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Studies of Dioxole Ring Substituted 3,4-Methylenedioxyamphetamine (MDA) Analogues

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NICHOLS, D. E., R. OBERLENDER, K. BURRIS, A. J. HOFFMAN AND M. P. JOHNSON. *Studies of dioxole ring substituted 3,4-methylenedioxyamphetamine (MDA) analogues*. PHARMACOL BIOCHEM BEHAV **34**(3) 571–576, 1989.—The 3,4-ethylidenedioxy and 3,4-isopropylidenedioxy analogues, EDA and IDA, respectively, of 3,4-methylenedioxyamphetamine (MDA) were compared to MDA in drug-stimulated [^{3}H]-serotonin overflow from prelabelled rat hippocampal slices, [^{3}H]-dopamine overflow from prelabelled rat caudate slices, in their ability to displace the 5-HT$_2$ agonist *R*-[^{125}I]-DOI from rat brain cortical binding sites. They were also compared in the two-lever drug discrimination assay in rats, utilizing *d*-LSD tartrate (0.08 mg/kg) or MDMA·HCl (1.75 mg/kg) as the training stimulus. MDA and EDA were nearly equipotent in inducing release of both [^{3}H]-monoamine transmitters, while IDA was considerably less potent. Pretreatment of hippocampal slices with the 5-HT-uptake inhibitor fluoxetine (3.2 μM) blocked the [^{3}H]-5-HT overflow induced by MDA. In the drug discrimination experiments, complete substitution occurred with all three drugs in both LSD- and MDMA-trained rats. The ED$_{50}$ values indicated that MDA had about twice the potency of EDA, and five times the potency of IDA in MDMA-trained rats. In the LSD-trained animals, MDA was about three times more potent than EDA and about seven times more potent than IDA. The K$_i$ values for displacement of *R*-[^{125}I]-DOI generally parallel the results of the LSD transfer tests.

LSD MDA MDMA Brain slices Serotonin Dopamine Drug discrimination Entactogens

THERE has been a good deal of recent interest in the pharmacology of the substituted amphetamine derivatives MDA and MDMA. This has largely developed as a concern over the recreational popularity of the latter. However, MDA has been a popular recreational drug for about twenty years, and earned a street reputation as the "love drug" (25). MDA, while classified as an hallucinogenic amphetamine, has been described as producing in users a desire to be with and talk to other people (9). Similarly, while MDMA seems to lack the hallucinogenic component that is present in MDA, it too produces unique psychoactive effects. In a recent survey of users of MDMA on one undergraduate campus, the effect most consistently described (90%) was a feeling of emotional closeness (22). The neuronal substrates of such a pharmacological response are of considerable interest, and both MDA (14,26) and MDMA (6) have been advocated as useful adjuncts for psychotherapy. Although a variety of substituted amphetamines, including MDA, have been categorized as hallucinogens, present pharmacological evidence regarding the mechanism of action of MDMA and certain related analogues supports their placement into a new pharmacological class, which we have called entactogens (15, 16, 20).

The evidence for this new classification is based on several structure-activity considerations, including stereochemical features, and N- and side chain-substitution (15,19). Furthermore, the pharmacological properties of MDMA differ from those of hallucinogenic amphetamines, in that the latter display a high affinity for serotonin 5-HT$_2$ receptor subtypes (3), while the current evidence suggests that the primary action of MDMA is related to effects on serotonin release and reuptake mechanisms (19,24).

In continuing efforts to explore the structure-activity relationships of psychoactive phenethylamine derivatives, analogs of 3,4-methylenedioxyamphetamine (MDA) were developed that had additional methyl groups attached to the dioxole ring (the methylene group of the methylenedioxy function). These compounds, ethylidenedioxyamphetamine (EDA) and isopropylidenedioxyamphetamine (IDA), have already been evaluated for their effects on spontaneous motor activity in mice (18). EDA had stimulant activity somewhat less than that of MDA, while IDA appeared inert until convulsant doses were reached. EDA and MDA both elicited a number of similar behaviors, while IDA failed to produce these effects. Thus, it was concluded that EDA had effects similar to MDA, but had a lower potency, while IDA

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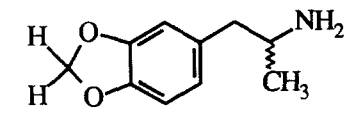
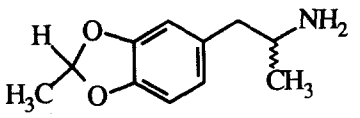
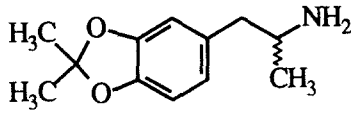


MDA



EDA



IDA

FIG. 1. Structures of dioxole-substituted MDA analogues.

lacked pharmacology similar to MDA.

The present study was prompted by interest in the mechanism of action for MDA (and MDMA), and the fact that these compounds had only been pharmacologically characterized for their effects on motor activity in mice. Further, we (16,21) and others (4,5) have shown that the complex discriminative stimulus properties of MDA include components that resemble those of the hallucinogens LSD and DOM, and of the novel psychoactive agent MDMA. The congeners EDA and IDA could thus be reexamined both for LSD-like and MDMA-like effects in the drug discrimination paradigm. In addition, in view of an earlier study in which we demonstrated that MDMA was a potent releaser of [^{3}H]-serotonin from prelabelled synaptosomes (19) or hippocampal slices (11), MDA, EDA, and IDA were compared for potency in that assay, for possible correlation with the in vivo drug discrimination data. Also, in view of the locomotor stimulant activity of MDA and EDA, it was decided to examine the ability of all three compounds to effect the release of [^{3}H]-dopamine from prelabelled rat caudate slices.

METHOD

Subjects

Male, Sprague-Dawley rats (n = 25 for LSD group; n = 20 for MDMA group), weighing approximately 200 g at the beginning of the study, were obtained from Murphy Breeding Labs, Inc., Plainfield, IN. For the first week, all rats were group housed, and for the remainder of the study the animals were housed individually in a temperature-controlled room (25°C) with an 0600–2000 lights on, 2000–0600 lights off schedule.

Immediately following scheduled discrimination sessions the animals were returned to their home cages. Food was provided to maintain each rat at about 80% of the free-feeding weight. On Sundays, no sessions were run and animals were allowed to feed at their regularly scheduled time. Water was available ad lib, except during the training and testing periods.

Apparatus

Six identical standard operant chambers (Coulbourn Instruments) equipped with two response levers separated by a food pellet delivery system were employed. Food pellets (Bioserve, 45 mg dustless) were used as reinforcement. Chambers contained a white house light and masking white noise and were enclosed in ventilated, sound-attenuated cubicles. The operant chambers were controlled by solid-state logic interfaced through a Coulbourn Instruments Dynaport to an IBM-PC located in an adjacent control room. Data acquisition and control were handled by the IBM-PC using locally developed software.

Drug Administration

The training dose of *d*-LSD tartrate (NIDA) (0.08 mg/kg; 185.5 nM/kg), (±)-MDMA·HCl (1.75 mg/kg) or appropriate test drug doses were administered in saline in a volume of 1.0 ml/kg of body weight. MDMA and the test drugs were synthesized in our laboratories by previously described methods (16,18). Dosages refer to the salt forms, and all test compounds except LSD were administered as hydrochloride salts. All injections were administered intraperitoneally 30 min prior to the start of discrimination sessions.

Lever-Pressing and Drug Discrimination Training

To avoid positional preference, half of the animals were trained to press TRAINING DRUG-L and SAL-R, while the other half were trained vice versa. Rats were trained on an FR50 schedule with 15-min maintenance sessions. No significant difference in responding rate was seen between either of the training drugs and saline ($p>0.05$, Student's *t*-test). The complete training procedure has been published in previous articles (17,21).

Tests of Stimulus Generalization

Testing sessions were run on Wednesdays and Saturdays only. Training sessions were held the rest of the week with Sundays off. On test days, the animal was placed into the operant chamber 30 min after injection. Test sessions lasted until the rat emitted 50 responses on either lever or until 5 min had passed, whichever came first. If the rat did not emit 50 responses on either lever within 5 min, he was scored as disrupted and was not included in the calculations. In either case, no reinforcement was given. In order to receive a test drug, the animals were required to satisfy the 85% correct lever response criterion on each of the two preceding training sessions. Also, following the procedure of Colpaert *et al.* (2), test data were discarded and the test condition later retested if the test session was followed by failure to meet the 85% criterion in either of the two subsequent training sessions. This procedure was employed to increase the reliability of the individual test data. It has been reported (2) that incorrect lever selections in trained rats typically occur in bursts of 1–3 sessions. This correction procedure assists in avoiding the contamination of test data that may occur during such bursts. If the animal was not included in the testing procedure on a given day, the session was used for training.

Several preliminary experiments to determine appropriate dosages for new compounds were carried out; those data were discarded. The drug treatments in this study were randomized within each group of rats tested (LSD-trained or MDMA-trained). At least eight animals were tested at each dose, except in cases where very high doses produced an excessive number of disruptions.

Data Analysis

Animals were scored as drug positive if they selected the LSD- or MDMA-appropriate lever, for LSD- or MDMA-trained rats,

respectively (i.e., if they emitted 50 responses on the drug lever). If generalization occurred (greater than 80% of the rats selecting the drug-appropriate lever at a given dose), these quantal data were analyzed by the method of Litchfield and Wilcoxon (12) to determine an ED_{50}.

Brain Slice Superfusion Procedure—Drugs and Buffer

All test drugs were dissolved in double-distilled water such that 50 μl of the stock solution added to 20 ml of buffer gave the desired drug concentration. Pargyline HCl (Sigma) was dissolved in 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; pargyline was not included in the superfusion buffer. ^{3}H-Serotonin (13.5 Ci/mmole, Amersham) was diluted in double-distilled water with 2% ethanol so that 50 μl in 4.0 ml buffer gave 0.1 μM ^{3}H-5-HT in the hippocampal slice incubation. Likewise, ^{3}H-dopamine (9.3 Ci/mmole, 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}H-DA in the caudate nucleus slice incubation. Throughout the experiment, slices were maintained in an artificial CSF buffer at 37.5°C, aerated with 95% O_2–5% CO_2. The buffer composition was, NaCl 118 mM, KCl 4.8 mM, $CaCl_2$ 1.3 mM, KH_2PO_4 1.2 mM, $MgSO_4$ 1.2 mM, $NaHCO_3$ 25 mM, glucose 10 mM ascorbic acid 0.06 mM, Na_2EDTA 0.03 mM, pH = 7.4.

Procedure and Apparatus

The procedure and apparatus used in these studies have been described previously (8,11). Male Sprague-Dawley rats (Lab Supplies, Indianapolis), weighing 180–220 grams, were sacrificed 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}H-neurotransmitter were added and the slices were incubated at 37.5°C for 45 min. The total time from sacrifice to incubation was consistently less than 4 min.

After the incubation period, the 4 slices with best appearance were transferred to the superfusion chambers. The chambers were then immersed in a constant temperature bath at 37.5°C and superfused at 0.5 ml/min with oxygenated buffer for a wash-out period of 15 min. Following the wash period, 15 one-minute 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. To each scintillation vial was added 3 ml of 2-ethoxyethanol and 7 ml of scintillation cocktail containing 5 g of 2,5-diphenyloxazole (PPO) per liter of toluene. All samples were stored in the dark at 4°C overnite before scintillation counting in a Packard Tri-Carb Model 4530 at approximately 27% counting efficiency. For analysis purposes the background dpm was subtracted from each fraction, and the net dpm for all fractions in a run, including the slice, was summed. Each fraction was simply expressed as a percent of total tissue tritium at the start of the fraction collection period.

Determination of [^{3}H]-Metabolite

The proportions of [^{3}H]-5HT and its deaminated metabolites released from rat hippocampal slices were determined using ion-exchange chromatography, following a modification of the procedure of Hammond *et al.* (7). In separate experiments (n = 8), slices were incubated and placed into the superfusion apparatus as previously described. Immediately after a 15-minute wash period with buffer solution, superfusate was collected at a rate of 0.5 ml/min for 5 minutes. Fifty μl of MDA stock solution (final MDA concentration = 10 μM) was then added to the superfusion chamber and superfusate was collected for 10 minutes. All superfusate samples were adjusted to pH 6.1 using dilute hydrochloric acid. A 1.75 ml aliquot of superfusate collected before addition of MDA was twice applied to a 9″ Pasteur pipet column containing 500 mg of Amberlite CG-50 (100–200 mesh) cation-exchange resin, followed by a wash with 3.25 ml of distilled H_2O. A 0.25 ml aliquot of the combined effluent, containing deaminated metabolites, was transferred to a scintillation vial. The [^{3}H]-5HT was then eluted from the column with 5.0 ml of 0.5 N acetic acid. A 0.25 ml aliquot of this eluate was transferred to a scintillation vial. Similarly, 3.75 ml of the superfusate collected after addition of the MDA was twice applied to a column, followed by a wash with 6.25 ml of distilled H_2O. A 0.25 ml aliquot of this effluent, containing neutral and acidic [^{3}H]-metabolites, was transferred to a scintillation vial. The [^{3}H]-5HT was then eluted from the column with 10.0 ml of 0.5 N acetic acid and a 0.25 ml aliquot of this was transferred to a scintillation vial. Scintillation fluid (10.0 ml) (Beckman Ready-Solv) was added to each vial. The vials were then counted for tritium. The individual activities of [^{3}H]-5HT and [^{3}H]-metabolites were expressed as a percent of the total released before and after MDA addition.

Data Analysis

Four separate studies were analyzed. The release of serotonin from rat hippocampus was examined with racemic mixtures of MDA, EDA and IDA at drug concentrations of 0.1, 1.0 and 10 μM. The effect of fluoxetine pretreatment (3.2 μM, 20 min) on release of [^{3}H]-serotonin by MDA and EDA at the 10 μM concentration was also measured. The third study examined the release of dopamine from rat caudate nucleus in response to racemic MDA, EDA and IDA at concentrations of 0.1, 1.0 and 10 μM. Finally, the percentage of the tritium efflux attributable to [^{3}H]-5-HT release from rat hippocampus in response to MDA (10 μM) was determined. Separate control runs were carried out for each study. Within each study, all treatments were randomized.

For each individual run, the percent of tritium released in fractions 4 through 6 was summed in order to determine the pretreatment rate (% released/3 min) for spontaneous tritium efflux. Fractions 10 through 12 were summed in a similar fashion to measure the effect of each treatment on the tritium efflux rate (% released/3 min). The ratio of posttreatment rate to pretreatment rate was determined in five separate experiments for each treatment, including distilled water control. Control ratios on the order of 0.8 to 0.9 reflect a steady decay in tritium efflux in the absence of a test drug. A grouped Student's *t*-test was used to determine a significant difference for the treatments from control.

R-[^{125}I]-DOI Radioligand Binding and Displacement

Experiments with *R*-[^{125}I]-DOI followed the procedures outlined earlier (10), with only minor modifications. Briefly, test

TABLE 1

SUBSTITUTION TESTING IN RATS TRAINED TO DISCRIMINATE LSD TARTRATE (0.08 mg/kg) FROM SALINE

Drug	Dose μM/kg	N*	D†	Result‡	ED_{50}§
Saline	1 ml/kg	8	0	0%	
LSD¶					0.025 μM/kg
					(0.0124–0.0498)
MDA¶					4.52 μM/kg
					(3.11–6.57)
EDA	2.32	8	0	0%	
	4.64	8	0	13%	
	9.28	9	1	50%	13.39 μM/kg
	18.56	9	1	50%	(7.13–25.12)
	37.12	17	9	88%	
IDA	4.64	8	0	0%	
	9.28	8	0	25%	29.25 μM/kg
	37.12	9	0	33%	(14.75–57.99)
	74.24	10	2	88%	

*N = Total number of rats tested.
†D = Number of disruptions (50 presses not completed in 5 min).
‡Percentage of responding (N-D) rats selecting the drug lever.
§95% CI in parentheses.
¶Previously published results (16).

TABLE 2

SUBSTITUTION TESTING IN RATS TRAINED TO DISCRIMINATE MDMA·HCl (1.75 mg/kg) FROM SALINE

Drug	Dose μM/kg	N*	D†	Result‡	ED_{50}§
Saline		8	0	0%	
MDMA	0.95	8	0	0%	
	1.9	8	0	25%	
	3.82	8	0	63%	3.40 μM/kg
	5.72	9	0	56%	(2.25–5.13)
	7.63	8	0	100%	
MDA	1.39	8	0	13%	
	2.78	9	1	25%	4.06 μM/kg¶
	5.57	9	1	50%	(2.59–6.38)
	8.35	10	2	100%	
EDA	2.18	8	0	13%	
	4.36	9	1	50%	8.09 μM/kg
	8.71	10	2	50%	(4.28–15.31)
	17.43	8	0	63%	
	26.14	8	0	75%	
	34.85	9	1	88%	
IDA	4.11	8	0	13%	
	12.32	8	0	13%	21.41 μM/kg
	36.96	8	0	63%	(12.51–36.66)
	49.28	9	1	88%	

*N = Total number of rats tested.
†D = Number of disruptions (50 presses not completed in 5 min).
‡Percentage of responding (n-D) rats selecting the drug lever.
§95% CI in parentheses.
¶Previously published value (21).

tubes containing rat prefrontal homogenate and appropriate ligands were allowed to come to equilibrium for 30 min at 24°C. Specific binding was defined as that displaceable with 100 nM unlabeled *R*-DOI, but similar results were obtained when specific binding was defined with 1 μM cinanserin. Under these conditions *R*-[^{125}I]-DOI was found to bind to a single site with a K_D of 1.84 ± 0.18 nM and a B_{max} of 111.6 ± 9.5 fmol/mg, and a Hill coefficient of 0.99 ± 0.02. The three test ligands were examined for ability to displace 0.25 nM *R*-[^{125}I]-DOI from its binding sites. Data were analyzed using LIGAND as implemented for the IBM PC by McPherson (13) and values reported represent the mean of three separate experiments.

RESULTS

Drug Discrimination

The results of the substitution testing are presented in Tables 1 and 2. Complete generalization of the LSD (0.08 mg/kg) stimulus to all three test compounds was observed. However, the ED_{50} values reflect a relatively large range of potency in substituting for this training stimulus, with potency ratios for MDA, EDA, and IDA of approximately 1:3:6.5. MDA was significantly more potent than both EDA and IDA, while there was no significant difference between EDA and IDA. Similarly, in the MDMA-trained group, the stimulus generalized to all three compounds. The relative potencies, based on the ED_{50} values for MDA, EDA, and IDA, were approximately 1:2:5.3. In this group, IDA was significantly less potent than either MDA or EDA, while MDA and EDA were not significantly different.

^{3}H-Serotonin Release From Hippocampal Slices

In studies of serotonin release from rat hippocampal slices, EDA and MDA at 10 μM showed similar efficacy (Fig. 2). IDA was about 10 times less potent than EDA and MDA, as shown by similar release potencies of IDA at 10 μM and MDA and EDA at 1 μM. MDA was the only treatment at 0.1 μM which tended to show any [^{3}H]-5-HT release above the basal rate. In a separate experiment, fluoxetine pretreatment (3.2 μM) for the 20 min prior to 10 μM drug significantly decreased the serotonin release induced either by MDA ($p<0.001$) or EDA ($p<0.02$), as compared to efflux ratios in the absence of fluoxetine. The postdrug/predrug tritium efflux ratios for these experiments were, for

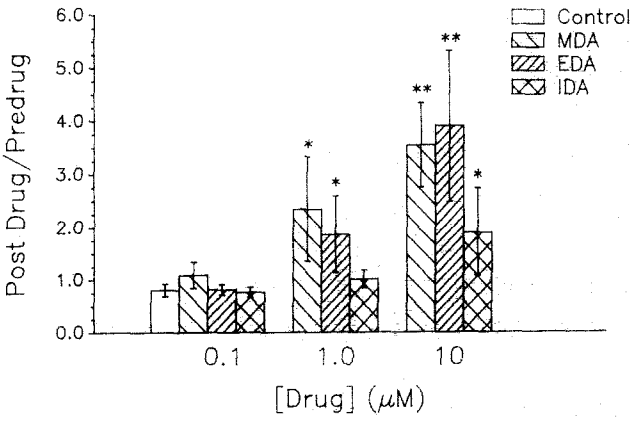


FIG. 2. Release of [^{3}H]-5-HT from superfused slices of rat hippocampus. Data are expressed as predrug/postdrug rate ratios (mean ± SEM), with differences tested between treatment and control rate ratios using Student's *t*-test. *$p<0.01$, **$p<0.001$.

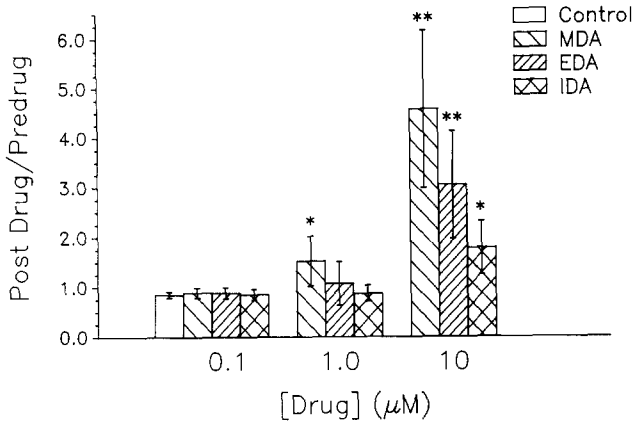


FIG. 3. Release of [^{3}H]-dopamine from superfused slices of rat caudate nucleus. Data are expressed as predrug/postdrug rate ratios (mean ± SEM), with differences tested between treatment and control rate ratios using Student's *t*-test. *$p<0.01$, **$p<0.001$.

control, MDA, and EDA, respectively: 0.88 ± 0.08, 1.34 ± 0.30, and 1.43 ± 0.53. Fluoxetine alone had no significant effect on [^{3}H]-5-HT efflux.

^{3}H-Dopamine Release From Rat Caudate Nucleus

In studies of dopamine release from rat caudate nucleus slices, MDA and EDA at 10 μM were similarly potent releasers, with MDA showing a trend toward slightly greater potency (Fig. 3). MDA was the only treatment at the 1 μM concentration to show a significant release, and was similar to IDA at 10 μM in potency. None of the 0.1 μM treatments were effective in eliciting ^{3}H-dopamine release.

Determination of Percent ^{3}H-5-HT in ^{3}H-Efflux

Analysis of tritium efflux from prelabelled rat hippocampal slices, using ion exchange chromatography in conjunction with liquid scintillation counting, provided an estimate of 35.6 ± 22.2% [^{3}H]-5-HT in the predrug release curve, and 79.0 ± 14.1% [^{3}H]-5-HT in the postdrug release curve in response to MDA at 10 μM. The total drug-induced [^{3}H]-efflux rate was increased approximately 2.8 times over predrug efflux, so these percentages indicate that the efflux rate of deaminated [^{3}H]-5-HT remains about the same both before and after drug, and that the increase in tritium is due predominantly to release of unmetabolized 5-HT.

R-*[^{125}I]-DOI Displacement*

The K_i values for displacement of 0.25 nM *R*-[^{125}I]-DOI from rat prefrontal cortical homogenate binding sites were (K_i in nanomolar ± SEM): 526 ± 33, 800 ± 98, and 3187 ± 421 for MDA, EDA and IDA, respectively. The corresponding Hill coefficients did not differ significantly from unity and were, respectively, 0.87 ± 0.04, 0.79 ± 0.05, and 0.95 ± 0.08.

DISCUSSION

Drug Discrimination

The complete substitution of MDA, EDA and IDA for LSD (0.08 mg/kg) suggests that all three share a mechanism of action which is in some way similar to that of LSD. Analysis of the discriminative stimulus properties of LSD in rats seems to indicate mediation by central serotonergic neuronal mechanisms and the lack of a significant role of the drug's dopaminergic properties (1,2). Most recently, it has been suggested that the LSD cue may be related to agonist activity at central serotonin receptors of the 5-HT_2 subtype (1). The present data would suggest that affinity and/or intrinsic activity for the receptor mediating this cue may be progressively decreased by methylation of MDA to give EDA and IDA.

The K_i values for displacement of the 5-HT_2 agonist ligand *R*-[^{125}I]-DOI are in qualitative agreement with the in vivo data. The difference in potency between MDA and IDA is very close to the difference in their affinity for the 5-HT_2 site. Although EDA has lower affinity for this receptor, it is not decreased quite to the extent that the ED_{50} in the LSD-trained animals would suggest.

Studies of the MDMA discriminative cue have provided evidence that it is mediated by a serotonin release and/or reuptake blockade mechanism (20). Again, methylation of the dioxole ring of MDA has a deleterious effect on this mechanism, with IDA being the least potent compound in the series. Although the potency of EDA is about half that of MDA, the relatively low potency of MDA in man (1–1.5 mg/kg) would indicate that EDA is not likely to possess significant clinical potency.

However, although EDA had only one-half the potency of MDA, it is important to point out that the position of methyl attachment in EDA is asymmetric, i.e., is chiral, and EDA is actually a mixture of four isomers. While MDA and IDA are simply racemic mixtures, by virtue of the chiral α-carbon in the side chain, the creation of a chiral center in the dioxole ring of EDA complicates analysis of biological activity. If the ligand-receptor binding process requires one face of the aromatic ring or the dioxole function to be sterically unencumbered, then only half of the EDA molecules can optimally undergo this interaction. Thus, one could speculate that if it were possible to resolve EDA into its four isomers (we were unsuccessful in many attempts to do this), that one or two of them might be as active as the enantiomers of MDA, depending on the bioassay used.

Brain Slice Experiments

MDA and EDA were nearly equipotent in release of [^{3}H]-5-HT and [^{3}H]-DA from brain slices and were more potent than IDA. Fluoxetine antagonized 5-HT release by MDA and EDA, indicating a possible competitive uptake and storage mechanism for serotonin release by these compounds. The [^{3}H]-5-HT release data at the highest drug concentrations might be interpreted as indicating that EDA should be more nearly comparable in potency to MDA in the MDMA-trained rats, if 5-HT release were the primary mechanism of the MDMA cue. However, the release data for the more biologically relevant 1 μM concentrations are more consistent with the observed in vivo data.

Although neither LSD nor MDMA produce primary discriminative cues that are mediated by dopamine release, both MDA and MDMA do appear to have secondary amphetamine-like dopaminergic effects (21). It seems possible that dopamine release may in some way also contribute to the in vivo behavioral effects of the MDA congeners. Again, the ordering of potencies based on the dopamine release data parallels that observed in the drug discrimination studies. Furthermore, the dopamine release data also parallel our earlier results, where the effect of these compounds was studied on locomotor activity in mice (18). That is, MDA was most potent, with EDA slightly less potent, and IDA lacking any ability to stimulate locomotor activity.

In summary, the progressive alkylation of the methylenedioxy function of MDA with methyl groups decreases potency with each

additional methyl group added. This decrease in potency is seen in all five assays, where the ability to induce release of [^{3}H]-5-HT or [^{3}H]-DA is decreased, affinity for the *R*-[^{125}I]-DOI-labelled 5-HT_2 receptor is decreased, as is potency in substitution tests in rats trained to discriminate saline from LSD or saline from MDMA. Even though a variety of macromolecules must be involved in all these responses, it is clear that none of the biological targets tolerate the addition of steric bulk to the dioxole ring in these analogues. It also seems unlikely from these results that congeners of MDA substituted on the methylenedioxy ring will possess significant human psychopharmacology.

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