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3,4-Methylenedioxymethamphetamine (MDMA): Stereoselective interactions at brain 5-HT_1 and 5-HT_2 receptors

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Abstract. 3,4-Methylenedioxymethamphetamine (MDMA), 3,4-methylenedioxy-amphetamine (MDA), and their optical isomers, were assayed for their affinities at radiolabeled brain serotonin (5-HT_1, 5-HT_2) and dopamine (D_2) binding sites. (R(−)-MDA and R(−)-MDMA displayed moderate affinities for ^{3}H-ketanserin-labeled 5-HT_2 sites (Ki = 3425 and 3310 nM, respectively) whereas the affinities for their S(+)-enantiomers were lower (Ki = 13,000 and 15,800 nM, respectively). Similar absolute and relative affinities were obtained at ^{3}H-serotonin-labeled 5-HT_1 sites; binding at D_2 sites was very low (Ki > 25,000 nM in each case). The (−) > (+) order of potency at 5-HT_2 sites is consistent with the observation that R(−)-MDA is a more potent psychoactive agent than its S(+)-enantiomer, but contrasts with the reported finding that S(+)-MDMA is more potent than R(−)-MDMA in humans. These results suggest that MDMA, unlike MDA and other hallucinogenic phenylisopropylamines, does not work primarily through a direct interaction at 5-HT sites.

Key words: 3,4-methylenedioxymethamphetamine – 3,4-methylenedioxyamphetamine – 5-HT receptors – dopamine receptors

3,4-Methylenedioxymethamphetamine (MDMA) has attracted the attention of psychopharmacologists because of its psychoactive actions and putative therapeutic utility (Anderson et al. 1978). Although this agent is structurally related to 3,4-methylenedioxyamphetamine (MDA) and certain other hallucinogenic phenylisopropylamines, its particular psychoactive properties (including minimal, if any, hallucinogenic effects) indicate that MDMA may possess a mechanism of action distinct from that of these other agents. We have recently reported that a significant correlation exists between the human hallucinogenic potencies, as well as discrimination derived ED_{50} values, of a series of substituted phenylisopropylamines and their brain 5-HT_2 receptor affinities (Glennon et al. 1984). In the present study, we wished to determine and compare the affinities of MDMA and MDA for brain 5-HT_1, 5-HT_2 and dopamine (D_2) binding sites in order to gain information on their mechanism of action.

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Materials and methods

^{3}H-Ketanserin (90.4 Ci/mmol), ^{3}H-serotonin (27.3 Ci/mmol) and ^{3}H-N-methylspiperone (76.7 Ci/mmol) were obtained from New England Nuclear. ^{3}H-N-methyl-spiperone is a new radioligand for D_2 dopamine receptors (Titeler et al. 1985). Serotonin HCl and pargyline were obtained from Sigma. Sulpiride and cinnanserin were gifts from Dr. M. Kuhar. MDMA and its isomers were synthesized as their HCl salts and were available from an earlier study; MDA and its isomers were obtained as their HCl salts from NIDA.

The membrane preparation and radioligand binding assays were performed as described previously (Glennon et al. 1984). Male Zivik-Miller Sprague Dawley rats (200 g) were decapitated and the brains were removed and placed in ice-cold 0.9% saline. Dissecting over ice, the striata and frontal-parietal cortices were removed and again placed in saline. The pooled tissues were weighed and quick frozen over dry ice before being stored at −25° C. The time between dissection and freezing was not longer than 30 min and tissue was stored for no longer than 1 week. Tissue was homogenized in 30 vol 50 mM Tris-HCl, 10 mM $MgCl_2$, and 0.5 mM Na_2EDTA (pH 7.7 at 25° C). Following homogenization the homogenate was centrifuged at 35000 *g* for 15 min. The pellets were resuspended and incubated at 37° C for 15 min followed by recentrifugation. The final incubation medium (2.0 ml) included 0.4 nM ^{3}H-ketanserin, 1.0 nM ^{3}H-serotonin, or 0.1 nM ^{3}H-N-methyl-spiperone for 5-HT_2, 5-HT_1, or D_2 dopamine receptor assays, respectively. Wet (4 mg and 8 mg wet weight) cortical tissue was used for the 5-HT^2 and 5-HT_1 assays respectively and 2.5 mg of striatal tissue was used for the D_2 receptor assays. Cinnanserin 10^{-6} M was used as the excess displacing agent for the 5-HT_2 assays and 10^{-6} M serotonin was the excess displacing agent for the 5-HT_1 assays. NaCl 140 mM was present in the D_2 receptor assays as the excess displacing agent, as sulpiride (10^{-5} M) is a sodium-dependent ligand. Pargyline (10^{-5} M) was present in all assays. Each compound was assayed in triplicate three times at 11 different concentrations. After incubation for 15 min at 37° C the incubation media was filtered over GF/B filters, washed with 10 ml ice-cold buffer, and placed in scintillation vials with 5 ml scintillation fluid (Scintiverse, Fisher). After equilibration the vials were counted on a Beckman 3801 at an efficiency of 50%. Competition experiments were ana-

Table 1. Ki values of MDA and MDMA for 5-HT_1, 5-HT_2 and D_2 binding sites[a]

Agent	KI (nM)[b]		
	5-HT_1	5-HT_2	D_2
(±)-MDA	7,150 (±1,300)	5,330 (± 600)	>100000
R(−)-MDA[c]	4,800 (± 800)	3,425 (± 740)	>100000
S(+)-MDA[c]	14,000 (±1,300)	13,000 (±3,000)	>100000
(±)-MDMA	6,850 (±1,300)	8,300 (±1,100)	41000 (±2,000)
R(−)-MDMA[c]	4,200 (± 500)	3,310 (± 140)	25400 (±1,200)
S(+)-MDMA[c]	11,300 (±1,600)	15,800 (±1,800)	>100000

[a] Assay conditions as per Materials and methods
[b] Values are the means of three experiments; (±) values are SEM
[c] Significant difference, between R(−) and S(+) isomers for 5-HT_1 and 5-HT_2 binding ($P<0.05$–0.02, paired t-test)

lyzed using a computer-assisted nonlinear least-squares regression analysis (McPherson 1984).

Results

Table 1 lists the Ki values for MDMA and related compounds at the 5-HT_1, 5-HT_2, and D_2 receptors. Racemic MDMA was found to possess a nearly equal affinity for 5-HT_1 and 5-HT_2 sites (Ki = 6850 and 8300 nM, respectively) and a rather low affinity for D_2 sites (Ki = 41250 nM). Binding was stereoselective at 5-HT_1 and 5-HT_2 sites in that R(−)-MDMA possesses about a 3-fold greater affinity than does its S(+)-enantiomer. Similar absolute and relative affinities were obtained with (±)-, R(−)-, and S(+)-MDA.

Discussion

The affinity of (±)-MDA for 5-HT_2 sites is in the same range as that of other hallucinogenic phenylisopropylamines of comparable human potency. In addition, as with other hallucinogenic phenylisopropylamines, the R(−)-isomer is several-fold more potent than its S(+)-enantiomer with respect to its human potency (Shulgin 1978) and 5-HT_2-site affinity (Table 1). Racemic MDMA, and its isomers, display a binding profile similar to that of MDA. However, it has been claimed that in human subjects (±)-MDMA produces an effect that is reminiscent of, but somewhat different from, that of MDA and, furthermore, (although only limited data are available) that S(+)-MDMA is several-fold more potent than R(−)-MDMA (Anderson et al. 1978). If, indeed, S(+)-MDMA is more potent than R(−)-MDMA, it is unlikely that this effect can be attributed to a direct 5-HT_2-mediated mechanism because of the greater potency of R(−)-MDMA for 5-HT_2 sites.

MDA produces both hallucinogen-like and amphetamine-like discriminative stimulus effects in animals; it has also been demonstrated that R(−)-MDA may be primarily responsible for the former effect, whereas it is the S(+)-isomer that produces the amphetamine-like effect (Glennon 1985). These results are consistent with those observed in human subjects; that is, there is a considerable stimulant component associated with S(+)-MDA (Shulgin 1978). Knowing that N-methylation decreases the potency of hallucinogenic phenylisopropylamines, but has essentially no effect on the potency of stimulant phenylisopropylamines (such as amphetamine) (Glennon 1985), it seems entirely possible that MDMA is more amphetamine-like than MDA. This would also explain the greater potency of S(+)-MDMA over R(−)-MDMA. Thus, the hypothesis that we are currently developing is that MDMA acts via a mechanism more closely related to the mechanism of action of amphetamine than to that of the hallucinogenic phenylisopropylamines. However, MDMA does bind to 5-HT binding sites and (in addition to any indirect or releasing properties that it might possess) may also be capable of producing effects that are serotonin related.

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