Pharmacologic Profile of Amphetamine Derivatives at Various Brain Recognition Sites: Selective Effects on Serotonergic Systems

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INTRODUCTION

The amphetamines have found widespread use in a number of clinical conditions, including narcolepsy, manic-depressive psychosis, orthostatic hypotension, nasal congestion, migraine, asthma, hyperactivity, and obesity. Although amphetamine (phenylisopropylamine) is structurally a simple molecule, modification of the compound at the aromatic ring, side chain, or terminal amino group can considerably change the pharmacological specificity of the resulting compound. Amphetamine itself is a potent central nervous system (CNS) stimulant and anorectic agent, which acts primarily by blocking catecholamine uptake and causing neurotransmitter release. The addition of a hydroxy group on the beta carbon atom reduces both the stimulant and anorectic effects of the compound, while addition of a second alpha methyl group to amphetamine preferentially attenuates the CNS stimulant properties. Anorectics with reduced stimulant and cardiovascular effects can be created by insertion of groups onto the side chain, terminal amino group, or aromatic ring. Aromatic ring substitution by a number of substituents, including methoxy groups, have been shown to markedly alter the pharmacologic specificity of the drug, from a catecholaminergic agent to one exerting effects primarily on serotonergic systems (Loh and Tseng 1971). For example, paramethoxylation of amphetamine was found to increase greatly the blockade of serotonin uptake and increase the release of [^{3}H]5-HT, while uptake and release of dopamine were found to be attenuated (Loh and Tseng 1971). Since a substantial amount of data have implicated the involvement of brain serotonergic systems in the mechanism of action of hallucinogenic agents (Downing 1964; Brawley and Duffield 1972; Freedman and Halaris 1978; Glennon and Rosecrans 1981; Glennon 1983), it would not be unexpected for a number of ring-substituted psychotomimetic amphetamines to elicit their behavioral and/or subjective effects via their preferential and potent interaction with central serotonin recognition sites.

The psychotomimetic mono- and dimethoxyamphetamines have been reported to produce a number of subjective effects similar to those elicited by agents such as LSD and mescaline (Shulgin et al. 1969; Snyder et al. 1969). Indeed, some of the most potent hallucinogens have been ring-substituted structural analogs of amphetamine. Since the actions of psychotomimetic amphetamines may be mediated at presynaptic serotonin recognition sites, as well as at one or more of the postsynaptic serotonin receptor subtypes (Titeler et al. 1987). it is important to develop a relative pharmacologic profile for these drugs at the various serotonin binding sites.

This chapter will (1) elucidate the serotonergic sites of action of various ring-substituted psychoactive derivatives of amphetamine with an emphasis on derivatives of 2,5-dimethoxyamphetamine (2,5-DMA); (2) describe a detailed pharmacological profile of the newer types of psychoactive methylenedioxy-substituted amphetamine derivatives, the so-called "designer drugs" such as 3,4-methylenedioxymethamphetamine (MDMA) and 3,4-methylenedioxyamphetamine (MDA); and (3) elucidate the role of various serotonergic brain recognition sites in mediating some of the behavioral and/or subjective effects of these methylenedioxy derivatives of amphetamine.

INTERACTIONS OF 2,5-DMA DERIVATIVES WITH SEROTONIN RECEPTORS

As mentioned, aromatic ring substituents can greatly enhance the serotonergic activity (Cheng and Long 1973; Cheng et al. 1974; Dyer et al. 1973; Nair 1974) of the amphetamines. Substitution of methoxy groups in the 2,5 position and further substitution of substituents in the para position of the phenyl ring of amphetamines markedly enhances their affinity for serotonin receptors. The advent of the drug discrimination paradigm and its application to the study of such hallucinogenic agents (Hirschhorn and Winter 1971; Silverman and Ho 1980; Glennon et al. 1982; Glennon 1983; Appel et al. 1982) has greatly enhanced our understanding of the putative sites of action of hallucinogenic agents and of the similarities among various hallucinogenic compounds. These studies have demonstrated a significant correlation between the potencies of numerous agents in eliciting intero ceptive hallucinogenic cues in animals and humans (Glennon et al. 1982; Glennon 1983). Drs. Glennon, Titeler, and their collaborators have carried out a series of behavioral and radioligand binding studies to elucidate the serotonin receptor subtype(s) that may be primarily responsible for the actions of these psychoactive agents. Specifically, these studies involved a detailed determination of the affinities of various 2,5-dimethoxy derivatives of amphetamine at 5-HT_1 and 5-HT_2 serotonin receptors (table 1) using radioligand binding techniques to directly label these sites.

The structures of some of these agents are shown in figure 1. Compounds listed in figure 1A are either hallucinogenic in man or produce

TABLE 1. *Affinities of 2,5-DMA derivatives for 5-HT$_1$ and 5-HT$_2$ serotonin receptors*

Agent	5-HT$_2$ Binding[a] K_i(nM)	(Hill Coefficient)	5-HT$_1$ Binding[b] K_i(nM)	Hill Coeficient	K_i(5-HT$_1$)/ K_i(5-HT$_2$)
R(-)-DOI	9.9	(0.72)	2,290	(0.98)	230
(±)-DOI	18.9	(0.73)	2,240	(0.86)	120
S(+)-DOT	35	(0.66)	920	(0.73)	26
R(-)-DOM		(0.71)	3,550	(0.84)	60
(±)-DOB	63	(0.80)	38,340	(0.77)	50
(±)-DOM	100	(0.71)	2,890	(0.82)	30
α-demethyl DOM	110	(0.77)	350	(0.72)	3
R(-)-DON	210	(0.75)	13,300	(0.85)	60
(±)-DON	300	(0.79)	14,100	(1.0)	45
R(-)-N-methyl DOM	260	(0.91)	4,300	(0.86)	15
(±)-N-methyl DOM	415	(0.83)	3,870	(0.86)	10
(±)-DOF	1,110	(0.76)	3,470	(0.97)	3
(±)-2,4,5-TMA	1,650	(0.68)	46,800	(0.49)	30
(±)-4-OEt2,5-DMA	2,220	(0.77)	35,500	(0.83)	15
(±)-4-Me PIA	3,360	(0.89)	14,800	(0.96)	4
(±)-2,5-DMA	5,200	(0.85)	1,020	(0.75)	< 1
(±)-PMA	33,600	(0.87)	79,400	(0.97)	2
(±)-3,4-DMA	43,300	(0.66)	64,600	(0.94)	1

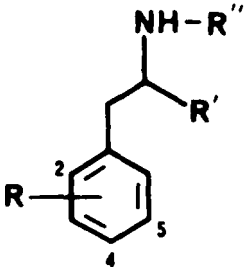
NOTE: [a]K_i values and Hill coefficients determined by competition experiments for 0.4 nM [^{3}H]ketanserin-labeled 5-HT$_2$ serotonin binding sites in rat frontal cortical homogenates. Data from Shannon et al. 1984.

[b]K_i values and Hill coefficents determined by competition experiments for 1.0 nM [^{3}H]LSD(+[10-^{3}M]Ketanserin)-labeled 5-HT^T sites in rat frontal cortical homogenates. Data from Shannon et al. 1984.

[c]Data from Glennon et al. 1986

hallucinogen-like responding in behavioral studies, while agents listed in figure 1*B* do not generalize to a hallucinogen cue. As shown in table 1, although 2,5-DMA itself exhibits higher affinity for 5-HT$_1$ versus 5-HT$_2$ serotonin receptors, all of the derivatives of 2,5-DMA exhibit substantially higher affinity for 5-HT$_2$ serotonin binding sites and appear to interact more selectively with this site than do tryptamine agonists such as serotonin. The selectivity of 2,5-DMA derivatives for 5-HT$_2$ serotonin receptors is particularly marked for compounds with substituents in the para position. For example, some of the para-halogenated compounds such as the iodinated (DOI) and brominated (DOB) derivatives demonstrate an extremely high affinity and degree of selectivity in their interactions with 5-HT$_2$ serotonin receptors.

Nearly all the derivatives of 2,5-DMA exhibited radioligand binding characteristics at 5-HT$_2$ serotonin receptors that were consistent with those of serotonin and other tryptamine agonists. It has been demonstrated



	R'	R"	R_2	R_4	R_5
(A) 2,5-DMA derivatives					
(±)-2,5-DMA	CH_3	H	OCH_3	H	OCH_3
(±)-2,4,5-TMA	CH_3	H	OCH_3	OCH_3	OCH_3
(±)-4-OEt 2,5-DMA	CH_3	H	OCH_3	OC_2H_5	OCH_3
(±)-DOF	CH_3	H	OCH_3	F	OCH_3
(±)-DOB	CH_3	H	OCH_3	Br	OCH_3
(±)-DOI	CH_3	H	OCH_3	I	OCH_3
R(−)-DOI	CH_3	H	OCH_3	I	OCH_3
(±)-DON	CH_3	H	OCH_3	NO_2	OCH_3
R(−)-DON	CH_3	H	OCH_3	NO_2	OCH_3
(±)-DOM	CH_3	H	OCH_3	CH_3	OCH_3
R(−)-DOM	CH_3	H	OCH_3	CH_3	OCH_3
α-Demethyl DOM	H	H	OCH_3	CH_3	OCH_3
(±)-N-Methyl DOM	CH_3	CH_3	OCH_3	CH_3	OCH_3
R(−)-N-Methyl DOM	CH_3	CH_3	OCH_3	CH_3	OCH_3
(B) Non-2,5-DMA derivatives					
(±)-PMA	CH_3	H	H	OCH_3	H
(±)-3,4-DMA	CH_3	H	H	OCH_3	OCH_3
(±)-4-Me PIA	CH_3	H	H	CH_3	H

FIGURE 1. *Structures of a series of 2,5 DMA and non-2,5 DMA derivatives*

SURCE: Shannon el at. 1984.

previously that classical serotonergic agonists of the hyptamine class interact with high- and low-affinity states of the 5-HT_2 serotonin receptor (Battaglia et al. 1984). Agonist-like properties of serotonin-related compounds were

initially revealed by Hill coefficient (n_H) values of less than one in radioligand binding studies. Values of less than one for n_H suggest interaction of the compound with multiple binding sites or multiple states of the receptor. As shown in table 1, nearly all the derivatives of 2,5-DMA exhibited n_H values of less than one at 5-HT_2, serotonin receptors, suggesting an agonist-like activity at this receptor. Data from competition experiments can be further quantitated using a computer-assisted two-site analysis program (Munson and Rodbard 1980). Computer-assisted two-site analysis for the interactions of a number of the 2,5-DMA derivatives with 5-HT_2 serotonin receptors indicates that these compounds do indeed interact with high- and low-affinity states of 5-HT_2 serotonin receptors, with the percentage of binding sites in the high-affinity state for 2,5-DMA derivatives being comparable to that observed for tryptamine agonists (table 2) (Battaglia et al. 1984). The agonist high-affinity state of 5-HT_2 serotonin receptors has also been shown to be sensitive to divalent cations and guanine nucleotides (Battaglia et al. 1984; Titeler et al. 1985), as previously demonstrated for agonists interacting with receptors coupled to a guanine nucleotide regulatory protein. Consistent with other agonist characteristics and, as shown in figure 2, DOI, the 4-iodo-DMA derivative, exhibited guanine nucleotide sensitivity. This is revealed by the decrease in overall affinity (K_I value) and increase in n_H closer to one in the presence of 5'-gyanylimidodiphosphate (Gpp(NH)p) (figure 2). Furthermore, derivatives such as DOI, DOB, and DOM exhibited substantially higher overall affinities (K_I) and higher affinities at the high-affinity component (K_H of 5-HT_2 serotonin receptors than did a number of tryptamine agonists at this site (table 2) (Battaglia et al. 1984; Shannon et al. 1984). With respect to stereospecificity, the *R*(-) isomers of DOI and other 2,5-DMA derivatives were the more potent isomers at 5-HT_2 serotonin receptors, while the *S*(+) isomers of methoxyamphetamines were more potent at presynaptic serotonin recognition sites. In the last few years, [^{125}I]-DOI (Glennon et al. 1988) and [^{3}H]-DOB (Titeler et al. 1985; Lyon et al. 1987) have proven to be highly selective agonist radiolabels for the high-affinity component of 5-HT_2 serotonin receptors.

Subsequent studies investigating the affinities of these and additional hallucinogenic phenylisopropylamines at 5-HT_2 serotonin receptors have clearly established a prominent role for 5-HT_2 serotonin receptors in the hallucinogenic process. Significant correlations were demonstrated between the *in vitro* affinities of a series of amphetamine derivatives at 5-HT_2 serotonin receptors and both their human hallucinogenic dose and their ED_{50} values in behavioral generalization to a hallucinogen cue (Glennon et al. 1984; Titeler et al. 1988).

Although initial studies indicated that the various derivatives of 2,5-DMA exhibited low affinity for 5-HT_1 serotonin receptors (Shannon et al. 1984). it was unclear from these studies what the affinities of the drugs were for the respective subtypes of 5-HT_1serotonin sites (i.e., 5-HT_{1A}, 5-HT_{IB}, and 5-HT_{1C} receptors). In subsequent studies (Titeler et al. 1988), the affinities

TABLE 2. *Two-site analysis of the interaction of tryptamine agonists and 2,5-DMA derivatives with 5-HT$_2$ serotonin receptors*

	K_H	K_L	$\%R_H$	K_I/K_H
2,5 DMA Derivatives[a]				
R(-)-DOI	1.5 ± 0.5	30.0 ± 5.1	40 ± 5	20
(±)-DOI	2.3 ± 1.0	47.4 ± 16.8	34 ± 9	21
R(-)-DOM	2.7 ± 1.6	190 ± 30	22 ± 5	71
		245 ± 90	50 ± 9	17
(±)-DOB	2.4 ± 0.7	100 ± 25	19 ± 1	42
a-demethyl DOM	35 ± 11	400 ± 110	52 ± 2	12
R(-)-DON	68 ± 29	900 ± 400	55 ± 18	13
(±)-DON	137 ± 49	1,500 ± 730	65 ± 19	11
(±)-2,4,5-TMA	200 ± 60	6,250 ± 1,200	41 ± 6	31
(±)3,4-DMA	3,100 ± 950	80,600 ± 19,000	25 ± 5	26
Tryptamine Derivatives[b]				
Serotonin	30 ± 3	1,173 ± 66	25 ± 4	39
5-Methoxytryptamine	130 ± 26	2,659 ± 550	45 ± 7	20
Bufotenine	96 ± 17	1,043 ± 220	35 ± 8	11
Tryptamine	302 ± 48	4,193 ± 570	15 ± 4	14

NOTE: Data were computer-analyzed with a two-site model (Munson and Rodbard 1980). K_H represents the dissociation constant of agonists clculated for the high-affinity component of [$_3$H]ketanserin binding. K_L is the dissociation constant calculated for the low-affinity component of [$_3$H]ketanserin competition curves. K_I/K_H is the ratio of the two dissociation constants. $\%R_H$ represents the percentage of sites in a high-form for the agonist.

SOURCE: Shannon et al. 1984; Battaglia et al. 1984.

of a comparable series of psychoactive amphetamine derivatives were compared at 5-HT$_{1A}$, 5-HT$_{1B}$, and 5-HT$_{1C}$ serotonin receptors. As shown in table 3, all derivatives exhibited relatively low affinities at 5-HT$_{1A}$ and 5-HT$_{1B}$ serotonin receptors but markedly higher affinities at the 5-HT$_{1C}$ serotonin receptor. Although these data suggest that 5-HT$_{1C}$ sites may contribute to some of the effects of these psychoactive amphetamines, the precise role of the 5-HT$_{1C}$ serotonin receptors in the hallucinogenic process or other effects of these drugs remains unclear at the present time.

PHARMACOLOGIC PROFILE OF MDMA AT VARIOUS BRAIN RECOGNITION SITES

Psychotomimetic amphetamines such as mescaline and DOM (STP) have experienced periods of popularity during the last two decades. In recent

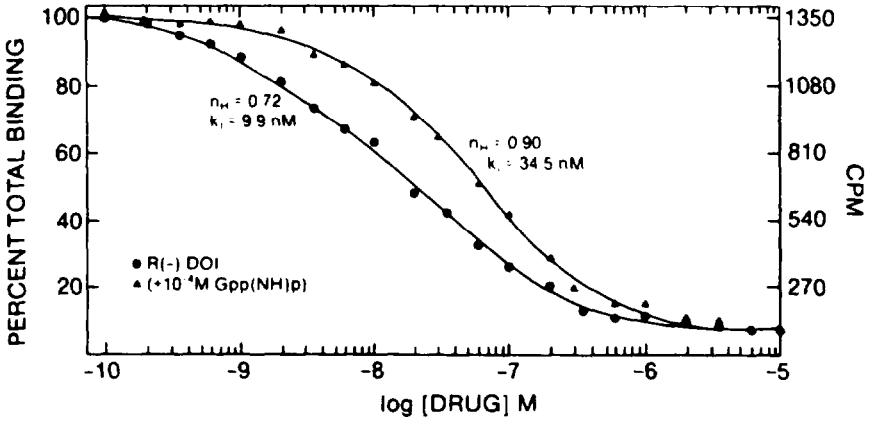


FIGURE 2. *Competition curves of R(-)-DOI for [^{3}H]ketanserin binding to 5-HT$_2$ serotonin receptors in rat frontal cortex membranes in the presence and absence of the guanine nucleotide analog Gpp(NH)p*

NOTE: Lines represent the best fit of the data acording to a model for tow biding sites [in the absence of Gpp(NH)p] and a model for one binding site [in the presence of Gpp(NH)p].

SOURCE: Shannon et al. 1984

years, a new class of designer drug, the methylenedioxyamphetamine derivatives, has received a great deal of attention. These compounds, which include MDMA, MDA, and MDA's N-ethyl derivative MDE, have been reported to elicit both moderate "amphetamine-like" stimulant and weak "LSD-like" hallucinogenic effects.

To elucidate the brain recognition sites through which MDMA might elicit its various behavioral, psychotomimetic, and neurotoxic effects. an extensive *in vitro* pharmacologic screening of MDMA was carried out at various brain neurotransmitter receptors and recognition sites. The relative potencies of MDMA at the various brain recognition sites were assessed from competition data in which affinities (K_i values) were determined using the nonlinear curve-fitting program LIGAND (Munson and Rodbard 1980). Details of the assay conditions and affinities of MDMA at the various recognition sites are reported in table 4. The pharmacologic profile of MDMA demonstrates a broad range of affinities of the drug for various brain recognition sites (Battaglia et al. 1988a). MDMA had the highest affinity for serotonin uptake sites (<1 μM) with lower but comparable affinities at 5-HT$_2$ serotonin, α_2-adrenergic, M-1 muscarinic cholinergic and H-1 histamine receptors (K_i values $\leq$ 5μM). The rank order of affinities of MDMA at

TABLE 3. *Affinities of 2,5-DMA derivatives at 5-HT$_1$ serotonin receptor subtypes*

Agent	5-HT$_{1A}$	5-HT$_{1B}$	5-HT$_{1C}$
R(-)-DOB	2,332 ± 188	683 ± 46	47 ± 10
DOI	2,355 ± 77	1,261 ± 105	30 ± 4
DOB	3,770 ± 188	831 ± 37	69 ± 16
DOPR	2,849 ± 170	2,330 ± 101	14 ± 1
R(-)-DOM	4,004 ± 107	1,840 ± 172	94 ± 17
DOET	3,930 ± 115	2,451 ± 226	101 ± 20
DOM	5,122 ± 140	2,063 ± 112	193 ± 20
S(+)-DOB	4,041 ± 156	883 ± 49	81 ± 7
DOBU	4,178 ± 165	1,211 ± 86	26 ± 5
2,4,5-TMA	>10,000	>10,000	2,666 ± 76
MEM	>10,000	>10,000	2,278 ± 90
2,5-DMA	1,131 ± 55	8,435 ± 668	1,217 ± 89
2,4-DMA	>10,000	>10,000	3,152 ± 83
3,4,5-TMA	>10,000	>10,000	5,710 ± 150

NOTE: The 5-HT$_{1A}$, 5-HT$_{1B}$, and 5-HT$_{1C}$ receptors were labeled with ^{3}H-OH-DPAT, ^{3}H-5-HT, and ^{3}H-mesulergine respectively.

SOURCE: Titeler et al. 1988.

various brain receptors and uptake sites were as follows: serotonin uptake > α_2-adrenergic = 5-HT$_2$ serotonin = M-1 muscarinic = H-1 histamine > norepinephrine uptake = M-2 muscarinic = α_1-adrenergic = β-adrenergic ≥ dopamine uptake = 5-HT$_1$ serotonin >> D-2 dopamine > D-1 dopamine. MDMA exhibited negligible affinities (>500 µM) at mu, delta, and kappa opioid, central-type benzodiazepine, and corticotropin-releasing factor receptors, as well as at choline uptake sites and at calcium channels. Although not shown here, the affinities of MDA were comparable (< two-fold difference) to those of MDMA at each of the respective brain recognition sites investigated. In general, the affinities of MDMA at the receptor sites investigated could be classified as high-, moderate-, and low-affinity interactions. These are summarized in table 5. MDMA appears to be most potent at a number of serotonin recognition sites as well as α_2-adrenergic and M-1 muscarinic receptors with affinity constants (K_i values) in the high nanomolar to low micromolar range. Affinities of MDMA in the micromolar range at the various recognition sites appear to be pharmacologically relevant, since similar brain concentrations of the drug have been detected in rats following systemic administration of a single dose of MDMA (20 mg/kg), which elicits behavioral as well as neurotoxic effects (Zaczek et al., unpublished observation).

TABLE 4. *Pharmacologic profile of MDMA at various brain recognition sites*

Brain Recognition Site	Affinity K_i(μM)	Radioligand/Displacer	Brain Region	Assay Time, Tempeature	Buffer
Uptake Sites					
Serotonin	0.61 ± .05	0.55nM[^{3}H]paroxetinel 1μM citalopram	1	120 min, Rm T	A
Norepinephrine	15.8 ± 1.7	4.OnM[^{3}H]mazindol/ 0.3μM desipramine	1	90 min, 4 °C	A
Dopamine	24.4 ± 1.9	1.0nM[^{3}H]GBR 12935/ 1μM mazindol	2	60 min, Rm T	A
Choline	>500	10nM[^{3}H]hemicholinium-3/ 10μM hemicholinium-3	2	30 min, 25 °C	B
Adrenergic Receptors					
α_1	18.4 ± 1.2	0.5nM[^{3}H]prazosin/ 10μM phentolamine	1	30 min, 37 °C	C
α_2	3.6 ± 0.8	0.5nM[^{3}H]para-aminocloni- dine/10μM phentolamine	1	30 min, 37 °C	
β	191 ± 21	0.5nM[^{3}H]dihydroalprenalol/ 1μM propranalol	1	30 min, 37 °C	C
Dopamine Receptior					
D-1	148 ± 14	0.2nM[^{3}H]SCH 23390/ 0.1μM flupenthixol	2	30 min, 37 °C	C
D-2	95 ± 15	0.2nM[^{3}H]spiperone/ 1μM (+)butaclamol	2	30 min, 37 °C	C
Serotonin Receptors					
5-HT_1	23 ± 1.5	2.5nM[^{3}H]serotonin/ 10μM serotonin	1	30 min, 37 °C	C
5-HT_2	5.1 ± 0.3	0.4nM[^{3}H]ketanserin/ 0.5μM cinanserin	1	30 min, 37 °C	C
Cholinergic Receptors					
M-1 muscarinic	5.8 ± 0.3	0.1nM[^{3}H](-)QNB/ 1μM atropone	1	90 min, Rm T	D
M-2 muscarinic	15.1 ± 0.1	0.1nM[^{3}H](-)QNB/ 1μM atropine	3	90 min, Rm T	D
Opioid Receptors					
Mu	>500	2nM[^{3}H]dihydromorphine/ 1μM levallorphan	4	45 min, 25 °C	E
Delta	>500	4nM[^{3}H]D-ala^2-D-leu^5- enkephalin (30nM morphine)/ 1μM levallorphan	4	45 min, 25 °C	E
Kappa	>500	1.6nM[^{3}H]ethylketazocine (30nM morphine + 100nM D-ala^2-D-leu^5-enkephalin)/ 1μM levallorphan	4	45 min, 25 °C	E

TABLE 4. (Continued)

Bran Recongnition Site	Affinity K_i(μM)	Radioligand/Displacer	Brain Region	Assay Time, Temperature	Buffer
Other Sites					
H-1 histamine Receptors	5.7 ± 2.4	2nM[^{3}H]mepyramine/ 1μM doxepin	1	60 min, Rm T	F
Benzodiazepin receptors	>500	0.2nM[^{3}H]flunitrazepam/ 1μM clonazepam	1	60 min, Rm T	G
Corticotropin-releasing factors (CFR) receptors	>500	0.1nM ^{125}I-Tyr^0-rat CRF/ 1μM ovine CCRF	5	120 min, Rm T	H
Calcium channels	>500	0.2nM[^{3}H]nitredipine/ 0.1μM nifedipine	1	60 min, Rm T	G

KEY: Assay buffers: A = 50 mM TRIS-HCl, 120 mM NaCl, 5 mM KCl (pH 7.4 at Rm T); B = 50 mM glycylglycine, 200 nM NaCl (pH 7.8 at 25 °); C = 50 mM TRIS-HCl, 10 mM MSO_4, 0.5 mM K_3HDTA (pH 7.4 aat 37 °C); D = 50 nM TRIS-HCl, 10 mM $MGSO_4$ (pH 7.7 at Rm T) E = 0.17 M TRIS HCl (pH 7.6 at 25 °C); G = 50 mM TRIS-HCl (pH 7.7 at Rm T); F = 50 nM Na^+K^+ phospate (pH 7.4 at Rm T); H = 50 mM TRIS-CHl, 10 mM $MgCl_2$, 2 mM EGTA 0.1% bovine serum albumin, 0.1 mM bacitracin sprotinin (100 KIU/mL) (pH 7.2 at 22 °C). Brain regions: 1 = frontal contex; 2 = striatum; 3 = brain stem; 4 = whole brain; and 5 = olfactory bulb.

NOTE: Data represent the mean ± SEM from three to five competition curves at each of the sites. K_i values were determined using the nonlinear least-sqares curve-fitting program LIGAND.

SOURCE: Battaglis et al. 1988.

TABLE 5. *Relative potencies of MDMA at various brain recognition sites*

High Affinity (0.6 to 6μM)	Moderate Affinity (10 to 100 μM)	Low Affinity (< 100 μM)
Serotonin uptake sites	Norepinephrine uptake sites	D-1 dopamine receptors
5-HT_2 serotonin receptors sites	Dopamine uptake sites	Choline uptake
α_2-adrenergic receptors	5-HT_1 serotonin receptors	Mu, delta, and kappa opioid receptors
M-1 muscarinic receptors	β-adrenergic receptors	Benzodiaepine receptors
		D-2 dopamine receptors

As shown in table 6, we have compared the affinities of a series of methylenedioxy derivatives with those of the parent compounds (amphetamine and methamphetamine) at some of the recognition sites in brain at which MDMA exhibited the highest affinities. These comparative studies indicate that addition of the methylenedioxy substituent in the 3,4 position increases their affinity at serotonin uptake, 5-HT_2 serotonin, and M-1 muscarinic receptors, while the unsubstituted parent compounds appear to be more potent at α_2-adrenergic receptors.

TABLE 6. *Relative potencies of amphetamine derivatives at selected brain recognition sites*

Compound	5-HT Uptake	5-HT_2 Serotonin	α_2 Adrenergic	M-1 Muscarinic
MDMA	1.0	1.0	1.0	1.0
MDA	1.8	0.5	0.5	1.4
MDE	0.4	3.5	3.3	1.8
Amphetamine	4.8	2.6	0.09	4.8
Meth-amphetamine	3.4	2.4	0.61	3.6

NOTE: Comparison of the affinities (K1 values) of amphetamine derivates at serotonin(5-HT) uptake sites, 5-HT_2serotonin, α_2 adrenergic, and M-1 muscarinic receptors with respect to the affinity of MDMA at these sites. Values smaller or larger than 1.0 indicate affinities higher or lower, respectively, than those of MDMA.

SOURCE: Battaglia et al. 1988a.

Interestingly, the anxiolytic-like effects of MDMA do not appear to be mediated through agonist actions at benzodiazepine receptors or antagonist effects at corticotropin-releasing factor receptors as evidenced by the low affinity of MDMA (>500 μM) at each of these receptors. In addition, the reinforcing, analgesic, and mood-altering properties of the drug do not appear to be mediated through interactions with any of the opioid receptor subtypes, since MDMA has relatively low affinities for these receptors.

INTERACTIONS OF MDMA WITH SEROTONIN RECOGNITION SITES

The previous data suggest that a number of the behavioral, psychotomimetic, and neurochemical effects of MDMA and other methylenedioxy derivatives of amphetamine may be explained by interactions of MDMA at multiple serotonin recognition sites in brain. MDMA may alter serotonergic transmission in brain through direct actions at postsynaptic as well as presynaptic serotonin recognition sites. As mentioned above, a number of hallucinogenic phenylisopropylamine derivatives exhibit potent agonist-like activity at brain 5-HT_2 serotonin receptors (Shannon et al. 1984) and the *in vitro* affinities of these hallucinogens at 5-HT_2 serotonin receptors significantly correlate with both their behavioral potencies in animals in generalization to other hallucinogens and with their human hallucinogenic potencies (Glennon et al. 1984; Titeler et al. 1988). Similar to previous observations for other ring-substituted amphetamines such as the derivatives at 2,5-DMA, we found that MDMA and other methylenedioxy derivatives of amphetamine also exhibited high-affinity agonist-like binding characteristics at 5-HT_2 serotonin receptors. The stereospecificity observed for methylene dioxy derivatives at 5-HT_2 receptors was consistent with that observed for other hallucinogenic compounds at this receptor (Lyon et al. 1986; Battaglia

et al. 1986). In addition, it was reported that MDMA interactions with the high-affinity state of 5-HT_2 serotonin receptors were sensitive to the effects of guanine nucleotides, similar to that observed for serotonin and other classical tryptamine agonists at this site (Battaglia et al. 1984) (figure 3). While the overall apparent affinity (K_1 value) of MDMA for [^{3}H]ketanserin-labeled 5-HT_2 serotonin receptors is in the low micromolar range, the authors have observed that the interactions of MDMA and other methylene-dioxyamphetamines with the high-affinity state of 5-HT_2 serotonin receptors labeled directly by [$_3$H]DGB (Lyon et al. 1987) are much more potent (K_1<300 nM). Since this high-affinity component of 5-HT_2 serotonin receptors represents the most potent site of action for MDMA in brain, it is likely that some of the "mood-altering" effects of MDMA may be mediated by direct agonist actions at 5-HT_2 serotonin receptors. A recent study demonstrating that the serotonin receptor antagonist methysergide can potentiate the MDMA-induced increases in locomotor activity (Gold and Koob 1988) further supports the claim for direct actions of MDMA at postsynaptic 5-HT_2 serotonin receptors. A comparison of the relative affinities of MDMA and MDA at postsynaptic 5-HT_2 serotonin receptors with those of other ring-substituted amphetamine hallucinogens suggests that MDMA and MDA would be much weaker hallucinogens at this site than would compounds such as DOM (STP) or DOI. This is not surprising, as the methylenedioxy class of designer drugs has been reported to have unique and subtle mood-enhancing subjective effects, rather than having the more vivid and disorienting sensations commonly attributed to very potent hallucinogens such as DOM or LSD.

In addition to the actions of MDMA and other derivatives at 5-HT_2 serotonin receptors. some of the effects on serotonergic systems could be mediated via 5-HT_{1A} receptors, at which MDMA has a moderate affinity. Direct agonist effects at this site might contribute to the mood-altering and calming effects of the drug, since similar effects have been reported for novel anxiolytics such as ipsaperone and buspirone, which interact with 5-HT_{1A} serotonin receptors.

In addition to its relatively high affinity at postsynaptic 5-HT receptors, MDMA exhibited high affinity for 5-HT uptake sites and has been shown to increase the release of [^{3}H]5-HT and block [^{3}H]5-HT uptake *in vitro*. These data suggest that some of the actions of MDMA may be mediated at presynaptic binding sites. With respect to [^{3}H]5-HT release, MDMA has been reported to increase the release of [^{3}H]5-HT from brain synaptosomes (Nichols et al. 1982) and hippocampal slices (Johnson et al. 1986). With respect to uptake blockade, MDMA has been reported to competitively inhibit ^{3}H-5-HT uptake *in vitro* (Shulgin 1986). Furthermore, the neurotoxic effects of *in vivo* administration of MDMA on serotonin terminals can be blocked by concomitant administration of the 5-HT uptake blocker citalo-pram (Battaglia et al. 1988b; Schmidt and Taylor 1987). Additional evidence in support of the hypothesis that MDMA produces some of its

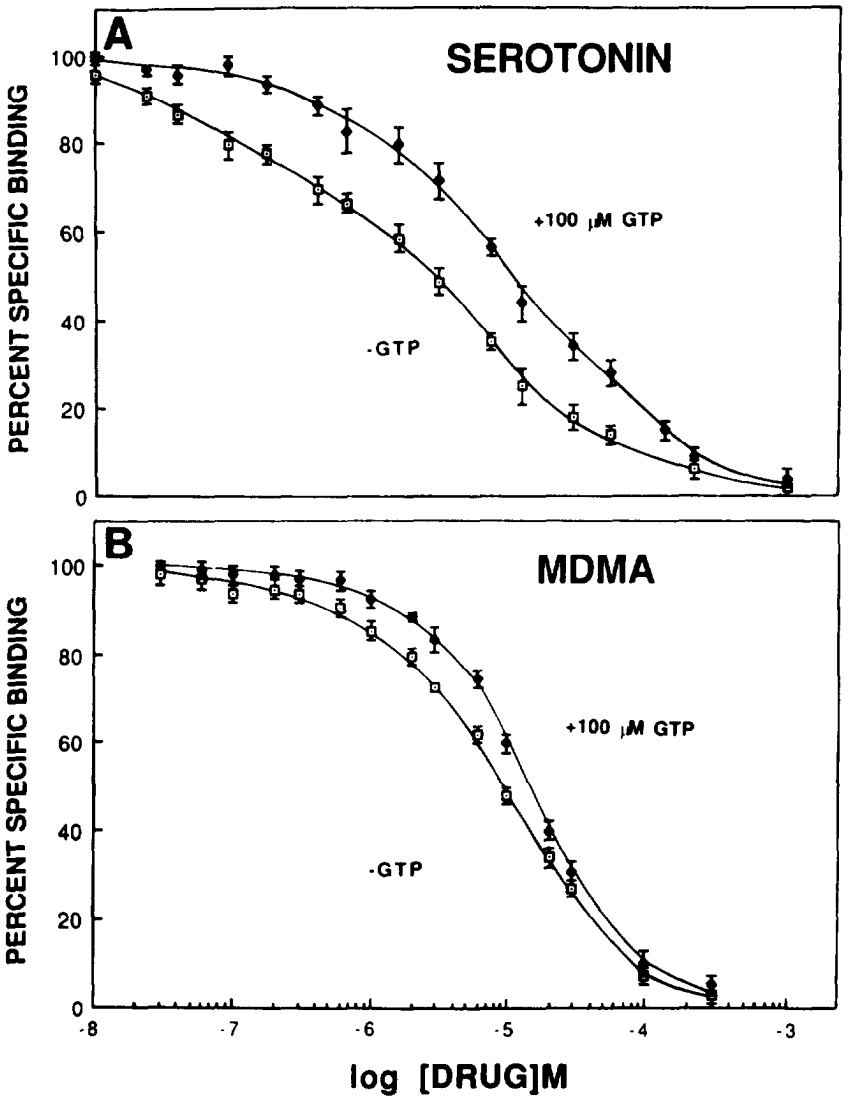


FIGURE 3. *Competition curves of (A) serotonin and (B) MDMA for [^{3}H] ketanserin binding to 5-HT^2 serotonin receptors in rat frontal cortex membranes in the presence and absence of the guanine nucleotide quanosine-5-triphosphate (GTP)*

effects through presynaptic serotonergic mechanisms is provided by data demonstrating that MDMA can generalize to a fenfluramine cue in stimulus discrimination studies (Schechter 1986).

Classic a-adrenergic receptor antagonists such as phentolamine have been reported to increase the release of [^{3}H]5-HT via effects on α_2-adrenergic receptors (Timmermans and Van Zwieten 1982). Thus, one might speculate that the serotonin-releasing effects of MDMA may be mediated, in part, by high-affinity antagonist-like effects at α_2-adrenergic receptors localized to presynaptic serotonin terminals. The relatively high affinity of MDMA at the serotonin uptake site and α_2-adrenergic receptor may contribute, in part, to the neurochemical, neurotoxic, and behavioral effects mediated at presynaptic serotonin terminals.

While brain serotonin systems may play a key role in mediating some of the effects of MDMA on analgesia and body temperature as well as in the reported anxiolytic-like and mood-altering subjective effects of the drug, additional neurotransmitter systems may contribute to some of the unique subjective experiences reported for MDMA and other drugs in this class.

SUMMARY AND CONCLUSIONS

Ring-substituted psychoactive derivatives of amphetamine exhibited high affinities for a number of serotonin recognition sites. Derivatives of 2,5-DMA exhibited preferential high affinity at 5-HT$_2$ serotonin receptors when compared to their relative affmities at 5-HT$_1$ serotonin receptors. Furthermore, 2,5-DMA derivatives exhibited agonist-like binding characteristics at 5-HT$_2$ serotonin receptors with the *R*(-) isomer being the more potent isomer. There were significant correlations between the *in vitro* affinities of 2,5-DMA derivatives at 5-HT$_2$ serotonin receptors and their human hallucinogenic potencies as well as with their potencies in behavioral generalization studies, suggesting the importance of 5-HT$_2$ serotonin receptors in mediating the hallucinogenic effects of the various 2,5-DMA derivatives.

A pharmacological profile of the methylenedioxy-substituted amphetamine derivatives indicates that MDMA and MDA exhibited highest affinity for serotonin uptake sites, 5-HT$_2$ serotonin, α_2-adrenergic and M-1 muscarinic receptors. The methylenedioxy amphetamine derivatives exhibited an inverse stereospecificity with respect to serotonin uptake sites versus postsynaptic 5-HT receptors with the *S*(+) isomer being more potent at the presynaptic recognition site, while the *R*(-) isomer was more potent at the postsynaptic recognition sites. Similar to the 2,5-DMA derivatives, MDMA and MDA exhibited agonist-like binding characteristics at 5-HT$_2$ serotonin receptors. Unlike 2,5-DMA derivatives, MDMA and MDA demonstrated little selectivity for 5-HT$_2$ versus 5HT$_1$ subtypes of receptors. The relatively weak hallucinogenic effects of the methylenedioxy-substituted

amphetamine derivatives (when compared to the 2,5-DMA derivatives) may be mediated through actions at 5-HT_2 serotonin receptors. In addition, the neurotoxic, psychotomimetic, analgesic, temperature regulation, and mood-altering effects of MDMA and other methylenedioxy-substituted derivatives may be mediated, in part, through their actions at other serotonin recognition sites in brain, including serotonin uptake sites and 5-HT_{1A} serotonin receptors. Other behavioral. cardiovascular, and toxic effects of MDMA and related derivatives may be mediated by actions at other central and/or peripheral recognition sites, including muscarinic cholinergic receptors and α_2-adrenergic receptors, for which these compounds exhibit relatively high affinity. The precise mechanisms for the various effects of the amphetamine derivatives remain to be determined.

DISCUSSION

QUESTION: In the absence of serotonin neurons, could MDMA still have a direct agonist action at postsynaptic receptors or is that 5-HT?

ANSWER: Yes. From the present data, one would expect direct agonist effects of MDMA at 5-HT_2 receptors in the absence of serotonin neurons. Based on the data that I showed you today, MDMA and other methylene dioxy amphetamine derivatives exhibit agonist-like binding properties that resemble those observed for 5-HT and other tryptamine agonists as well as for other hallucinogenic amphetamines. We would expect the effects of MDMA at 5-HT_2 receptors to be somewhat weaker compared to those of other amphetamine derivatives, since MDMA-like compounds exhibit substantially lower affinity than the 2,5-DMA derivatives at 5-HT_2 sites. In addition, unlike what we Observe with the 2,5-DMA derivatives, MDMA and the other methylenedioxy compounds do not exhibit the preferential affinity for 5-HT_2 sites over 5-HT_1 subtypes as observed for the more potent hallucinogens. The comparable affinity of MDMA for multiple 5-HT receptors may contribute to the comparatively weak hallucinogen-like properties of this class of compounds. With respect to the second part of the question, it would be expected that, in the presence of an intact serotonergic system, MDMA-induced release of 5-HT via presynaptic sites of action would also have some postsynaptic 5-HT_2 receptor consequences. I inferred that MDMA may have antagonist-like effects at $alpha_2$ adrenergic receptors, and this may be responsible for increased 5-HT release. However, the only recognition site where we have tried to discern agonist versus antagonist characteristics is at the 5-HT_2 serotonin receptors.

QUESTION: Not the presynaptic?

ANSWER: No, only the postsynaptic 5-HT_2 receptors.

COMMENT: It seems to me that, in the absence of the serotonin input, the 5-HT_2 serotonin receptors downregulate instead of upregulate. So if you

took away the serotonin input, you would expect to see a decreased potency.

RESPONSE: Downregulation of 5-HT_2 receptors, which has been observed following treatment with antagonists, may be viewed as due to compensatory changes in response to tbe absence of 5-HT input. In order to assess the hypothesis that there was modulation in the absence of serotonin, we looked at 5-HT_2 serotonin receptors following lesion with MDMA. We chose to look at a time point 2 weeks after treatment in order to allow time for postsynaptic receptor changes to occur. Although we did not see any changes in the density of sites, we have not investigated whether there may have been changes in second messenger systems coupled to these receptors.

QUESTION: Would you expect the direct effects of MDMA on the 5-HT_2 receptor to have any significance in the presence of this massive 5-HT release that it is causing?

ANSWER: When we are dealing with the effects of the methylenedioxy-substituted derivatives on serotonergic systems, we are dealing with a multiplicity of effects.

Our data indicate that the racemates of these compounds most likely mediate effects on both presynaptic as well as postsynaptic 5-HT sites. Furthermore, there is an inverse stereospecificity associated with these actions. For example, the dextro isomers of MDMA and other drugs in this class exhibit higher affinity than the levo isomer for the presynaptic 5-HT uptake and also appear to be more potent in causing 5-HT release. This is the opposite of the isomer affinities at postsynaptic receptors. The levo isomers of MDMA-like compounds exhibit preferentially higher affinity than the dextro isomers for both 5-HT_1 and 5-HT_2 serotonin receptor subtypes. Similar stereospecificity is observed with the parent compounds, amphetamine and methamphetamine, as well for the hallucinogenic 2,5-DMA derivatives. While we can discern the agonist properties of these compounds at 5-HT_2 receptors, it is unclear whether these drugs are acting as agonists or antagonists at the various subtypes of 5-HT_1 receptors. With respect to the original question, if MDMA exhibits simultaneous 5-HT_2 agonist and 5-HT_1 antagonist activity, then one may speculate that these effects can significandy influence the fmal response, even in the presence of massive 5-HT release by these agents.

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