Effects of the enantiomers of MDA, MDMA and related analogues on \([^{3}\text{H}]\)serotonin and \([^{3}\text{H}]\)dopamine release from superfused rat brain slices

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The primary amines 3,4-methylenedioxyamphetamine (MDA), and 1-(1,3-benzodioxol-5-yl)-2-butanamine (BDB) were measured for efficacy in release of \([^{3}\text{H}]\)serotonin (5-HT) from rat hippocampal slices, and release of \([^{3}\text{H}]\)dopamine (DA) from rat caudate nucleus slices. The N-methyl derivatives of MDA and BDB, MDMA and MBDB, respectively, and the optical antipodes of these four agents were compared in this paradigm. All of the test compounds demonstrated a similar efficacy of \([^{3}\text{H}]\)5-HT release in the micromolar concentration range. No significant stereoselectivity was seen in measurements of 5-HT release. However, striking differences were found between the test compounds when \([^{3}\text{H}]\)DA release was studied. N-methylation of racemic MDA resulted in a decreased ability to release DA, while side chain extension from \(\alpha\)-methyl to \(\alpha\)-ethyl completely abolished this activity. Stereoselectivity for the S\((+)-\)isomers of MDA and MDMA was also demonstrated in the DA release studies. Correlation of these biochemical findings with human subjective reports indicates that serotonin release may play a more important role in the mechanism of action than does dopamine release.

Amphetamine analogues; Brain slices; \([^{3}\text{H}]\)Dopamine release; Entactogens; \([^{3}\text{H}]\)Serotonin release

1. Introduction

As a result of the recent recreational popularity of 3,4-methylenedioxyamphetamine (MDMA), the United States Drug Enforcement Administration placed this compound into Schedule I of the Controlled Substances Act of 1970. Although 3,4-methylenedioxyamphetamine (MDA) and now MDMA have been classified as hallucinogens, these agents have an unusual behavioral profile compared to the classical psychedelics. It has been reported that MDA has a decreased potential to produce the severe sensory distortion typical of phenylamine hallucinogens such as 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane (DOM) (Naranjo et al., 1967).

MDMA apparently exhibits an even lower potential for sensory disruption than MDA (Shulgin and Nichols, 1978). On the illicit market, MDA and MDMA have been labelled the "love drug" and "ecstasy" respectively, emphasizing the purported effects of these agents on emotion and affect (Weil, 1976; Cohen, 1985). It has been suggested that these compounds reduce the anxiety or fear that normally accompanies the discussion of emotionally painful events. In this context they have been utilized as psychotherapeutic adjuncts (Greer, 1983; Yensen et al., 1975).

As part of our continuing studies of the structure-activity relationships of mind-altering substances, we have recently reported the synthesis and behavioral pharmacology of the \(\alpha\)-ethyl analogues of MDA and MDMA, BDB and MBDB (Nichols et al., 1986). Drug discrimination studies

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**Fig. 1.** S-\((+)^{-}\) isomers of the title compounds.

using lysergic acid diethylamide (LSD) trained rats (0.08 mg/kg i.p.) indicated that no stimulus generalization occurred with the racemates or enantiomers of MDMA or MBDB. In addition, other workers have shown that racemic MDMA does not substitute for the DOM (1.0 mg/kg i.p.) training stimulus (Glennon et al., 1982). The unique pharmacological profile demonstrated by MBDB prompted us to designate it as the prototype of a new therapeutic class to be called 'entactogens' (Nichols et al., 1986).

The goal of this study was to evaluate the dopamine and serotonin releasing properties of the title compounds in a brain slice assay using a newly designed superfusion chamber (Hoffman et al., 1986). We have previously shown that the isomers of MDA and MDMA stimulate release of \([3\text{H}]5\text{-HT}\) from rat whole brain synaptosomes (Nichols et al., 1982). The present study represents a more complete analysis of the direct and indirect releasing properties of some new MDMA-like compounds on central dopaminergic as well as serotonergic systems. Therefore, we compared the effects of the racemates and enantiomers of MDA, MDMA, BDB and MBDB (fig. 1) in the release of \([3\text{H}]5\text{-HT}\) from rat hippocampal slices and the release of \([3\text{H}]\text{DA}\) from rat caudate nucleus slices.

### 2. Materials and methods

#### 2.1. Drugs and buffer

All test drugs were dissolved in double-distilled water so that 50 μl of the stock solution added to 20 ml of buffer gave the desired drug concentration. Pargyline HCl (Sigma) was dissolved in double-distilled water so that 50 μl of the solution in 4.0 ml of buffer gave a final concentration of 1.0 μM pargyline in the incubation. \([3\text{H}]\text{Serotonin}\) (19.6 Ci/mmol, Amersham) was diluted in double-distilled water with 2% ethanol so that 50 μl in 4.0 ml buffer gave 0.1 μM \([3\text{H}]5\text{-HT}\) in the hippocampal slice incubation. Likewise, \([3\text{H}]\text{dopamine}\) (15 Ci/mmol, Amersham) was diluted with 0.02 M ascorbic acid and ethanol (9:1) such that 50 μl in 4.0 ml of buffer gave 0.1 μM \([3\text{H}]\text{DA}\) in the caudate nucleus slice incubation. Throughout the experiment, slices were maintained in an artificial cerebrospinal fluid (CSF) buffer at 37.5°C, aerated with 5% CO\(_2\)-95% O\(_2\). The buffer composition (mM) was: NaCl 118, KCl 4.8, CaCl\(_2\) 1.3, KH\(_2\)PO\(_4\) 1.2, MgSO\(_4\) 1.2, NaHCO\(_3\) 25, glucose 10, ascorbic acid 0.06, Na\(_2\)EDTA 0.03, pH = 7.4. In calcium deficient experiments the same buffer omitting CaCl\(_2\) was used.

#### 2.2. Procedure and apparatus

The procedures and apparatus used in these studies have been described previously (Hoffman et al., 1986). Male Sprague–Dawley rats (Harlan, Indianapolis) weighing 175-200 g were killed by decapitation. The brain was quickly removed and placed on a filter paper lightly moistened with chilled buffer. Either the caudate nucleus or hippocampus was dissected out and the middle one-third (longitudinally) was placed on the platform of a McIlwain tissue chopper set for a 0.30 mm thickness. The tissue was sliced and gently transferred to a test tube half-filled with chilled buffer, and the slices were coaxed apart using a vortex on a low setting. The slices and buffer were transferred to a petri dish and 10-12 intact slices were visually selected and placed into an incubation vessel containing 3.9 ml of oxygenated buffer. Then, 50 μl of pargyline HCl and 50 μl of the appropriate \(^3\text{H}\) neurotransmitter were added and allowed to incubate with the slices at 37.5°C for 30 min. The total time from killing to incubation was consistently less than 4 min.

After the incubation period, the four slices with the best appearance were transferred to the superfusion chambers. The chambers were then immersed in a constant temperature bath and
superfused at 0.5 ml/min with oxygenated buffer for a wash-out period of 30 min. Following the wash period, 15 1 min fractions were collected directly into scintillation vials. After the fifth fraction was collected, the appropriate drug stock solution (or double distilled water, as a control) was added to 20 ml of the buffer in the oxygenated reservoir for each chamber. At the end of the experiment each slice was removed from the superfusion chamber and transferred to a scintillation vial containing 0.5 ml of buffer. Aqueous Counting Solution (10 ml, Amersham) was added to each fraction, the vial was tightly capped and shaken, and then counted for tritium on a Minivax B tri-CARB 4000 series liquid scintillation counter (United Technologies Packard) at 40.0% counting efficiency.

2.3. Data analysis

Five separate studies were undertaken. The release of serotonin from rat hippocampal slices was examined with racemic mixtures of MDA, MDMA, BDB and MBDB at drug concentrations of 1 and 10 μM. The release of [3H]serotonin by the optical antipodes of the four test drugs was studied at the same two concentrations. The third study examined the release of dopamine from rat caudate nucleus slices in response to racemic MDA, MDMA, BDB and MBDB at concentrations of 0.1-10 μM. Each optical isomer was examined in the fourth study for dopamine releasing efficacy at drug concentrations of 1 and 10 μM. Finally, the release of [3H]5-HT and [3H]DA by the isomers of the test drugs (10 μM) was measured for calcium dependency using a calcium deficient buffer. Separate control runs were carried out for each study. Within each study, all treatments were randomized.

Two methods were used to analyze and present the data. First, tritiated overflow versus fraction curves were utilized to determine efflux significance above control (point-by-point Student’s t-test, P < 0.05). We have previously determined that only about 35% of the tritium overflow in the predrug curve is due to unmetabolized [3H]5-HT. This increases to 77% throughout the drug-induced release phase. Analysis of [3H]DA release revealed a quite similar metabolic profile (unpublished results). The first 5 1 min fractions served as a control to develop a baseline for spontaneous overflow. Experimental runs were not significantly different from control during this initial period. Following addition of the drug after fraction 5, the amount of tritium released in each fraction was compared to control for significance above that of spontaneous release (figs. 2, 3). Second, in order to present the results in a concise form, a table was prepared by integrating the area under the curve for the drug-induced percent released (i.e. fraction 7-15) and expressing it as a fraction relative to control release for the same time period (table 1). This fraction approaches one in the limit of zero drug effect. Analysis of variance yielded P values less than 0.05 for each study.

3. Results

3.1. [3H]Serotonin release from rat hippocampal slices

As seen in fig. 2, treatment with racemic MDA, MDMA, BDB and MBDB at a concentration of 10 μM resulted in [3H]5-HT efflux significantly above control. Significant release also occurred at 1 μM (not shown). The primary amines (+)-MDA and (+)-BDB, at 10 μM induced release significantly above that at 1 μM. However this was not true for the methylated derivatives (+)-MDMA and (+)-MBDB. In other words, the primary amines exhibited steeper dose-response relationships than their N-methylated counterparts. This trend was also observed in studies of the optical isomers (table 1).

With the exception of R-(−)-MDA and R-(−)-BDB at 1 μM, both enantiomers of each test compound stimulated release significantly above control (table 1). It should be noted that no significant difference in [3H]5-HT release could be detected between the optical isomers of the individual test drugs, with the exception of S-(+)-BDB, which gave release significantly greater that its R-(−) isomer at 10 μM. Although trends were apparent, no significant difference between the 1 and 10 μM concentration could be detected with
Fig. 2. The drug-induced release of [3H]serotonin from superfused hippocampal slices. All drugs were added after the fifth 1 min fraction at a final concentration of 10 μM. Each point represents the mean ± the 95% confidence limits of four determinations. P < 0.05 vs. control (point by point Student's t-test) for all drug fractions past number 7. (-----) Control; (-----) (±)-MDA; (-----) (±)-MDMA; (-----) (±)-BDB; (-----) (±)-MBDB.

Fig. 3. The drug-induced release of [3H]dopamine from superfused rat caudate nucleus slices. All drugs were added after the fifth 1 min fraction at a final concentration of 10 μM. Each point represents the mean ± 95% confidence limits of four determinations. P < 0.05 vs. control (point by point Student's t-test) for (±)-MDA and (±)-MDMA fractions past number 7. (-----) Control; (-----) (±)-MDA; (-----) (±)-MDMA; (-----) (±)-BDB; (-----) (±)-MBDB.
S-(+)-MBDB and only a slight difference was observed for S-(+)-MDMA. In a separate series of experiments the 5-HT release produced by each of the isomers was found to be calcium independent (data not shown).

3.2. \([3^2]H\)dopamine release from rat caudate nucleus slices

As illustrated in fig. 3, only the \(\alpha\)-methyl analogues facilitated \([3^2]H\)dopamine release above control, at any concentration. Racemic MDA at 10 \(\mu\)M resulted in greater \([3^2]H\)DA efflux than any of the other racemates. N-methylation of (±)-MDA to give (±)-MDMA apparently diminished dopamine releasing efficacy but did not abolish it. Racemic MDA at 1 \(\mu\)M (not shown) induced release slightly above control, while the effect of (±)-MDMA at 1 \(\mu\)M was similar to control.

<table>
<thead>
<tr>
<th>Compound ((\mu)M)</th>
<th>Fraction released relative to control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>([3^2]H)serotonin</td>
</tr>
<tr>
<td>Control</td>
<td>1.00 ± 0.03</td>
</tr>
<tr>
<td>S-MDA 10</td>
<td>2.94 ± 0.58 (\star)</td>
</tr>
<tr>
<td></td>
<td>1.25 ± 0.09 (\star)</td>
</tr>
<tr>
<td>R-MDA 10</td>
<td>2.48 ± 0.27 (\star)</td>
</tr>
<tr>
<td></td>
<td>1.09 ± 0.05</td>
</tr>
<tr>
<td>S-MDMA 10</td>
<td>2.01 ± 0.18 (\star)</td>
</tr>
<tr>
<td></td>
<td>1.59 ± 0.20 (\star)</td>
</tr>
<tr>
<td>R-MDMA 10</td>
<td>2.21 ± 0.35 (\star)</td>
</tr>
<tr>
<td></td>
<td>1.25 ± 0.13 (\star)</td>
</tr>
<tr>
<td>S-BDB 10</td>
<td>2.60 ± 0.40 (\star)</td>
</tr>
<tr>
<td></td>
<td>1.30 ± 0.13 (\star)</td>
</tr>
<tr>
<td>R-BDB 10</td>
<td>1.79 ± 0.27 (\star)</td>
</tr>
<tr>
<td></td>
<td>1.03 ± 0.08</td>
</tr>
<tr>
<td>S-MBDB 10</td>
<td>1.74 ± 0.15 (\star)</td>
</tr>
<tr>
<td></td>
<td>1.46 ± 0.33 (\star)</td>
</tr>
<tr>
<td>R-MBDB 10</td>
<td>1.87 ± 0.22 (\star)</td>
</tr>
<tr>
<td></td>
<td>1.14 ± 0.04 (\star)</td>
</tr>
</tbody>
</table>

\(\star\) \(P < 0.05\), point by point Student’s t-test against control.

Neither of these two agents were significantly different from control at 0.1 \(\mu\)M. Treatment with the \(\alpha\)-ethyl derivatives, (±)-BDB and (±)-MBDB, did not result in significant \([3^2]H\)DA release at any concentration tested.

Both the R-(−) and the S-(+) isomers of the \(\alpha\)-methyl derivatives, MDA and MDMA, were found to give significant release of \([3^2]H\)DA at a concentration of 10 \(\mu\)M, but only S-(+)-MDA and S-(+)-MDMA gave efflux significantly above control at 1 \(\mu\)M (table 1). No significant differences were found between S-(+)-MDA and S-(+)-MDMA at either concentration. Similarly, there was no difference between the effects of R-(−)-MDA and R-(−)-MDMA at either concentration. However, both S-(+)-MDA and S-(+)-MDMA were substantially more potent than their R-(−) isomers at 1 and 10 \(\mu\)M. Most notably, only R-MDMA was found to be calcium dependent, indicating a marked difference between the R and S isomer of this secondary amine in DA release.

4. Discussion

The brain slice paradigm used in these studies allows for several theoretical mechanisms of \(3^2\)H-neurotransmitter release. In this model, a drug may have a direct postsynaptic receptor-mediated excitatory action, resulting in stimulus-coupled neurotransmitter release at its associated nerve terminus. Conversely, a compound may act indirectly by displacement of presynaptic stores, requiring entry into the terminal via the normal neurotransmitter uptake mechanism. Furthermore, an agent may act as a simple competitive uptake inhibitor, resulting in increased extracellular \(3^2\)H-neurotransmitter concentrations following spontaneous release. The first mechanism requires calcium while the latter two mechanisms are calcium independent. Any of these processes could account for an increase in \(3^2\)H-neurotransmitter efflux from the brain slice. However, experimental evidence to date suggests that only one or two of the aforementioned possibilities could be responsible for the neurotransmitter release seen in the present study.
Recently, Lyon et al. (1986) have shown that the isomers of both MDA and MDMA have very low affinity for both 5-HT_1 and 5-HT_2 receptor subtypes in rat cortex. Furthermore, the R(-) isomers of both compounds display higher affinity than do the S(+) isomers, a finding in stark contrast to that observed in in vivo studies, where the (+) isomer of MDMA is more active (Anderson et al., 1978). In addition, our earlier study using rat whole-brain synaptosomes provides evidence that intact neurons are not required for [3 H]5-HT release by the enantiomers of MDA and MDMA (Nichols et al., 1982). These data, coupled with our finding that the release of serotonin and dopamine from brain slices by the (+) isomers of the test compounds is calcium independent, renders it unlikely that these compounds act by a direct postsynaptic receptor mechanism.

We have shown that [3H]serotonin release by MDA is blocked in the presence of the specific serotonin uptake inhibitor fluoxetine (3 μM) (unpublished results). Other investigators (Gehlert et al., 1985) have found that the reduction of striatal 5-HT levels produced by MDMA in vivo is inhibited by citalopram, another serotonin reuptake inhibitor, and have proposed that (+)-[3H]-MDMA is probably not actively accumulated in striatal synaptosomes. These data indicate that the likely mechanism for neurotransmitter release in brain slices may be by a simple blockade of the uptake carrier protein. However, an alternative mechanism that cannot presently be discounted is that these compounds promote the release of [3H]5-HT by affecting the uptake carrier. Blockade of the carrier with fluoxetine or citalopram could inhibit the releasing effect. Such a mechanism does not necessarily imply that the test compounds compete for the uptake site on the carrier.

Specific serotonin uptake inhibitors have proven therapeutically useful in the treatment of affective disorders. Clinical studies utilizing fluoxetine, citalopram and zimelidine have demonstrated that these compounds are at least as effective as classical tricyclic antidepressants (Lemberger et al., 1985; Bjerkenstedt et al., 1985). The present findings indicate that use of MDMA in an appropriate dose range might be similarly effective in the treatment of depression.

In the present study, no significant difference was found between the optical isomers of the test drugs with respect to [3H]5-HT release. However, upon close examination of the dose-response relationships subtle differences are evident. Specifically, the effect of N-methylation appears to be enantiospecific. That is, N-methylation of the S-(+) isomers substantially decreases the magnitude of dose related response, while N-methylation apparently has less of an effect on the R(-) isomers.

With respect to [3H]dopamine release, our results clearly show that (+)-MDA induced a significantly greater effect than (+)-MDMA. In contrast, when R(-)-MDA is compared with R(-)-MDMA, or S(+)-MDA is compared with S(+)-MDMA, no significant difference in efflux is seen. This apparent discrepancy may be the result of the existence of competitive mechanisms for the S and R isomers of MDMA. This suggestion follows from the observations of Steele (1986) of non-parallel log dose-response curves for the isomers of MDMA in synaptosomal [3H]DA uptake inhibition experiments. Further, Lyon et al. (1986) found that of the isomers of MDA and MDMA, only R-MDMA had a K_i less than 100,000 nM for binding to the dopamine D_2 receptor. This correlates with the finding that R-MDMA was the only calcium-dependent dopamine releaser. Therefore, the evidence to date suggests the possibility of different mechanisms of action for the isomers of MDMA on dopaminergic systems. If these mechanisms were antagonistic, racemic MDMA would show a decreased effect, as observed in the present study. This implies that N-methylation of the MDA enantiomers does not decrease the efficacy of the S isomer but may have a more pronounced effect on the mechanism of the R isomer.

Significantly, no increase in [3H]dopamine efflux was seen with either the racemic mixtures or the optical isomers of the α-ethyl analogues BBD and MBDB. This dopamine release process seems to exhibit a steric intolerance for an α substituent larger than a methyl group. The lack of dopamine release may have relevance to the report that the α-ethyl derivative, MBDB, produces less euphoria than its α-methyl analog, MDMA (Nichols et al.,
MBDB may lack the reinforcing qualities that are characteristic of such drugs as amphetamine and cocaine, where an increase in dopamine turnover probably plays a significant role (Wise, 1984).

The results reported herein are in general agreement with those obtained in previous studies using rat brain synaptosomes. A slight stereoselectivity for the S isomers of MDA and MDMA was evident in \([3H]5\)-HT release studies from whole brain synaptosomes (Nichols et al., 1982). However, a 3-fold stereoselectivity for the S isomers of MDA, MDMA and MBDB was demonstrated by Steele (1986) in \([3H]5\)-HT uptake inhibition experiments using hippocampal synaptosomes. In that study, the stereoselectivity and stereotolerance observed in \([3H]DA uptake inhibition using striatal synaptosomes paralleled the findings in the present study. The stereoselectivity observed is similar to that demonstrated by the enantiomers of amphetamine in caudate slices (unpublished results), corresponding to their central stimulant potencies. The isomers of MDA have also been studied for \([3H]\)norepinephrine uptake inhibition and an approximately 2-fold selectivity for the S isomer has been demonstrated (Marquardt et al., 1978; Steele, 1986).

In summary, N-methylation and \(\alpha\)-ethylation in the 3,4-methylenedioxyphenethylamine series has profound effects on the drug-induced efflux of \([3H]\)dopamine and \([3H]\)serotonin from rat brain slices. N-methylation did not appear to significantly decrease dopamine or serotonin release. While \(\alpha\)-ethylation did not affect the efficacy of serotonin release, it completely abolished dopamine release. These results indicate that the combination of \(\alpha\)-ethylation and N-methylation of MDA may substantially decrease its dopaminergic component while retaining the ability to modulate serotonergic systems.

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