in a volume of 1 ml/kg. The dialysis studies were begun at 09:00 h and drugs were administered between 12:00-13:00 h.

![Molecular structures of MDA, MDMA, MDE and MBDB.](image)

**Fig. 1.** Molecular structures of MDA, MDMA, MDE and MBDB.

2.2. Animals

Male Sprague-Dawley rats weighing between 220-260 g were purchased from Zivic Miller (Allison Park, PA) and used in all experiments. Animals were housed two per cage in a temperature controlled room (23 °C) with a 12/12 h lighting schedule (lights on at 06:00 h). Food and water were available ad libitum.

2.3. In vivo microdialysis

Under chloral hydrate (400 mg/kg i.v.) anesthesia, each rat was placed in a stereotactic frame. A U-shaped dialysis probe (Nash, 1990) was inserted into the ventralateral striatum using the following coordinates: A: + 1.0, L: + 3.5, V: -3.3, according to the atlas of Paxinos and Watson (1982). The dialysis probe was secured using dental cement and a skull screw. The dialysis probe was flushed with a modified Ringer solution (mM: KC1 1.7, KH2PO4 1.0, CaCl2 1.2, NaCl 136.0, Na2HPO4 6.0 at pH 7.4) and the animals were placed in clear plastic cages. On the following day, the dialysis probe was connected to a Harvard infusion pump (Harvard Instruments, South Natick, MA) set to deliver Ringer solution at a rate of 1.8 μl/min. Dialysate samples were collected every 30 min. Baseline samples were collected for 2-3 h, after which an equimolar dose (87 μM/kg; approximately 20 mg/kg) of either MDA, MDMA, MDE, MBDB or vehicle was administered and samples were collected over a 3 h time period. At the end of each study, the rats were returned to their home cage. Each rat was killed 7 days following drug administration and the brain was rapidly removed from the skull. The location of the dialysis probe was confirmed in each rat and the contralateral striatum was removed and rapidly frozen on dry ice. The tissue samples were stored at -80 °C until the time of assay.

2.4. Biochemical measurements

The acute concentrations of DA and its major metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) were determined in dialysate samples using previously established HPLC methods (Nash, 1990; (50 μl) was nomenex, RA LC-4B ampe about 20. The s dissolved with a 12 min solution a

2.5. Statistical analysis

The effect of the vehicle on the extracellular concentrations of HVA, 5-HT and 3,4-methylenedioxyamphetamine HCl (MDA) and N-ethyl-3,4-methylenedioxyamphetamine HCl (MDE) were assayed for in vivo using one-way ANOVA. The area under the HPLC peak was determined with a 12 min solution. The statistical analysis included a 12 h lighting schedule (lights on at 06:00 h). Food and water were available ad libitum.

Post-mortem

HVA, 5-HT and 3,4-methylenedioxyamphetamine HCl (MDA) and N-ethyl-3,4-methylenedioxyamphetamine HCl (MDE) were assayed for in vivo using one-way ANOVA. The area under the HPLC peak was determined with a 12 min solution. The statistical analysis included a 12 h lighting schedule (lights on at 06:00 h). Food and water were available ad libitum.

The maximal effect of the vehicle on the extracellular concentration of HVA was determined using one-way ANOVA. The area under the HPLC peak was determined with a 12 min solution. The statistical analysis included a 12 h lighting schedule (lights on at 06:00 h). Food and water were available ad libitum.

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The maximal increase in DA concentrations occurred MDMA > MDE > MBDB. There were no differences between the treatment groups in the basal concentrations of DA and its metabolites (table 1). Vehicle administration had no effect on extracellular concentrations of DA, DOPAC or HVA (data not shown).

All four compounds significantly (P < 0.01) decreased the extracellular concentrations of DOPAC after administration. The rank order of potency for increasing DA concentrations was MDA > MDMA > MDE > MBDB.
and HVA (fig. 2). There was no difference in the effect of MDA as compared to MDMA with respect to the decrease in the concentrations of the DA metabolites. Similarly, MBDB and MDE produced the same magnitude of effect on HVA and DOPAC. Both MDMA and MDA produced a significantly (P < 0.05) greater decrease in the concentrations of HVA and DOPAC when compared to MDE or MBDB.

The effect of a single administration of MDA, MDMA, MDE, MBDB or vehicle on the concentrations of 5-HT and 5-HIAA in the striatum 7 days following a single dose is presented in table 2. MDA, MDMA and, to a lesser extent, MDE significantly (P < 0.05) reduced the concentrations of 5-HT and 5-HIAA in the striatum as compared to vehicle-treated rats. MBDB had no effect on the concentrations of 5-HT or 5-HIAA. Neither MDA nor the other analogues studied had a significant effect on the concentration of DA or its metabolites 7 days following a single dose of administration (table 2).

Figure 3 illustrates the correlation between the concentration of 5-HT or 5-HIAA, measured 7 days following drug administration, and the acute (3 h) increase in the extracellular concentration of DA as measured by in vivo microdialysis (fig. 2). There was a significant (P < 0.001) negative correlation (r = -0.79) between the concentration of 5-HT or 5-HIAA and the AUC

4. Discussion

MDA and its structural analogues increased the extracellular concentration of DA in the striatum as measured by in vivo microdialysis. All four compounds significantly decreased the extracellular concentration of the DA metabolites, DOPAC and HVA. Addition of an ethyl group onto the amine nitrogen or the α-carbon significantly diminished the ability of these compounds to increase the concentration of DA. These data are consistent with in vitro studies of the release of DA from striatal slices as well as the inhibition of synaptosomal DA uptake.

The increase and decrease in the extracellular concentrations of DA and DOPAC or HVA, respectively, produced by MDA, MDMA, MDE, MBDB or vehicle on the concentrations of 5-HT and 5-HIAA in the striatum 7 days following a single dose is presented in table 2. MDA, MDMA and, to a lesser extent, MDE significantly (P < 0.05) reduced the concentrations of 5-HT and 5-HIAA in the striatum as compared to vehicle-treated rats. MBDB had no effect on the concentrations of 5-HT or 5-HIAA. Neither MDA nor the other analogues studied had a significant effect on the concentration of DA or its metabolites 7 days following a single dose administration (table 2).

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TABLE 2
Effect of MDA, MDMA, MDE and MBDB on the concentration of DA, 5-HT and their metabolites in the striatum 7 days following administration. Each value represents the mean ± S.E. of four rats. MDA, MDMA, MDE MBDB (87 μM/kg i.p.) or vehicle was administered 7 days prior to killing.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>DA</th>
<th>DOPAC</th>
<th>HVA</th>
<th>5-HT</th>
<th>5-HIAA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>100.9 ± 5.3</td>
<td>9.0 ± 0.5</td>
<td>6.8 ± 0.8</td>
<td>4.30 ± 0.77</td>
<td>4.78 ± 0.85</td>
</tr>
<tr>
<td>MDA</td>
<td>96.0 ± 2.3</td>
<td>8.1 ± 0.3</td>
<td>5.5 ± 0.5</td>
<td>1.64 ± 0.54 b</td>
<td>1.87 ± 0.42 b</td>
</tr>
<tr>
<td>MDMA</td>
<td>94.2 ± 5.8</td>
<td>10.1 ± 0.9</td>
<td>7.6 ± 0.6</td>
<td>1.53 ± 0.20 b</td>
<td>2.22 ± 0.38 b</td>
</tr>
<tr>
<td>MDE</td>
<td>88.8 ± 6.7</td>
<td>9.8 ± 0.7</td>
<td>6.9 ± 0.7</td>
<td>1.99 ± 0.14 b</td>
<td>3.17 ± 0.38 b</td>
</tr>
<tr>
<td>MBDB</td>
<td>106.2 ± 3.8</td>
<td>8.1 ± 0.8</td>
<td>7.1 ± 0.7</td>
<td>3.62 ± 0.26</td>
<td>4.39 ± 0.26</td>
</tr>
</tbody>
</table>

*a pg/mg protein. b P < 0.05 as compared to vehicle-treated rats.

produced by MDMA administration in the present study are consistent with the results obtained elsewhere (Hiramatsu and Cho, 1990; Nash, 1990). The prolonged increase in the extracellular concentrations of DA produced by these 3,4-methylenedioxyphenethyllamines could be the result of carrer-and/or vesicle-mediated DA release as well as uptake inhibition. The decrease in the extracellular concentrations of the DA metabolites could be attributed to the inhibition of monoamine oxidase and/or a decrease in the intraneuronal concentration of DA as a result of release and uptake inhibition of DA. Additional studies are necessary to determine the mechanism(s) responsible for the effects observed in the present study.

Previous studies conducted in our laboratories have found that the α-ethyl homologue of p-chloroamphetamine has significantly reduced pharmacological effects (Nichols et al., 1990). Specifically, the release of DA in the striatum and the 5-HT-depleting effects of p-chloroamphetamine are significantly attenuated by extension of the α-methyl to an ethyl group. These data are in agreement with the present findings in that MBDB is significantly less potent than MDA or the other analogues with respect to DA release and 5-HT depletion.

It has been suggested that 3,4-methylenedioxyphenethylamines may represent a novel class of pharmacological agents (Nichols et al., 1986). These compounds which include MDA, MDMA, MDE and MBDB do not fit the pharmacological profile of either phenethyllamine hallucinogens (i.e. 1-(2,5-dimethoxy-5-iodophenyl)-2-amino propane; DOI) or psychostimulants such as amphetamine. For example, with the exception of the R(+) enantiomer of MDA, these compounds do not substitute for the potent hallucinogen, LSD, in two lever drug discrimination studies (Nichols et al., 1986). Unlike hallucinogens, the S(-) isomer of the 3,4-methylenedioxyphenethylamines is more potent than the R(-) isomer in rodent as well as human studies (for review see: Nichols and Glennon, 1984). Thus, these compounds differ in stereoselectivity from phenethylamine hallucinogens.

The fact that S(+) MDMA is more potent than the R(-) isomer resembles the stereoselectivity of amphetamine. In this regard, the S(+) isomer of amphetamine will completely substitute for MDMA in drug discrimination studies (Oberlender and Nichols, 1988). However, in animals trained to discriminate amphetamine from saline, MDMA only partially substituted for amphetamine, whereas neither MBDB nor MDE produced amphetamine lever selection (Oberlender and Nichols, 1988; Glennon and Misenheimer, 1989). These results may be related to the differential effect of the compounds on the release of DA as presented in fig. 2. Nevertheless, these data are suggestive that although MDMA and its structural analogues share some similarities with amphetamine, there are significant differences.

MDA, MDMA, MDE and MBDB have nearly equivalent effects on the release of 5-HT and on 5-HT uptake inhibition (Johnson et al., 1986; Steele et al., 1987; Hekmatpanah and Peroutka, 1990). However, these compounds differ in their ability to produce long-lasting decreases in the concentrations of 5-HT and 5-HIAA in brain following single or repeated administration (Schmidt, 1987; Ricaurte et al., 1987; Johnson et al., 1987; Johnson and Nichols 1989). Therefore, it seems unlikely that the release of 5-HT alone can account for the 5-HT depleting effects of these compounds.

Conversely, these structurally related compounds differ in their ability to release DA and block its reuptake (Johnson et al., 1986; Steele et al., 1987). Moreover, in the present study the increase in the extracellular concentration of DA following the acute administration of these compounds was negatively correlated with the concentration of 5-HT or 5-HIAA in the same brain region in the same animal 7 days later. Although these data do not demonstrate a cause-effect relationship, they are supportive of the hypothesis that DA plays a significant role in the 5-HT depleting effects of these compounds (Stone et al., 1988; Nash et al., 1990).

The rank order of potency for these compounds to increase the extracellular concentration of DA parallels their euphoriant properties as well as their abuse
potential. For example, neither MDE nor MBDB produce the degree of euphoria associated with MDMA administration (Braun et al., 1980; Nichols et al., 1986). Based on the results obtained in the present study, it is tempting to speculate that the euphoriant effects of these compounds are mediated, in part, via DA release. A similar hypothesis could be offered regarding the abuse liability of these compounds since, so far, neither MDE nor MBDB appear to have become popular recreational drugs. Clearly, the use of microdialysis may provide the answer to some of these questions.

In summary, alkyl substitutions longer than methyl on the nitrogen or α-carbon of MDA significantly diminish the potency of these compounds with respect to the release of DA in the striatum, as measured by in vivo microdialysis. In addition to their attenuated effect on DA release, the ability of these compounds to produce long-lasting depletion of brain 5-HT is also reduced.

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References


