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Ring-substituted amphetamine interactions with neurotransmitter receptor binding sites in human cortex

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The binding affinities of 3 ring-substituted amphetamine compounds were determined at 9 neurotransmitter binding sites in human cortex. (±)-3,4-Methylenedioxyamphetamine (MDA), (±)-3,4-methylenedioxyethamphetamine (MDE), and (±)-3,4-methylenedioxymethamphetamine (MDMA or 'Ecstasy') all display highest affinity (approximately 1 μM) for the recently identified 'DOB binding site' labeled by [^{77}Br]R(−)4-bromo-2,5-dimethoxyphenylisopropylamine ([^{77}Br]R(−)DOB). MDA displays moderate affinity (4–5 μM) for the 5-hydroxytryptamine$_{1A}$ (5-HT$_{1A}$), 5-HT$_{1D}$, and α_2-adrenergic sites in human cortex. MDE and MDMA display lower affinity or are inactive at all other sites tested in the present study. These observations are discussed in relation to the novel psychoactive effects of the ring-substituted amphetamines.

A series of ring-substituted amphetamine derivatives exist which are structurally related to both amphetamines and hallucinogens. However, drugs such as (±)-3,4-methylenedioxyamphetamine (MDA), (±)-3,4-methylenedioxyethamphetamine (MDE), and (±)-3,4-methylenedioxymethamphetamine (MDMA or 'Ecstasy') appear to produce unique psychoactive effects which are distinct from the effects of both amphetamines and hallucinogens [13, 15]. These agents have been reported to induce a mild euphoria with a sense of enhanced insight in human users. As a result of these properties, ring-substituted amphetamines have been used in both psychotherapeutic [4] and recreational [8] settings. The human use of these compounds is of concern due to the neurotoxic effects of ring-substituted amphetamines on serotonergic nerve terminals in laboratory animals [2, 10, 11, 16].

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The mechanism of action by which ring-substituted amphetamines produce their unique psychoactive effects remains unknown. In the present study, the potencies of MDA, MDE, and MDMA at 8 neurotransmitter binding sites in human cortex were analyzed. In addition, drug potencies were determined at the recently identified binding site radiolabeled by 4-bromo-2,5-dimethoxyphenylisopropylamine (DOB), a putative serotonergic receptor. Titeler and colleagues first labeled this site in rat brain with [^{3}H]DOB [7, 17]. Recently, and in the present study, our laboratory has used the novel radioligand [^{77}Br]R(−)DOB to label the 'DOB binding site' [19]. The present study represents the first report of ring-substituted amphetamine interactions with neurotransmitter binding sites in human cortex.

Radioligand binding studies were performed as described previously [9, 5, 19]. Briefly, human cortex samples were thawed in Tris-HCl buffer. Tissues were homogenized in 20 vols. Tris-HCl buffer (pH 7.7 at 25°C) and then centrifuged at 49,000 *g* for 10 min. The supernatant was discarded and the pellet was resuspended and incubated at 37°C for 10 min prior to a second centrifugation at 49,000 *g* for 10 min. The final pellet was resuspended in 80 vols. of Tris-HCl buffer containing 10 μM pargyline, 4 mM calcium chloride and 0.1% ascorbic acid. The buffer used for the DOB site analysis consisted of 50 mM Tris-HCl, 0.5 mM EDTA, 10 mM $MgCl_2$, 0.1% ascorbate, and 10 μM pargyline. Radioligand binding studies consisted of 0.1 ml [^{3}H]radioligand (final concentrations were 0.3 nM [^{3}H]8-OH-DPAT; 1.4 nM [^{3}H]5-HT; 0.4 nM [^{3}H]spiperone; 0.5 nM [^{3}H]WB 4101; 1.5 nM [^{3}H]rauwolscine; 0.2 nM [^{3}H]dihydroalprenolol hydrochloride (DHA); 12–32 pM ^{77}Br-R(–)DOB; 0.08 nM [^{3}H]quinuclidinyl benzilate (QNB); 0.1 nM [^{3}H]flunitrazepam), 0.1 ml buffer or displacing drug and 0.8 ml tissue suspension. The presence of 0.1 μM 8-OH-DPAT when labeling the 5-HT_{1D} site with [^{3}H]5-HT and the α_1-adrenergic site with [^{3}H]WB-4101 eliminated radioligand binding to 5-HT_{1A} sites. Specific binding was defined as the excess taken over blanks in the presence of 10^{-5} M 5-HT for 5-HT_{1A} and 5-HT_{1D} sites, 10^{-6} M cinanserin for 5-HT_2 sites, 10^{-6} M 4-iodo-2,5-dimethoxyphenylisopropylamine (DOI) for DOB sites, 10^{-6} M prazosin for α_1-adrenergic sites, 10^{-4} M yohimbine for alpha$_2$-adrenergic sites, 10^{-6} M propranolol for β-adrenergic sites, 10^{-6} M scopolamine for muscarinic cholinergic sites, and 10^{-6} M diazepam for benzodiazepine sites.

The K_D values for the various radioligands in general have not been determined in human cortex so that calculating K_i values is not possible at this time. Final radioligand concentrations used in the assays were less than one-half the K_D values reported for the radioligands in rat brain.

Radiolabeled drugs were obtained from Dupont-New England Nuclear except [^{3}H]8-OH-DPAT from Research Products International Corp. and [^{77}Br]R(−)DOB (1875 Ci/mmol), a generous gift from Dr. Chester A. Mathis. Drugs were obtained from commercial sources except MDMA, MDE, MDA, and DOI which were obtained from the National Institute on Drug Abuse, Bethesda, MD.

Drug interactions with 5-hydroxytryptamine binding site subtypes. Drug competition studies were performed to determine the IC_{50} values of 3 ring-substituted amphetamine compounds at 5-HT binding sites in human cortex (Table I). All 3 of the psy-

TABLE I

RING-SUBSTITUTED AMPHETAMINE INTERACTIONS WITH HUMAN 5-HT BINDING SITE SUBTYPES

Radioligand binding assays were performed using human cortex membranes as described in the text. IC_{50} values were determined using log probit analysis. Values shown are means ± S.E.M. of 3–5 experiments, each performed in triplicate.

Human binding site	Radioligand	IC_{50} values (μM)		
		MDA	MDE	MDMA
DOB	[^{77}Br]R(−)DOB	1.2 ± 0.3	0.88 ± 0.2	1.4 ± 0.1
5-HT_{1A}	[^{3}H]8-OH-DPAT	4.7 ± 0.3	11 ± 2	11 ± 1
5-HT_{1D}	[^{3}H]5-HT	4.1 ± 0.6	70 ± 10	11 ± 1
5-HT_2	[^{3}H]Spiperone	33 ± 3	36 ± 7	18 ± 6

choactive agents display the highest affinity for the 'DOB binding site' labeled by [^{77}Br]R(−)DOB. MDA is at least 3-fold more potent at the DOB site (1.2±0.3 μM) than at any of the other sites tested. MDE (0.88±0.2 μM) and MDMA (1.4±0.1 μM) are essentially equipotent with MDA at the 'DOB binding site' and are at least 10-fold more potent at this site than at the other sites tested.

MDA displays an affinity below 5 μM for both the 5-HT_{1A} site labeled by [^{3}H]8-OH-DPAT and the 5-HT_{1D} site labeled by [^{3}H]5-HT in the presence of 0.1 μM 8-OH-DPAT. Both MDE and MDMA display affinities greater than 10 μM for the 5-HT_{1A} and 5-HT_{1D} sites. MDE is notably weak at the 5-HT_{1D} site (70 μM). All 3 agents are relatively weak at the 5-HT_2 site labeled by [^{3}H]spiperone, displaying affinities of $\geqq$ 18 μM.

Drug interactions with other neurotransmitter binding sites. The affinities of MDA, MDE, and MDMA for various other neurotransmitter binding sites in human cortex are shown in Table II. MDA displays moderate affinity (5.2±0.1 uM) for the α_2-adrenergic site labeled by [^{3}H]rauwolscine. MDA is weaker at the α_1-adrenergic site (38±9 μM) labeled by [^{3}H]WB-4101 in the presence of 0.1 μM 8-OH-DPAT and essentially inactive at the β-adrenergic site labeled by [^{3}H]DHA, the muscarinic cholinergic site labeled by [^{3}H]QNB, and the benzodiazepine site labeled by [^{3}H]flunitrazepam. MDE and MDMA are relatively weak (>10 μM) at all of the above sites and are inactive at the benzodiazepine site.

The major finding of the present study is that the ring-substituted amphetamine compounds, MDA, MDE, and MDMA all display relatively high affinity (approximately 1 μM) for the recently identified 'DOB binding site' in human cortex. MDA is at least 3-fold, and MDE and MDMA at least 10-fold, more potent at the 'DOB binding site' than at any of the other 8 sites analyzed in this study. Although previous studies have reported MDA and MDMA interactions with brain 5-HT receptors in the rat [1, 6], the present study is the first report of ring-substituted amphetamine interactions with neurotransmitter binding sites in human cortex. These results are

TABLE II

RING-SUBSTITUTED AMPHETAMINE INTERACTIONS WITH HUMAN NEUROTRANSMITTER BINDING SITES

Radioligand binding assays were performed using human cortex membranes as described in the text. IC_{50} values were determined using log probit analysis. Values shown are the means ± S.E.M. of 3–5 experiments, each performed in triplicate.

Human binding site	Radioligand	IC_{50} values (μM)		
		MDA	MDE	MDMA
α_1-Adrenergic	[^{3}H]WB-4101	38 ± 9	36 ± 6	18 ± 5
α_2-Adrenergic	[^{3}H]rauwolscine	5.2 ± 0.1	22 ± 2	13 ±2
β-Adrenergic	[^{3}H]DHA	>100	33 ± 2	37 ± 3
Muscarinic cholinergic	[^{3}H]QNB	92 ± 4	65 ± 10	24 ± 2
Benzodiazepine	[^{3}H]flunitrazepam	>100	>100	>100

of potential interest since the mechanism of action of ring-substituted amphetamines remains unknown.

The results of the present study suggest the 'DOB binding site' might mediate some of the psychoactive effects of the ring-substituted amphetamines. The 3 compounds are essentially equipotent at the 'DOB binding site' in human cortex. Similarly, the usual human doses of these 3 drugs are also equivalent: i.e. 100–150 mg [12, 14]. Micromolar blood levels of these agents would be expected from such doses since a single oral dose of 50 mg MDMA has been reported to result in a plasma MDMA level of 0.45 μM (106 μg/l) [18]. A plasma level of 30 μM (7000 μg/l) MDMA has also been documented in a recreational MDMA user although the exact amount of MDMA ingested by the individual was not known [3]. These data suggest that recreational human doses of the ring-substituted amphetamines interact with the 'DOB binding site' in human brain whereas, at higher doses, the drugs are likely to interact with multiple other neurotransmitter receptors. Clearly, further studies are needed to determine the functional significance of the 'DOB binding site' in the human central nervous system and its possible relationship to the psychoactive effects of MDA, MDE and MDMA.

The mechanism of action by which ring-substituted amphetamines produce their psychoactive effects is of importance due to their neurotoxic effects in laboratory animals and their recent recreational popularity [8]. Conceivably, their unique psychoactive effects may be independent of their toxic effects on serotonergic nerve terminals. If so, then the possibility exists for the development of non-toxic analogues that may have useful psychotherapeutic applications. On the other hand, the neurotoxic effects of the currently available agents suggests that human use of these compounds should be avoided.

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