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STEREOCHEMICAL EFFECTS OF 3,4-METHYLENEDIOXYMETHAMPHETAMINE (MDMA) AND RELATED AMPHETAMINE DERIVATIVES ON INHIBITION OF UPTAKE OF [^{3}H]MONOAMINES INTO SYNAPTOSOMES FROM DIFFERENT REGIONS OF RAT BRAIN

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Abstract—3,4-Methylenedioxymethamphetamine (MDMA) is a recently popularized recreational drug, although some have advocated its psychotherapeutic potential. Since the pharmacology of MDMA is largely uncharacterized, the stereochemical profiles of MDMA and some of its homologs were derived on inhibition of synaptosomal uptake of [^{3}H]monoamines and compared to those of amphetamine and the hallucinogenic phenylisopropylamine 2,5-dimethoxy-4-methylamphetamine (DOM). In contrast to the 5-fold stereoselectivity observed with amphetamine, only the *S*-(+) enantiomer of MDMA and 3,4-methylenedioxyamphetamine (MDA) inhibited [^{3}H]dopamine uptake into striatal synaptosomes. Neither stereoisomer of the α-ethyl homolog of MDMA, *N*-methyl-1-(1,3-benzodioxol-5-yl)-2-butanamine (MBDB), inhibited [^{3}H]dopamine uptake. The two stereoisomers of amphetamine and the MDMA-related compounds were equipotent in inhibiting [^{3}H]norepinephrine uptake into hypothalamic synaptosomes. Both steroisomers of MDMA, MDA and MBDB were potent inhibitors of [^{3}H]serotonin uptake into hippocampal synaptosomes, but only *S*-(+)-amphetamine produced an appreciable inhibition of [^{3}H]serotonin uptake. Neither steroisomer of DOM inhibited synaptosomal uptake of any [^{3}H]monoamine. These results suggest that MDMA and its homologs may be more closely related to amphetamine rather than to DOM in their biochemical mode of action. The pronounced effects of the methylenedioxy-substituted compounds on [^{3}H]serotonin and [^{3}H]norepinephrine uptake implicate these neurotransmitters in the pharmacological effects of these drugs.

A variety of chemical derivatives of amphetamine possess hallucinogenic activities similar to that of lysergic acid diethylamide (LSD) [1, 2]. Substitution with a 3,4-methylenedioxy group on the aromatic ring of amphetamine results in a compound, 3,4-methylenedioxyamphetamine (MDA), that has effects similar to both LSD and amphetamine in the chronic spinal dog [3, 4]. The qualitative nature of MDA intoxication in humans also appears to possess characteristics of both drugs [5]. In addition, MDA induces a state of enhanced perceptual awareness and a sense of empathy which led to its experimental use as an adjunct to psychotherapy [5, 6]. The *N*-methyl analog of MDA, 3,4-methylenedioxymethamphetamine (MDMA, "Ecstasy", "XTC", "Adam"), is the latest amphetamine derivative to appear on the illicit drug market. Effects of MDMA are reported to be similar to those of MDA, but of shorter duration and with little or no hallucinogenic character [7]. Anecdotal reports by psychiatrists suggest that MDMA may be useful as an adjunct to psychotherapy [8, 9], but the pharmacology of this compound is relatively uncharacterized.

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The diverse behavioral effects of substituted amphetamine derivatives may be due to biochemical actions similar to those of amphetamine itself or, as in the case of 2,5-dimethoxy-4-methylamphetamine (DOM), similar to those of LSD. The central stimulant activity of amphetamine is attributed to a functional elevation of central catecholamines via indirect release or inhibition of reuptake of norepinephrine and dopamine and, at high doses, an inhibition of monoamine oxidase activity [10]. The *S*-(+) (dextro) isomer of amphetamine is more potent than the *R*-(−) (levo) enantiomer on these biochemical mechanisms and, also, in eliciting the typical behavioral effects of the drug [10]. The psychotropic effects of LSD, DOM, and other hallucinogens appear to depend on interactions with central serotonin (5-HT) receptors, resulting in altered central serotonergic activity [11, 12]. Within this class of compounds, the *R*-(−) enantiomers possess the highest affinity for 5-HT$_2$ receptors and are more potent in animal and human assays [13, 14]. Drug discrimination studies have indicated that the *S*-(+) isomer of MDA is responsible for the amphetamine-like effects of the drug in rats [15] and that the *R*-(−) enantiomer is responsible for hallucinogen-appropriate responding [16]. These findings parallel *in*

vitro biochemical data, which have shown a greater effect of *S*-(+)-MDA in releasing and inhibiting the uptake of [^{3}H]norepinephrine in hypothalamic synaptosomes [17], suggestive of the amphetamine character of the compound, whereas *R*-(−)-MDA possesses a greater affinity for 5-HT_2 receptors than its antipode [18].

As with MDA, the amphetamine stimulus generalizes to MDMA [15]. In contrast, neither racemic nor the individual enantiomers of MDMA substitute for the discriminative stimulus cues of DOM [16] or LSD [19]. Although N-methylation reduces the hallucinogenic activity associated with the *R*-(−) isomer, MDMA retains the ability to induce subjective effects in humans similar to those of MDA. Indeed, human studies have indicated that *S*-(+)MDMA is substantially more active than the *R*-(−) enantiomer in eliciting the unique subjective effects of the racemate [20]. Despite the evidence suggesting that the diverse behavioral effects of MDA and MDMA may be attributable to stereoselective actions, few biochemical studies have addressed this aspect of the pharmacology of these drugs.

In the present study, we investigated potential stereoselective effects of MDMA, MDA and a recently developed homolog of MDMA, *N*-methyl-1-(1,3-benzodioxol-5-yl)-2-butanamine (MBDB) [19], on a neurochemical mechanism that is sensitive to the stereoselective effects of amphetamine, inhibition of synaptosomal uptake of [^{3}H]monoamines. The data obtained should indicate whether these compounds are more similar to amphetamine than to DOM with respect to their biochemical modes of action. By correlating *in vitro* data with findings in behavioral studies, it was anticipated that insight might be gained as to the neurotransmitters involved in the diverse behavioral actions and psychotropic effects of MDMA and its homologs.

METHODS

Preparation of synaptosomes. Male Sprague–Dawley rats weighing 175–200 g were decapitated. Brains were rapidly removed and placed on a chilled, glass plate, and the hypothalamus, striatum, and hippocampus were dissected according to the methods of Glowinski and Iversen [21]. Pooled tissue from three to five rats was homogenized in 30 vol. of ice-cold 0.32 M sucrose in a chilled glass mortar with a motor-driven pestle at 850 rpm for 30 sec. The homogenate was centrifuged at 1086 *g* (3000 rpm) for 10 min in a Sorvall RC2-B refrigerated centrifuge at 4°. The supernatant containing the crude synaptosomal fraction was decanted into a glass vessel and placed on ice.

Incubation conditions. Incubations were carried out in a shaking incubator under an atmosphere of 95% O_2/5% CO_2 at 37° or 0–2° to measure total tissue uptake and non-specific uptake respectively. Uptake of 1-[^{3}H]noradrenaline (Amersham Corp., 37 Ci/mmol) was measured in hypothalamic tissue, [^{3}H]dopamine HCl (Amersham Corp., 15 Ci/mmol) uptake in striatal tissue, and [^{3}H]-5-hydroxytryptamine creatinine sulfate (Amersham Corp., 19.6 Ci/mmol) uptake in hippocampal tissue. A 5-min preincubation was begun by adding 0.2 ml of the synaptosomal preparation to test tubes containing 1.65 ml of Krebs–Henseleit buffer (118 mM NaCl, 4.8 mM KCl, 1.3 mM $CaCl_2$, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 25 mM $NaHCO_3$, 10 mM glucose, 0.6 mM ascorbic acid and 0.03 mM disodium EDTA), 50 μl of drug, and 50 μl of pargyline HCl (final concentration of 1 μM). Radiolabeled monoamines (final concentration 0.01 μM) were added in 50-μl aliquots to the sample mixture, and incubation was continued for 10 min. Incubations were terminated by rapid filtration through 0.65 μm membrane filters (Millipore Corp., type DAWP) on a filtration device equipped with a vacuum pump. The filters containing the synaptosomes and retained monoamines were rinsed with 4 ml of buffer and placed in glass scintillation vials, and then 4 ml of 2-ethoxyethanol and 10 ml of 5% 2,5-diphenyloxazole (PPO)/toluene were added. The vials were allowed to sit overnight prior to liquid scintillation counting. Data were derived from counts of tissue disintegrations per minute (dpm).

Data analysis. Specific tissue uptake (SU) of radiolabeled neurotransmitter was determined from the difference in the mean dpm of three samples run at 37° and 0°. Percent inhibition was calculated from the following equation:

$$\% \text{ Inhibition} = \frac{\text{control-SU (dpm)} - \text{drug-SU (dpm)}}{\text{control-SU (dpm)}} \times 100$$

Each drug was tested at three different concentrations in three independent trials. The IC_{50} values and 95% confidence intervals, and slope comparisons to reflect deviations from parallelism, were determined by regression analysis [22].

Drugs. The hydrochloride salts of the isomers of MDA, MDMA, and DOM, and the α-ethyl homolog of MDMA, MBDB, were synthesized in our laboratory by previously described methods [19]. SKF Laboratories provided *d*- and *l*-amphetamine sulfate, and pargyline HCl was purchased from the Sigma Chemical Co.

RESULTS

Inhibition of [^{3}H]dopamine uptake by striatal synaptosomes. The stereoselectivity exhibited by the optical isomers of amphetamine toward inhibition of synaptosomal uptake of [^{3}H]dopamine is illustrated in Fig. 1 (upper panel). The difference between the slopes of the log dose–response lines derived for the two isomers was not significant ($P > 0.05$). The mean IC_{50} determinations for the stereoisomers of amphetamine indicated that the dextro (*S*-[+]) isomer was approximately five times more potent than its antipode as a [^{3}H]dopamine uptake inhibitor in striatal synaptosomes (IC_{50}: *S*-(+) = 0.38 μM, *R*-(−) = 2.05 μM; Table 1). The IC_{50} value for each isomer was excluded from the confidence interval of its antipode, but overlap of the intervals was evident.

More pronounced stereoselectivity was observed with MDA and MDMA than for amphetamine itself, as shown for MDMA in Fig. 1 (middle panel). The *S*-(+) isomers of both MDA and MDMA were effective as inhibitors of synaptosomal uptake of [^{3}H]dopamine at concentrations in the micromolar

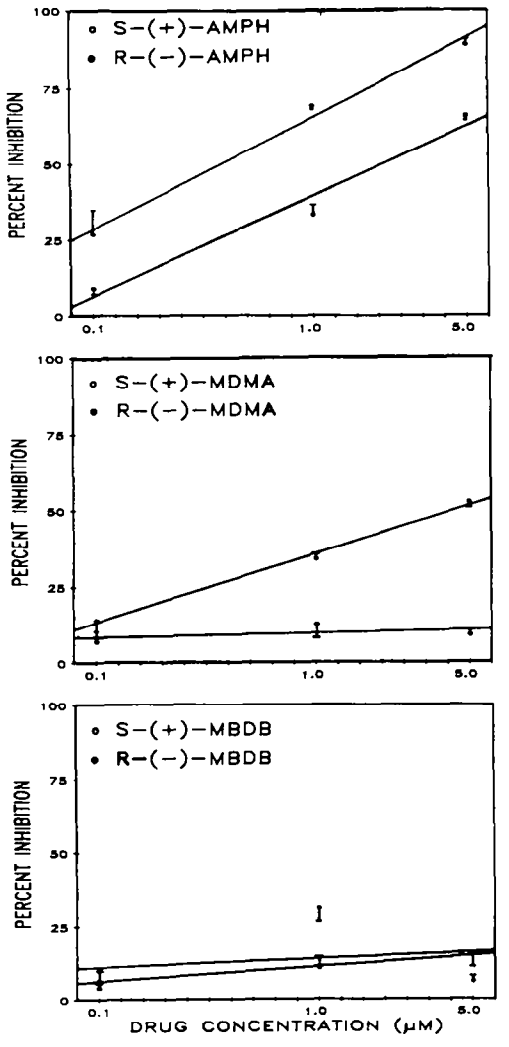

Fig. 1. Inhibition of [^{3}H]dopamine uptake into striatal synaptosomes by the stereoisomers of amphetamine (AMPH), MDMA and MBDB. Drugs were preincubated with the synaptosomal preparation for 5 min prior to the addition of 0.01 μM [^{3}H]dopamine. Each point represents the mean (± SEM) of three independent trials.

range (IC_{50}: *S*-(+)-MDA = 1.96 μM, *S*-(+)-MDMA = 4.20 μM; Table 1). In contrast to amphetamine, the *R*-(−) enantiomers of these compounds were virtually devoid of inhibitory effects at the highest drug concentrations tested (5 μM). Neither stereoisomer of the α-ethyl homolog of MDMA, MBDB, inhibited uptake of [^{3}H]dopamine to 50% of controls at the highest concentrations tested (5 μM). Interestingly, the peak inhibitory effect for *S*-(+)-MBDB was noted consistently at the 1 μM drug concentration, and an "inverted-U" shaped dose–response curve was obtained (Fig. 1, lower panel).

The IC_{50} determinations with their 95% confidence limits and the slopes of the regression lines for the stereoisomers of the five compounds tested are presented in Table 1. It was not possible to determine the relative potencies of particular isomers [e.g. *S*-(+)] for all drugs due to non-parallelism of the regression lines. The trend observed was that inhibitory potency progressively diminished with increasing substitution of the *S*-(+) enantiomers [i.e. *S*-(+)-amphetamine > *S*-(+)-MDA > *S*-(+)-MDMA > *S*-(+)-MBDB). Neither stereoisomer of DOM produced an appreciable inhibition of [^{3}H]dopamine uptake at the highest concentration tested (10 μM).

Inhibition of [^{3}H]norepinephrine uptake into hypothalamic synaptosomes. No significant stereoselectivity was exhibited by amphetamine in inhibiting the uptake of [^{3}H]norepinephrine into hypothalamic synaptosomes. The log dose–response curves for the stereoisomers of amphetamine, shown in Fig. 2 (left panel), were nearly superimposable, resulting in similar slopes and IC_{50} values. Both *S*-(+)- and *R*-(−)-amphetamine were potent inhibitors of synaptosomal uptake of [^{3}H]norepinephrine (IC_{50}: *S*-(+) = 0.07 μM, *R*-(−) = 0.10 μM; Table 2).

Stereoselectivity with MDA and MDMA was suggested by the mutual exclusion of the IC_{50} values for either isomer from the confidence interval of their respective antipode (Table 2). However, the slope of the log dose–response curves was steeper for the *S*-(+) enantiomers, as shown for MDMA in Fig. 2 (right panel). In the case of MBDB, the regression lines for the enantiomers were parallel ($P > 0.05$). The IC_{50} for *S*-(+)-MBDB was three times lower

Table 1. IC_{50} Determinations with 95% confidence limits and regression slopes for inhibition of [^{3}H]dopamine uptake into striatal synaptosomes

	Isomer					
	S(+)			*R*(−)		
Drug	IC_{50} (μM)	*r*	Slope	IC_{50} (μM)	*r*	Slope
AMPH	0.38 (0.10–1.36)	0.96	37.0	2.05 (0.69–6.66)	0.97	32.9
MDA	1.96 (0.60–7.13)	0.97	26.8*†	>5	0.03	0.3*
MDMA	4.20 (1.52–13.36)	0.98	22.5*†	>5	0.18	1.3*
MBDB	>5	0.16	2.9*†	>5	0.62	5.1*
DOM	>10	0.54	3.3*	>10	0.72	3.9*

Amphetamine (AMPH), MDA, MDMA and MBDB were tested at concentrations of 5.0, 1.0, and 0.1 μM and DOM was tested at 10.0, 1.0, and 0.1 μM. The final concentration of [^{3}H]dopamine was 0.01 μM.

* Slope is different from corresponding isomer of amphetamine ($P < 0.05$).

† Slopes of the two stereoisomers are different ($P < 0.05$).

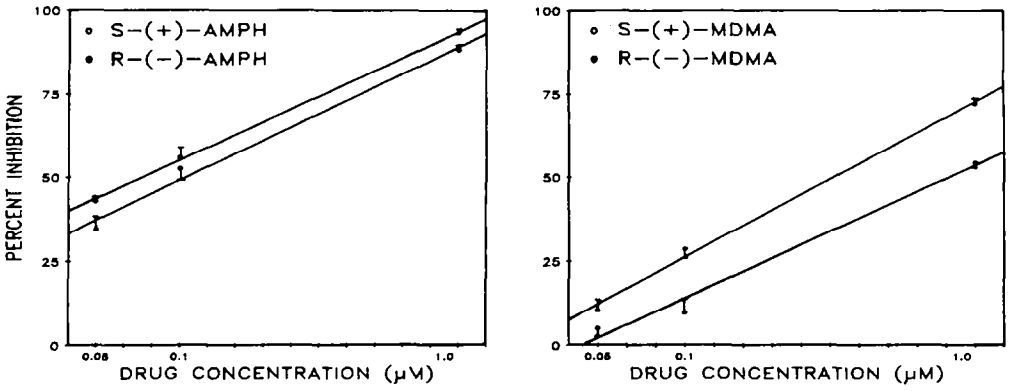

Fig. 2. Inhibition of [^{3}H]norepinephrine uptake into hypothalamic synaptosomes by the stereoisomers of amphetamine (AMPH) and MDMA. Drugs were preincubated with the synaptosomal preparation for 5 min prior to the addition of 0.01 μM [^{3}H]norepinephrine. Each point represents the mean (± SEM) of three independent trials.

than that determined for *R*-(−)-MBDB (0.64 and 2.22 μM respectively).

The data obtained for inhibition of [^{3}H]norepinephrine uptake into hypothalamic synaptosomes by the stereoisomers of the five compounds tested are summarized in Table 2. Only the regression slopes for the *S*-(+) isomers of MDA and MDMA were sufficiently similar to allow comparison of their potencies, which were also similar. The regression line for [^{3}H]norepinephrine uptake inhibition by the *R*-(−) isomer of amphetamine was parallel to the regression lines obtained for the *R*-(−) isomers of MDA and MDMA. When compared to *R*-(−)-amphetamine, significantly lower inhibitory potencies were obtained for *R*-(−)-MDA (4-fold) and *R*-(−)-MDMA (8-fold). As was observed with [^{3}H]dopamine uptake, neither stereoisomer of DOM had an appreciable inhibitory effect on [^{3}H]norepinephrine uptake.

Inhibition of [^{3}H]serotonin uptake into hippocampal synaptosomes. The stereochemical profile of amphetamine on inhibition of [^{3}H]serotonin uptake into hippocampal synaptosomes is shown in Fig. 3 (left panel). Since the log dose–response plot for *S*-(+)-amphetamine was not linear, the data were converted to probits. Probit analysis also failed to provide a linear relationship. The IC_{50} value and its confidence interval derived for *S*-(+)-amphetamine using probits (Table 3) were comparable to those derived by linear regression. The IC_{50} value was in the micromolar range (2.65) but suggests a weaker inhibitory effect on synaptosomal uptake of [^{3}H]serotonin than that exerted on [^{3}H]catecholamine uptake. *R*-(−)-Amphetamine had a minimal inhibitory effect on synaptosomal uptake of [^{3}H]serotonin, and an IC_{50} value could not be obtained.

Both enantiomers of the three methylenedioxy-substituted compounds were potent inhibitors of synaptosomal uptake of [^{3}H]serotonin. The log dose–response curves for the enantiomers of MDMA on inhibition of [^{3}H]serotonin uptake also deviated significantly from linearity, and the data for the series were analyzed using probits. This analysis yielded

Table 2. IC_{50} Determinations with 95% confidence limits and regression slopes for inhibition of [^{3}H]norepinephrine uptake into hypothalamic synaptosomes

	Isomer					
	S(+)			*R*-(−)		
Drug	IC_{50} (μM)	*r*	Slope	IC_{50} (μm)	*r*	Slope
AMPH	0.07 (0.04–0.12)	0.99	38.14	0.10 (0.04–0.25)	0.97	40.4
MDA	0.27 (0.17–0.44)	0.99	47.9*†	0.46 (0.27–0.81)	0.99	39.0
MDMA	0.32 (0.19–0.56)	0.99	46.4*†	0.81 (0.35–2.15)	0.97	39.7
MBDB	0.64 (0.19–2.09)	0.97	29.7*	2.22 (0.47–12.92)	0.95	31.3*
DOM	>10	0.76	9.8*	>10	0.72	6.2*

Amphetamine (AMPH), MDA and MDMA were tested at concentrations of 1.0, 0.1, and 0.05 μM, MBDB at 5.0, 1.0, and 0.1 μM, and DOM at 10.0, 1.0, and 0.1 μM. The final concentration of [^{3}H]norepinephrine was 0.01 μM.

* Slope is different from corresponding isomer of amphetamine ($P < 0.05$).

† Slopes of the two stereoisomers are different ($P < 0.05$).

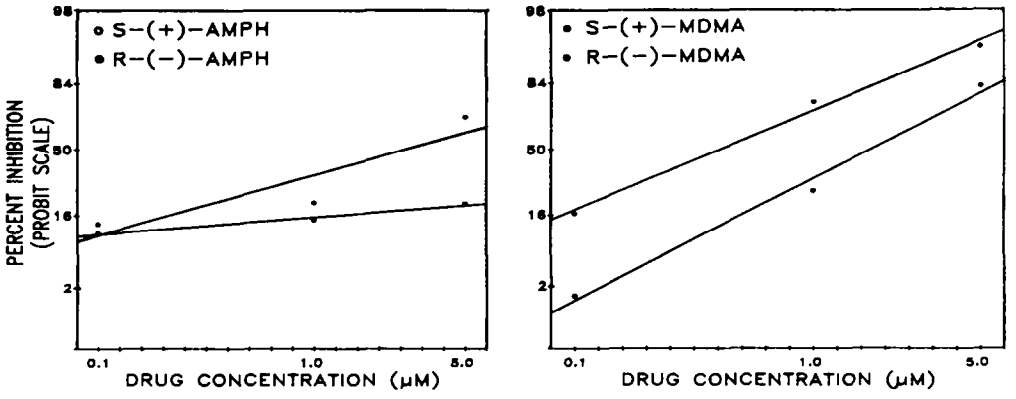

Fig. 3. Inhibition of [^{3}H]serotonin uptake into hippocampal synaptosomes by the stereoisomers of amphetamine (AMPH) and MDMA. Drugs were preincubated with the synaptosomal preparation for 5 min prior to the addition of 0.01 μM [^{3}H]serotonin. Data were analyzed using probits and are plotted on a probit scale. Each point represents the mean of three independent trials. Standard errors were 5% or less.

linear plots in all instances except for *R*-(−)-MDMA (Fig. 3, right panel). The curves for the *S* enantiomers of MDMA and MDA were parallel, but the slope of the curve for *S*-(+)-MBDB was diminished in comparison. The IC_{50} values determined for either the *S*-(+) or the *R*-(−) isomers of all three compounds were similar (Table 3) and suggest that these compounds have potent inhibitory effects on [^{3}H]serotonin uptake into hippocampal synaptosomes. The ratios of the IC_{50} values for the *R* and *S* enantiomers were approximately 3- to 4-fold with the greater degree of inhibition being produced by the *S*-(+) isomer, but no significant degree of stereoselectivity was observed for any of the compounds.

The data obtained for the drug-induced inhibition of [^{3}H]serotonin uptake into hippocampal synaptosomes by the stereoisomers of the five compounds tested are summarized in Table 3. Note that S-(+)-amphetamine had an apparent 5-fold lower potency and a lower regression line slope than the corresponding enantiomer of any of the methylenedioxy-substituted compounds. Despite the inability of either enantiomer of DOM to achieve a 50% inhibition of synaptosomal uptake of [^{3}H]serotonin, this was the only instance in which a greater degree of inhibition was produced by the *R*-(−) enantiomer ($P < 0.05$; two-way ANOVA).

DISCUSSION

Much controversy has arisen surrounding MDMA as a consequence of its street use and its claimed psychotherapeutic potential. Dissociation of these aspects as they pertain to MDMA could open new psychotherapeutic avenues. Based on the results of the present study and previous reports, arguments will be presented that suggest (1) a distinction between MDMA and its homologs and the classical hallucinogens and stimulants and (2) the potential neuromediators of the psychotherapeutic or subjective effects of the methylenedioxy-substituted compounds.

Studies with the parent compound amphetamine correlate the *in vitro* effects of its stereoisomers with their observed behavioral effects [10]. Our findings of a more pronounced inhibition of [^{3}H]dopamine

Table 3. IC_{50} Determinations with 95% confidence limits from probit analysis of the data for inhibition of [^{3}H]serotonin uptake into hippocampal synaptosomes

	Isomer					
	S(+)			*R*(−)		
Drug	IC_{50} (μM)	*r*	Slope	IC_{50} (μM)	*r*	Slope
AMPH	2.65 (0.15–122.0)	0.86	0.91*	>5	0.57	0.26
MDA	0.49 (0.23–1.02)	0.99	1.29†	1.62 (0.69–3.87)	0.98	1.50†
MDMA	0.41 (0.22–0.74)	0.98	1.50*†	1.73 (1.00–3.07)	0.99	1.84*†
MBDB	0.41 (0.07–1.90)	0.95	1.11†	1.88 (0.76–4.88)	0.98	1.14*†
DOM	>10	0.78	0.44*†	>10	0.59	0.24*†

Amphetamine (AMPH), MDA, MDMA, and MBDB were tested at concentrations of 5.0, 1.0, and 0.1 μM and DOM was tested at 10.0, 1.0, and 0.1 μM. The final concentration of [^{3}H]serotonin was 0.01 μM.

* Slopes of the two stereoisomers are different ($P < 0.05$).

† Slope is different from corresponding isomer of amphetamine ($P < 0.05$).

uptake into striatal synaptosomes by *S*-(+)-amphetamine, but a lack of stereoselectivity for inhibition of [^{3}H]norepinephrine uptake into hypothalamic synaptosomes are consistent with previous reports [23–26]. This evidence implicates dopamine in the mediation of the behavioral responses of the drug [10]. It also appears that a dopaminergic component underlies the discriminative stimulus cue of amphetamine [27, 28]. Only the *S*-(+) isomer of amphetamine significantly inhibited [^{3}H]serotonin uptake into hippocampal synaptosomes, but much higher drug concentrations were required than for inhibition of synaptosomal uptake of [^{3}H]catecholamines.

In contrast to amphetamine, inhibition of [^{3}H]dopamine uptake into striatal synaptosomes by MDMA and MDA was stereospecific, as the *R*-(−)enantiomers were virtually inactive. In a concurrent investigation, parallel results suggesting stereospecific release of [^{3}H]dopamine from rat striatal slices by *S*-(+)-MDMA and *S*-(+)-MDA have been obtained [29]. Marquardt *et al.* [30, 31] have reported similarities in the behavior of animals treated with *S*-(+)-MDA and either *S*-(+)- or *R*-(−)-amphetamine, but animals treated with *R*-(−)-MDA displayed a behavioral profile resembling that of animals treated with LSD or mescaline. In drug discrimination paradigms, *S*-(+)-amphetamine generalizes to *S*-(+)-MDA, but not to the *R*-(−) enantiomer [15]. Since no significant degree of stereoselectivity was observed with any of the methylenedioxy-substituted compounds in inhibiting synaptosomal uptake of [^{3}H]norepinephrine, it seems likely that a dopaminergic component underlies the amphetamine-like behavioral effects of MDA, and possibly those of MDMA. In *in vivo* assays, dopamine antagonists block some of the pharmacological effects of racemic MDA [3, 32], supporting the involvement of a dopaminergic component in the actions of these drugs.

The potent inhibition of [^{3}H]norepinephrine uptake into hypothalamic synaptosomes by the enantiomers of MDA and MDMA suggests the possible involvement of central noradrenergic mediation of some of the psychotropic effects of these compounds. These data also raise the question of whether similar effects on noradrenergic mechanisms in the periphery contribute to the cardiovascular toxicities of these agents [32, 33].

The finding that neither stereoisomer of DOM had significant inhibitory effects on synaptosomal uptake of [^{3}H]catecholamines was not unexpected. Horn [34] had suggested the unlikelihood of hallucinogenic methoxylated amphetamine derivatives producing their characteristic effects by inhibiting the reuptake mechanism for catecholamines, although others have reported weak inhibitory effects of DOM on synaptosomal uptake of [^{3}H]monoamines [35] and in releasing [^{3}H]catecholamines [36]. However, those studies did not address possible stereochemical differences in these actions. Although *S*-(+)-DOM had a somewhat greater effect than the *R*-(−)enantiomer on inhibition of synaptosomal uptake of [^{3}H]dopamine and [^{3}H]norepinephrine (data not shown), neither isomer produced a 50% inhibition of uptake at concentrations up to 10 μM. Considering the approximately 10-fold greater potency of racemic DOM in humans in comparison to racemic MDA [37], it is apparent that the latter compound and its analogs have a distinctly different action than DOM on brain catecholamines.

Both isomers of MDA and MDMA were potent inhibitors of [^{3}H]serotonin uptake into hippocampal synaptosomes. The IC_{50} values for the *S* enantiomers were approximately three times lower than for the corresponding *R* enantiomers, although there was no significant degree of stereoselectivity. N-Methylation of MDA had no effect on potency as an inhibitor of [^{3}H]serotonin uptake. These two drugs exhibit stereochemical profiles in the release of [^{3}H]serotonin from rat hippocampal slices [29] that parallel those reported here for inhibition of synaptosomal uptake of [^{3}H]serotonin. The pronounced effects of the stereoisomers of MDMA and MDA on hippocampal serotonergic mechanisms, in comparison to those of amphetamine, suggest a prominent role for serotonergic mediation of the subjective effects of MDMA and MDA. Reports of selective serotonin neurotoxicity induced by racemic MDA [38] and MDMA [39] support the notion that central serotonergic neurons may be primary targets of the compounds and potential mediators of their central effects.

Our data do not explain why the *R*-(−) enantiomer of MDA, but not *R*-(−)-MDMA, substitutes for hallucinogens in drug discrimination paradigms [16, 19]. Since the *S*-(+) enantiomer of both drugs was more potent in our *in vitro* assays, and the *R*-(−) enantiomers of the two drugs were equipotent inhibitors of synaptosomal uptake of [^{3}H]serotonin, the neurotransmitter primarily implicated in hallucinogenesis [11, 12], potential differences in the hallucinogenic capacity of the two drugs may be due to some other neurochemical alteration.

The rationale for the synthesis of MBDB, based on known structure–activity relationships for phenylalkylamine hallucinogens [37], was that extension of the α-methyl group by one carbon in this series would abolish the hallucinogenic character. MBDB does not induce stimulus-generalization in LSD-trained rats but has subjective effects similar to MDMA in humans [19]. The most surprising effect of this chemical modification noted in this study was the lack of inhibitory effect of *S*-(+)-MBDB on [^{3}H]dopamine uptake into striatal synaptosomes. A similar reduction in the dopamine-releasing capability of *S*-(+)-MBDB in rat striatal slices has been observed [29]. Thus, this compound may have fewer amphetamine-like characteristics, including abuse potential, than MDA or MDMA. The IC_{50} determinations for *S*-(+)- and *R*-(−)-MBDB were similar to those of the corresponding enantiomers of MDA and MDMA for inhibition of [^{3}H]serotonin uptake in hippocampal synaptosomes, and slightly higher for inhibition of [^{3}H]norepinephrine uptake. The apparent lack of activity of MBDB on dopaminergic mechanisms, and retention of inhibitory effects on [^{3}H]norepinephrine and [^{3}H]serotonin uptake suggest that only the latter two systems are necessary components of the psychotropic effects of the methylenedioxy-substituted compounds. Compared to *S*-(+)-amphetamine, both stereoisomers of the three MDMA homologs were more potent inhibitors

of [^{3}H]serotonin uptake, supporting our contention for the involvement of this neurotransmitter system in mediating the subjective, including those potentially therapeutic, effects of the methylenedioxy-substituted phenylalkylamines.

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