European Journal of Pharmacology, 149 (1988) 159–163
Elsevier

EJP 20117

Short communication

Pharmacologic profile of MDMA (3,4-methylenedioxymethamphetamine) at various brain recognition sites

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Received 23 February 1988, accepted 1 March 1988

We report here an in vitro pharmacologic profile for MDMA (3,4-methylenedioxymethamphetamine) at various brain recognition sites. The rank order of affinities of MDMA at various brain receptors and uptake sites are as follows: 5-HT uptake > α_2-adrenoceptors = 5-HT_2 serotonin = M-1 muscarinic = H-1 histamine > norepinephrine uptake = M-2 muscarinic = α_1-adrenoceptors = β-adrenoceptors ≥ dopamine uptake = 5-HT_1 serotonin ≫ D-2 dopamine > D-1 dopamine. MDMA exhibited negligible affinities (> 500 μM) at opioid (μ, δ and κ), central-type benzodiazepine, and corticotropin-releasing factor receptors, and at choline uptake sites and calcium channels.

3,4-Methylenedioxymethamphetamine (MDMA); 3,4-Methylenedioxyamphetamine (MDA); N-Ethylmethylenedioxyamphetamine (MDE); Amphetamine; Receptors; Uptake sites

1. Introduction

MDMA (3,4-methylenedioxymethamphetamine), a ring substituted derivative of methamphetamine, has received a great deal of recent attention since it represents one of a number of 'designer drugs' which is being increasingly abused among certain segments of the population. MDMA has been reported to produce both stimulant- and hallucinogen-like effects in man (Shulgin, 1986). In experimental animals, MDMA produces a variety of effects on motor, autonomic and central nervous system (CNS) function (reviewed in Shulgin, 1986). Neurochemical studies in rodents have demonstrated that MDMA exerts marked and preferential effects on serotonergic systems in brain including blockade of active uptake and stimulation of release of [^{3}H]serotonin in vitro, decreases in the content of serotonin (5-HT), and 5-hydroxyindoleacetic acid, and decreases in the maximal uptake of 5-HT and tryptophan hydroxylase activity following in vivo administration of MDMA (Shulgin, 1986; Battaglia et al., 1987; in press). In order to assess more directly the sites in brain which may mediate some of the psychotomimetic, behavioral and neurotoxic effects of MDMA, we have carried out in vitro radioligand binding studies to determine the relative potencies (i.e. affinities) or MDMA at various brain recognition sites.

2. Materials and methods

All radioligand binding assays were carried out using standard procedures. The details of the radioligand concentrations, buffer and incubation conditions, and drugs used to define specific binding are described in table 1. Initially, we screened

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the effects of a 500 μM concentration of MDMA at the various brain recognition sites. Brain recognition sites at which MDMA inhibited greater than 50% of the specific binding at the 500 μM concentration were further screened using at least 9 concentrations of the drug. All data were analyzed using the iterative program 'LIGAND' (Munson and Rodbard, 1980) which provides objective data analysis of ligand binding giving estimates of the relative affinities (K_i). All radio-

TABLE 1

Pharmacologic profile of MDMA at various brain recognition sites. Affinities of MDMA at various brain recognition sites. Data represent the mean and S.M. from 3-5 competition curves at each of the sites. K_i values were determined using the nonlinear least-squares curve fitting program LIGAND. Assay buffers were as follows: (A) 50 mM Tris-HCl, 120 mM NaCl, 5 mM KCl (pH 7.4 at Rm T); (B) 50 mM glycylglycine, 200 mM NaCl (pH 7.8 at 25°C); (C) 50 mM Tris-HCl, 10 mM $MgSO_4$, 0.5 mM K_2EDTA (pH 7.4 at 37°C); (D) 50 mM 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 mM Na^+ K^+ phosphate (pH 7.4 at Rm T); (H) 50 mM Tris-HCl, 10 mM $MgCl_2$, 2 mM EGTA, 0.1% bovine serum albumin, 0.1 mM bacitracin, aprotinin (100 KIU/ml) (pH 7.2 at 22°C). Brain regions were as follows: (1) frontal cortex; (2) striatum; (3) brain stem; (4) whole brain; and (5) olfactory bulb.

Brain recognition site	Affinity K_i (μM)	Radioligand/displacer	Brain region	Assay time, temperature	Buffer
Uptake sites					
Serotonin	0.61 ± 0.05	0.25 nM [^{3}H]Paroxetine, 1 μM citalopram	1	120 min at Rm T	A
Norepinephrine	15.8 ± 1.7	4.0 nM [^{3}H]Mazindol, 0.3 μM desipramine	1	90 min, 4°C	A
Dopamine	24.4 ± 1.9	1.0 nM [^{3}H]GBR 12935, 1 μM mazindol	2	60 min, Rm T	A
Choline	> 500	10 nM [^{3}H]Hemicholinium-3, 10 μM hemicholinium-3	2	30 min, 25°C	B
Adrenoceptors					
α_1	18.4 ± 1.2	0.5 nM [^{3}H]Prazosin, 10 μM phentolamine	1	30 min, 37°C	C
α_2	3.6 ± 0.8	0.5 nM [^{3}H]p-Aminoclonidine, 10 μM phentolamine	1	30 min, 37°C	C
β	19.2 ± 2.1	0.5 nM [^{3}H]Dihydroalprenolol, 1 μM propranolol	1	30 min, 37°C	C
Dopamine receptors					
D-1	148 ± 14	0.2 nM [^{3}H]SCH 23390, 0.1 μM flupenthixol	2	30 min, 37°C	C
D-2	95 ± 15	0.2 nM [^{3}H]Spiperone, 1 μM (+)butaclamol	2	30 min, 37°C	C
Serotonin receptors					
5-HT_1	23 ± 1.5	2.5 nM [^{3}H]Serotonin, 10 μM serotonin	1	30 min, 37°C	C
5-HT_2	5.1 ± 0.3	0.4 nM [^{3}H]Ketanserin, 0.5 nM cinanserin	1	30 min, 37°C	C
Cholinergic receptors					
M-1 muscarinic	5.8 ± 0.3	0.1 nM [^{3}H](−)QNB, 1 μM atropine	1	90 min, Rm T	D
M-2 muscarinic	15.1 ± 0.1	0.1 nM [^{3}H](−)QNB, 1 μM atropine	3	90 min, Rm T	D
Opioid receptors					
μ	> 500	2 nM [^{3}H]Dihydromorphine, 1 μM levallorphan	4	45 min, 25°C	E
δ	> 500	4 nM [^{3}H][D-Ala^2,D-Leu^5]enkephalin (30 nM morphine), 1 μM levallorphan	4	45 min, 25°C	E
κ	> 500	1.6 nM [^{3}H]Ethylketazocine (30 nM morphine + 100 nM [D-Ala^2,D-Leu^5]enkephalin), 1 μM levallorphan	4	45 min, 25°C	E
Other sites					
H-1 histamine receptors	5.7 ± 2.4	2 nM [^{3}H]Mepyramine, 1 μM doxepin	1	60 min, Rm T	F
Benzodiazepine receptors	> 500	0.2 nM [^{3}H]Flunitrazepam, 1 μM clonazepam	1	60 min, Rm T	G
Corticotropin-releasing factor (CRF) receptors	> 500	0.1 nM ^{125}I-Tyr^0-rat CRF, 1 μM ovine CRF	5	120 min, Rm T	H
Calcium channels	> 500	0.2 nM [^{3}H]Nitredipine, 0.1 μM nifedipine	1	60 min, Rm T	G

ligands were purchased from New England Nuclear (Boston, MA). Most of the materials were purchased from Sigma Chemical (St. Louis, MO). MDMA, MDA (3,4-methylenedioxyamphetamine), MDE (N-ethylmethylenedioxyamphetamine), amphetamine and methamphetamine were kindly provided by National Institute on Drug Abuse.

3. Results

The relative potencies of MDMA at the various brain recognition sites are summarized in table 1. MDMA had highest affinity for 5-HT uptake sites (< 1 μM) with lower but comparable affinities at 5-HT_2 serotonin, α_2-adrenoceptors, M-1 muscarinic cholinergic and H-1 histamine receptors (K_i values $\leqslant 6$ μM). The rank order of affinities of MDMA at various brain receptors and uptake sites were as follows: 5-HT uptake > α_2-adrenoceptors = 5-HT_2 serotonin = M-1 muscarinic = H-1 histamine > norepinephrine uptake = M-2 muscarinic = α_1-adrenoceptors = β-adrenoceptors $\geqslant$ dopamine uptake = 5-HT_1 serotonin $\gg$ D-2 dopamine > D-1 dopamine. MDMA exhibited negligible affinities (> 500 μM) at μ, δ and κ opioid, central type benzodiazepine, and corticotropin-releasing factor receptors and at choline uptake sites and calcium channels. The affinities of MDA were comparable (< 2-fold difference) to those of MDMA at each of the respective brain recognition sites investigated.

TABLE 2

Relative potencies of amphetamine derivatives at selected brain recognition sites. Comparison of the affinities of amphetamine derivatives at 5-HT uptake sites, 5-HT_2 serotonin, α_2-adrenoceptor and M-1 muscarinic receptors with respect to the affinity of MDMA at these sites (see table 1).

Compound	5-HT uptake	5-HT_2 serotonin	α_2-Adrenoceptor	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
Methamphetamine	3.4	2.4	0.61	3.6

To assess the effect of methylenedioxy substitution on the relative potencies of the amphetamine derivatives, the affinities of MDMA, MDA MDE and their parent compounds (amphetamine and methamphetamine) were compared at binding sites with high affinity for MDMA such as the 5-HT uptake site, 5-HT_2 serotonin receptor, α_2 adrenoceptor and M-1 muscarinic receptor (see table 2). Methylenedioxy substitution of the parent compounds increased the overall affinity (2-5-fold) at 5-HT uptake sites, 5-HT_2 serotonin and M-1 muscarinic receptors. In contrast, the parent compounds amphetamine and methamphetamine were 2-5-fold more potent at α_2-adrenoceptors than the methylenedioxy substituted derivatives.

4. Discussion

The pharmacologic profile of MDMA demonstrates a broad range of affinities of the drug for various brain recognition sites. MDMA is most potent at serotonin recognition sites and at α_2-adrenoceptors with affinity constants in the high nanomolar to low micromolar range. These affinities appear to be pharmacologically relevant since brain concentrations of MDMA in the high micromolar range 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, Battaglia and De Souza, unpublished).

Some of the behavioural, psychotomimetic and neurochemical effects of MDMA may be explained by interactions of MDMA at serotonin recognition sites. MDMA may alter serotonergic transmission in brain both through direct as well as indirect actions at post- as well as pre-synaptic 5-HT recognition sites. A number of hallucinogenic phenylisopropylamine derivatives exhibit potent agonist-like activity at brain 5-HT_2 serotonin receptors. The in vitro affinities of these hallucinogens at 5-HT_2 serotonin receptors are significantly correlated with both their behavioral potencies in animals in generalization to other hallucinogens and in their human hallucinogenic potencies (Glennon et al., 1984). Similarly, MDMA and other methylenedioxy derivatives of

amphetamine exhibit high affinity agonist-like binding characteristics at 5-HT_2 serotonin receptors and a stereospecificity consistent with that observed for other hallucinogenic compounds at this receptor (Battaglia et al., 1986). Thus, it is feasible that a component of the 'mood-altering' effects of MDMA may be mediated through 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 amphetamine hallucinogens suggests that MDMA and MDA would be weak hallucinogens. The relatively high affinity of MDMA at the 5-HT uptake site may relate in part to the neurochemical and neurotoxic effects of this drug on presynaptic 5-HT terminals. MDMA has been reported to competitively inhibit [^{3}H]serotonin uptake in vitro (Shulgin, 1986) and the neurotoxic effects of in vivo administration of MDMA on 5-HT terminals can be blocked by concomitant administration of the 5-HT uptake blocker citalopram (Battaglia et al., in press). The potent effects of MDMA on brain serotonergic systems may help explain other effects of MDMA on analgesia and body temperature, as well as the 'anxiolytic-like' properties of the drug (Shulgin, 1986). Additional evidence in support of the hypothesis that MDMA produces its effects through brain serotonergic mechanisms is provided by data demonstrating that MDMA generalizes to a fenfluramine cue in discrimination studies (Schechter, 1986).

MDMA has relatively high affinity for α_2-adrenoceptors. In humans, MDMA has been reported to produce large increases in both systolic and diastolic pressure (Shulgin, 1986); these effects on cardiovascular function may be mediated through 'antagonist-like' effects at α_2-adrenoceptors in brain. Likewise, since α-adrenoceptor antagonists such as phentolamine have been reported to increase the release of [^{3}H]serotonin via effects on α_2-adrenoceptors (Timmermans and Van Zwieten, 1982) and since MDMA has been reported to increase the release of [^{3}H]serotonin from brain slices (Johnson et al., 1986), one might speculate that the [^{3}H]serotonin releasing effects of MDMA may be mediated, in part, by high affinity antagonist-like effects at α_2-adenoceptors. In addition to α_2-adrenoceptors, MDMA has relatively high affinity for M-1 muscarinic receptors. MDMA has been reported to produced intense salivation (see Shulgin, 1986), an effect known to be mediated through actions of agonists at muscarinic cholinergic receptors. While MDMA has relatively high affinity for H-1 histamine receptors, the effects of MDMA at these receptors remain to be determined.

In drug discrimination studies in rats, the stimulus cue for MDMA has been reported to generalize to that of dopamine agonists such as apomorphine and 1-cathinone (Schechter, 1986). These agonist-like properties of MDMA are probably produced through indirect effects of MDMA on the presynaptic release of dopamine (Johnson et al., 1986) since MDMA has relatively low affinity for postsynaptic D-1 and D-2 dopamine receptors. The anxiolytic-like effects of MDMA do not appear to be mediated through agonist actions at benzodiazepine receptors or antagonist effects at corticotrophin-releasing factor receptors as evidenced by the low affinity of MDMA (> 500 μM) at these receptors. In addition, neither the reinforcing, analgesic and other mood-altering properties of the drug appear to be mediated through interactions with the opioid receptors since MDMA has relatively low affinities for these binding sites.

In summary, the data we present here suggest that the neurotoxic, psychotomimetic, analgesic, temperature regulation and mood-altering effects reported for MDMA may be mediated in part through its actions at serotonin recognition sites in brain. Other behavioral, cardiovascular and toxic effects of MDMA may be mediated by actions at other central and/or peripheral recognition sites including muscarinic cholinergic receptors and α_2-adrenoceptors for which MDMA exhibits relatively high affinity. The precise mechanisms for the various effects of MDMA remain to be determined.

Acknowledgements

We would like to thank Theresa Kopajtic for excellent technical assistance and Terrie Pierce and Mary Flutka for manuscript preparation.

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