NOVEL SEROTONERGIC AGENTS

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The preliminary structure-activity relationships are described for a series of substituted phenethylamines that induce the release of neuronal serotonin. Structures that also have the ability to release neuronal catecholamines are toxic to serotonin neurons, leading to long-term reductions in serotonin markers in rat brain. Conversely, compounds that are selective for the serotonin uptake carrier, and do not affect catecholamines, are not neurotoxic. It is suggested that these novel, selective serotonin releasing agents may find therapeutic utility in disorders where serotonin uptake inhibitors are currently employed.

KEY WORDS: Serotonin uptake; serotonin release; neurotoxicity; drug discrimination; brain slices

INTRODUCTION

Although most of the known serotonergic therapeutic agents act through a direct receptor mechanism, indirectly-acting serotonergic agents may produce serotonergic effects through elevation of synaptic serotonin levels, thereby presumably increasing serotonergic transmission. Examples of such agents might be the antidepressant agents fluoxetine (Prozac), or chlorimipramine, and the anorectic agent fenfluramine. Experimentally, para-chloroamphetamine (PCA) has been used to induce behavioral effects through the release of neuronal serotonin. It should be noted, however, that the latter two agents produce serotonin neurotoxicity, the destruction of serotonin neuronal terminals.

Our interest in indirectly-acting serotonergic agents was stimulated about ten years ago by studies of the psychoactive phenethylamine 3,4-methylenedioxymeth-amphetamine (MDMA, **1**).[1,2] This substance later became popular as a recreational drug, available on the street under the name "ecstasy". The drug produces unique psychoactive effects, which differ both from classical psychostimulants and from hallucinogenic agents, and we subsequently named this psychopharmacological class "entactogens". However, similar to fenfluramine and PCA, MDMA was shown to
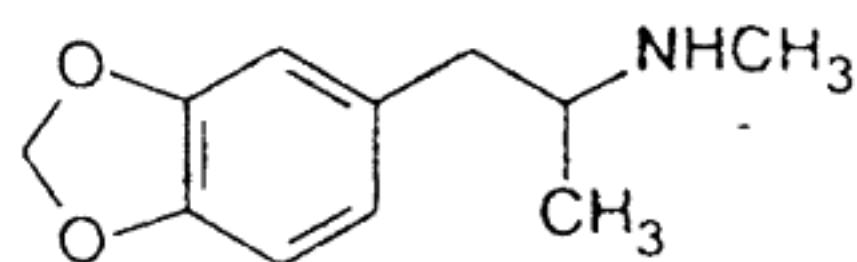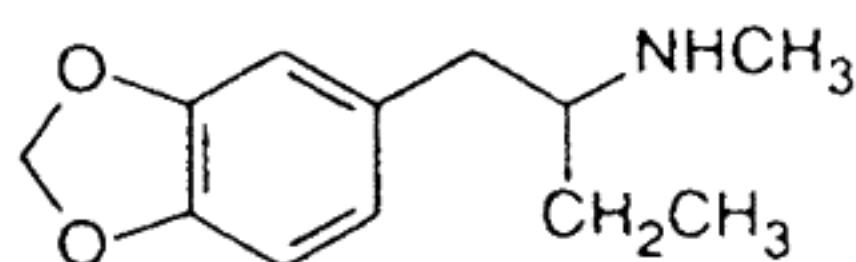
lead to serotonin neuron degeneration in rodents and in non-human primates (for a review, see Ref. 3). This was a major finding which led to concerns regarding the public health aspects of widespread abuse of this substance, and which continues to thwart the possibility of clinical studies with MDMA in the United States.

With the widespread recognition that modulation of serotonin function in the CNS might have a variety of therapeutic applications, we began to explore entactogens, and other compounds related to MDMA. After the central serotonin neurotoxic properties of MDMA were described, this work was carried out with a view toward the identification of molecules which might lack serotonin neurotoxic effects.

Our approach to the design of these molecules is two-fold. (1) The initial screen employs the two-lever drug discrimination assay in rats. Our early training drug was (+)-MDMA, but we later used several other analogs, including MBDB (2) and MMAI (see later discussion). (2) Following the identification of a molecule with an MDMA-like behavioral cue, the structure is studied for a serotonin neurotoxic effect following acute or subacute dosing. In conjunction with this latter assay, the drug is evaluated for its ability to inhibit the uptake of [^{3}H]-labeled monoamines into rat brain synaptosomes and/or for ability to release monoamines from superfused slices of rat brain.

The following discussion will highlight these efforts, and will identify a variety of structures that are potent releasers of neuronal serotonin, many of which lack serotonin neurotoxicity. Although the therapeutic potential of these compounds presently remains untapped, it is difficult to believe that this novel pharmacological category will not ultimately lead to useful therapeutic agents.

Side chain modifications

Our initial efforts involved an examination of the alpha-ethyl homologue of MDMA, MBDB (2). The rationale for this was the fact that MDMA was first described as an "hallucinogenic amphetamine", despite user's descriptions that it differed significantly from classical hallucinogens such as LSD or mescaline. It was already known that the homologation of the alpha-methyl of hallucinogenic amphetamines to an alpha ethyl completely abolished their hallucinogenic properties. Thus, it was reasoned, extension of the alpha methyl of MDMA to an ethyl should lead to a compound that lacked hallucinogenic activity. If the molecule retained psychopharmacology similar to MDMA, by all known structure-activity arguments, it would not be an hallucinogen.

In fact, MBDB proved to have effects in man similar to that of MDMA.[4] In addition, EEG field potentials recorded in freely-moving rats showed marked differences in the recordings made from rats treated with hallucinogenic amphetamine derivatives, and those from rats treated with MDMA or MBDB.[5] Furthermore, in contrast to the hallucinogenic amphetamines, such as R-DOM (3) where the R enantiomer is more potent, it was the S isomer of 2 that had higher activity.

While possessing effects similar to MDMA in a two-lever drug discrimination paradigm in rats,[6] MBDB has reduced serotonin neurotoxicity when compared with MDMA. When administered in a single acute dose, there was no long-term reduction in serotonin markers.[7] However, a subacute dosing regimen did reduce serotonin neuron markers, but not to the extent that MDMA did.[8] This was an early indication that the psychoactive properties of MDMA could be "dissected" from its neurotoxic effect.

S-(+)-2

R-(-)-3
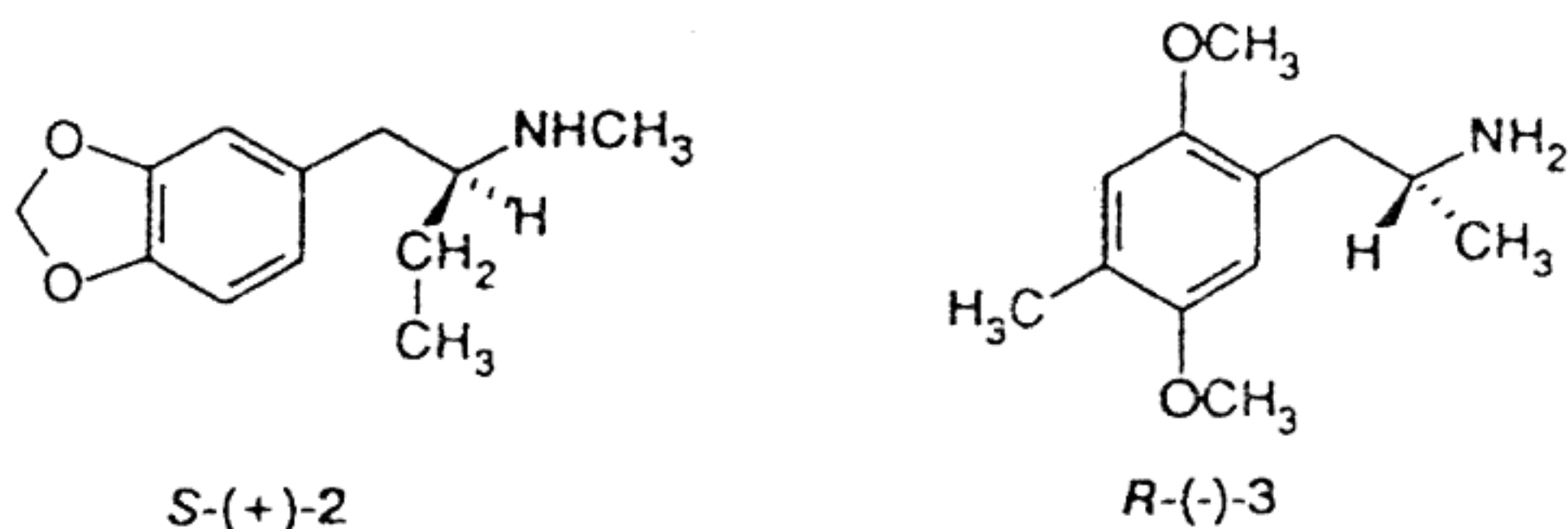
A number of studies in our laboratory, as well as a recent report by Nash and Nichols[7] using *in vivo* microdialysis, have demonstrated that a major salient feature of MBDB which distinguishes it from MDMA is its relative lack of effect on dopaminergic systems. In rat brain synaptosome preparations, in superfused rat brain slices, and using *in vivo* microdialysis techniques, MBDB has been shown to have little impact on dopaminergic neurons.[7,9,10]

The replacement of the alpha-methyl with an alpha-ethyl moiety markedly attenuates the catecholamine-releasing activity characteristics of the amphetamines, for all ring substitutions that we have so far examined. In fact, in drug discrimination experiments in animals trained to discriminate (+)-amphetamine sulfate from saline, the alpha-ethyl analogue of amphetamine itself failed to produce complete substitution, and was more than 1 order of magnitude less potent in producing a behavioral effect.[11] The interoceptive cue of (+)-amphetamine is generally accepted to be mediated by dopamine release, so this argues for the attenuating effect of an alpha-ethyl on this process. Carrying out this facile structural transformation on any phenethylamine derivative possessing dopaminergic activity would thus be expected to attenuate its dopaminergic component.

Our interest in the dopaminergic effects of MDMA was centered primarily on the possibility that dopamine systems were involved in the expression of serotonin neurotoxicity induced by MDMA, PCA (4), and possibly by fenfluramine. Numerous studies had shown a correlation between the relative ability of MDMA-like drugs to induce dopamine release *in vitro* and their ability to induce a neurotoxic *in vivo* response. These included studies of PCA,[12,13] the enantiomers of MDA and MDMA,[9,10,12,14,15] and the N-ethyl[14,16,17,18] and alpha-ethyl[8,9,10] analogues of MDMA.

PCA

CAB

4

5
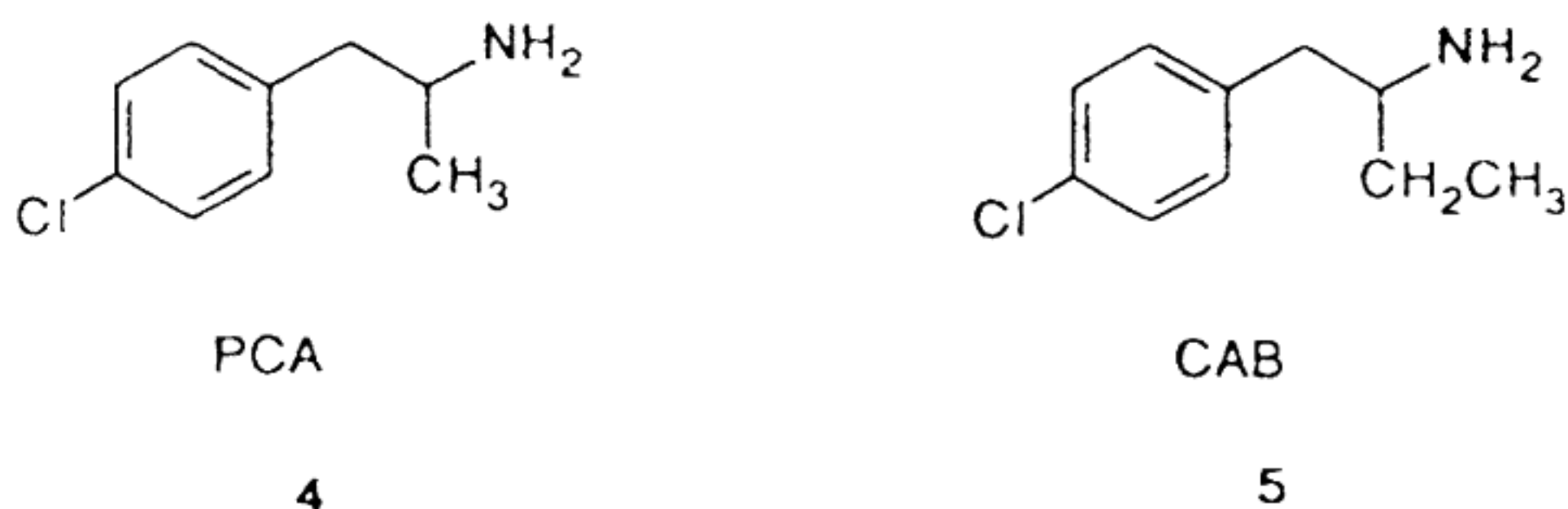
It was decided to test this hypothesis by assessing the behavioral and neurotoxic properties of the alpha-ethyl homologue of PCA, CAB (5). Since PCA is by far the most neurotoxic of the phenethylamine-derived serotonin neurotoxins, this appeared to be a fairly stringent test. Indeed, the potency of CAB to induce dopamine release *in vivo*, as well as to inhibit uptake of dopamine into whole brain synaptosomes was dramatically attenuated. CAB likewise had less than half the potency of PCA in the induction of long-term deficits in serotonin markers in both rat cortex and hippocampus.[19]

At this point it seemed interesting to identify unrelated structures that had pharmacological properties consisting of both serotonin release and dopamine release. One might hypothesize that such a drug would possess neurotoxic properties. At about this time, the report of a death attributed to the use of alpha-ethyltryptamine (Etryptamine; Monase) came to our attention.[20] While alpha-methyltryptamine was known to be a hallucinogen, Monase had been marketed as a non-hallucinogenic antidepressant in the late 1950s. Furthermore, published descriptions of the mood-elevating effects of Monase, and the production of a "talkative intoxication" at higher doses, suggested similarities to the psychopharmacology of MDMA. Although drug discrimination studies of Monase in rats trained to discriminate (+)-MBDB from saline have not been completed, 5/7 (71%) of rats tested at 0.83 mg/kg responded on the drug lever, while 3/4 (75%) of rats responded on the drug lever at 1.24 mg/kg. Although not conclusive, these data suggest the possibility that Monase might fully substitute at higher dosages, indicating an MBDB-like cue. More importantly, as anticipated, subacute dosing led to long-term reductions in serotonin markers and serotonin uptake sites.[21]

In view of the large number of compounds that gave substitution in MDMA or MBDB-trained animals, there was concern that we lacked a good animal model for the psychoactive effects of MDMA. This concern was heightened by the finding that fenfluramine gave symmetrical substitution with both MDMA and MBDB in drug discrimination tests,[22,23] but the human psychopharmacology of fenfluramine is quite different from the latter two agents. Furthermore, PCA also substituted in MDMA and MBDB-trained rats. It is a potent serotonin neurotoxin, and displays *in vitro* effects on monoamine uptake and release similar to MDMA. Unfortunately, nothing is known of its psychoactive properties in man, other than that its N-methyl derivative was briefly tested as an antidepressant agent. We thus had several drugs that apparently produced a behavioral cue in drug discrimination experiments similar to that of MDMA, but it was unknown whether this was relevant to the human psychopharmacology of this new class of drugs.

Eventually, however, the idea of identifying structures that would possess clinical psychopharmacology similar to MDMA was simply abandoned and instead the focus was placed on identifying drugs which were selectively or specifically able to induce the release of endogenous neuronal serotonin. As discussed later, many of these drugs have proven to lack serotonin neurotoxicity. Thus we have for the first time developed, we believe, pharmacological entities which affect serotonin neurons in a way analogous to that through which amphetamine affects catecholamine neurons. These compounds lack the neurotoxicity that is characteristic of earlier prototypes such as PCA and MDMA.

Side chain conformation

Early on, we wished to define the active side chain conformation of serotonergic phenethylamines. Fuller *et al.*[24,25] had shown that 6-chloro-2-aminotetralin, a cyclic analogue of PCA, had acute serotonin releasing effects, but did not produce long-term deficits in serotonin markers. For MDMA, there were two possible isomeric aminotetralins that could be envisioned; 5,6-methylenedioxy-2-aminotetralin (**6**), and 6,7-methylenedioxy-2-aminotetralin (**7**). Indeed, while **7** and the ring-contracted analogue 5,6-methylenedioxy-2-aminoindan (**8**) gave full substitution in drug discrimination assays using MDMA as the training drug, the isomeric **6** and its

6

7

8

aminoindan analogue did not have similar behavioral effects.[12] More importantly, acute administration of either **7** or **8** to rats did not lead to reductions in serotonin markers upon sacrifice at one week following drug administration.

These studies established for the first time that molecules could be developed that possessed MDMA-like effects in behavioral models, but which lacked serotonin neurotoxicity. In addition, they also established the active side chain conformation of phenethylamines for inducing these behavioral effects.

As a further test of the effect of tethering the side chain into an aminoindan, we investigated 5-iodo-2-aminoindan (IAI, **9**) as an analogue of para-iodoamphetamine (PIA, **10**).[26] In drug discrimination studies, both PIA and IAI completely substituted in either MDMA-trained or (+)-MBDB-trained animals. A single acute administration of PIA led to significant reductions in all three rat brain markers, serotonin, 5-HIAA, and number of [³H]-paroxetine binding sites one week later. IAI gave slight, but significant, reductions in number of cortical serotonin uptake sites and hippocampal serotonin level, but did not produce other significant changes. Thus, while the effects were not as dramatic as with the methylenedioxy compounds, the iodoaminoindan induced less neurotoxicity than did its amphetamine counterpart.

9

10

At this point, we felt that the structure-activity relationships were too narrowly constrained to amphetamine analogues with a para-halogen or a methylenedioxy substituent. We began to seek compounds that would expand the SAR and allow greater understanding of the mechanism of action of these substances, as well as the basis for their neurotoxicity.

Aromatic ring substituents

The methylenedioxy group of MDMA or MBDB itself cannot tolerate much modification. Addition of even a single methyl group to the carbon of the methylenedioxy moiety seems to destroy clinical activity[4] while the substitution on this carbon of two geminal groups abolishes activity *in vitro* and in *in vivo* animal models.[27] Effects on *in vitro* monoamine release are dramatically altered by repositioning the methylenedioxy group on the aromatic ring, adding another methoxy substituent in any position, expanding the methylenedioxy to an ethylenedioxy, or substituting one of the methylenedioxy oxygens with a sulfur

atom.[28] Thus, with the exception of para-halogen-substituted compounds, it seemed that very few aromatic substituent groups were tolerated within the structure-activity relationships of MDMA-like compounds.

However, Carlsson et al.[29] had shown that 4-methyl-meta-tyramine with an alpha-methyl (H77/77) or an alpha-ethyl (H75/12) had the ability acutely to deplete central dopamine, norepinephrine, and serotonin. These compounds seemed to have certain pharmacological similarities to MDMA and, although they were 3,4-disubstituted it was not with a methylenedioxy group. Furthermore, O-methylation of H77/77 gives a compound which could also be invisioned as the 2-desmethoxy analogue of the hallucinogenic amphetamine DOM 3. This substance had previously been shown to have effects in mice similar to DOM.[30] Thus, we prepared 3-methoxy-4-methylamphetamine (MMA, 11) and its enantiomers. In addition, we synthesized the 2-aminoindan analogue, 5-methoxy-6-methyl-2-aminoindan (MMAI, 12).[31]
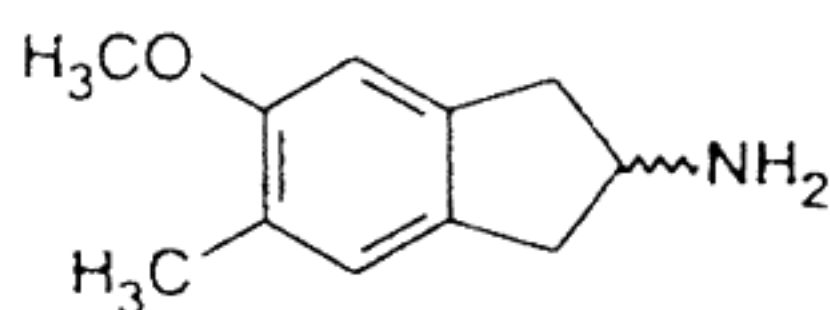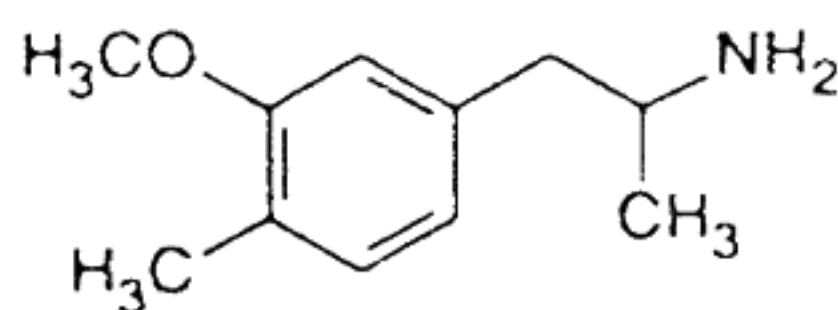
12   11

Racemic MMA proved to be as potent as MDMA or (+)-MBDB in drug discrimination assays in rats trained to discriminate MDMA or (+)-MBDB, respectively.[31] MMAI had nearly equal potency to MMA. Surprisingly, both enantiomers of MMA had nearly identical potencies, similar to the racemate, in substitution tests in (+)-MBDB-trained rats.

Perhaps most exciting was the finding that both 11 and 12 lacked serotonin neurotoxicity. Indeed, of all the compounds examined in our laboratory, 11 and 12 are the only ones to be given by the subacute dosing schedule (20 mg/kg given twice daily for four days) that produced absolutely no reduction in serotonin markers one week later. Yet, these are two of the most potent of the serotonin releasing agents we have identified. Furthermore, examination of brain monoamine levels 3 hours following drug administration showed that serotonin levels were depleted to approximately the same extent as after MDMA. It certainly seemed clear that serotonin release alone was not sufficient to induce neurotoxicity. The major difference in the pharmacology of MMAI, to be discussed below, seemed to be its relative lack of effect on catecholamine transporters.

We have also carried out preliminary studies on 5-methoxy-2-aminoindan, and 5-methoxy-6-bromo-2-aminoindan, where the aromatic methyl of 12 has been replaced with a hydrogen or bromine, respectively. The bromo compound was virtually equipotent to 12 in monoamine uptake inhibition studies, while the analogue possessing only the methoxy had about one-fourth the potency of 12 as an inhibitor of serotonin uptake into synaptosomes (unpublished data).

A final substituted compound that has been examined is P-methylthioamphetamine (MTA, 13). We reasoned that a larger, less electronegative group than a halogen to replace the chlorine of PCA might be interesting for study, and this led to the synthesis of 13. In vitro, this compound proved to be the most potent of all the serotonin releasing agents we had studied to date.[32] It also had good selectivity for the serotonin
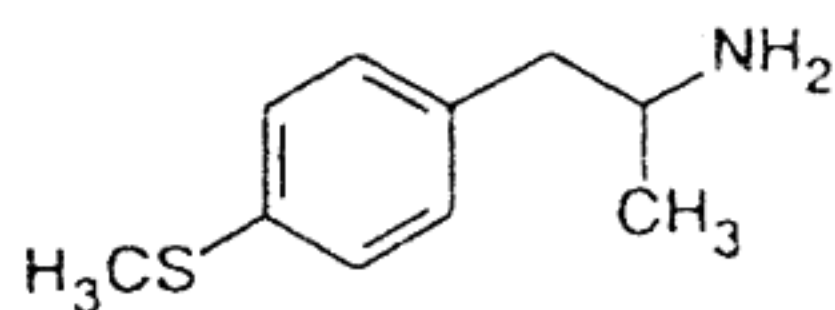
MTA

13

carrier over the other monoamine carriers, and lacked serotonin neurotoxicity after a single high dose. Drug discrimination studies in (+)-MBDB-trained animals showed it to be virtually equipotent to PCA, the most active of all the analogues previously studied. Figure 1 illustrates the efflux of radioactivity from superfused rat frontal cortex slices, preincubated with [^{3}H]-serotonin. It is evident from a comparison of the 10 micromolar efflux curves for both MTA and PCA that the two are virtually equipotent.

Serotonin neurotoxicity

Whilt it is not yet known whether all of these compounds possess actions at receptors or targets other than the monoamine uptake carriers, it was clear that the reported serotonin neurotoxicity of PCA and MDMA prevented investigations of their clinical potential. With our goal of developing a specific serotonin releasing agent, this was a serious toxicity problem to be avoided for any potential clinical candidates.

Initially, it was unclear whether serotonin release alone was sufficient to elicit serotonin neuron destruction. The evidence now suggests that serotonin release is probably a necessary, but not sufficient condition to induce neurotoxicity. There
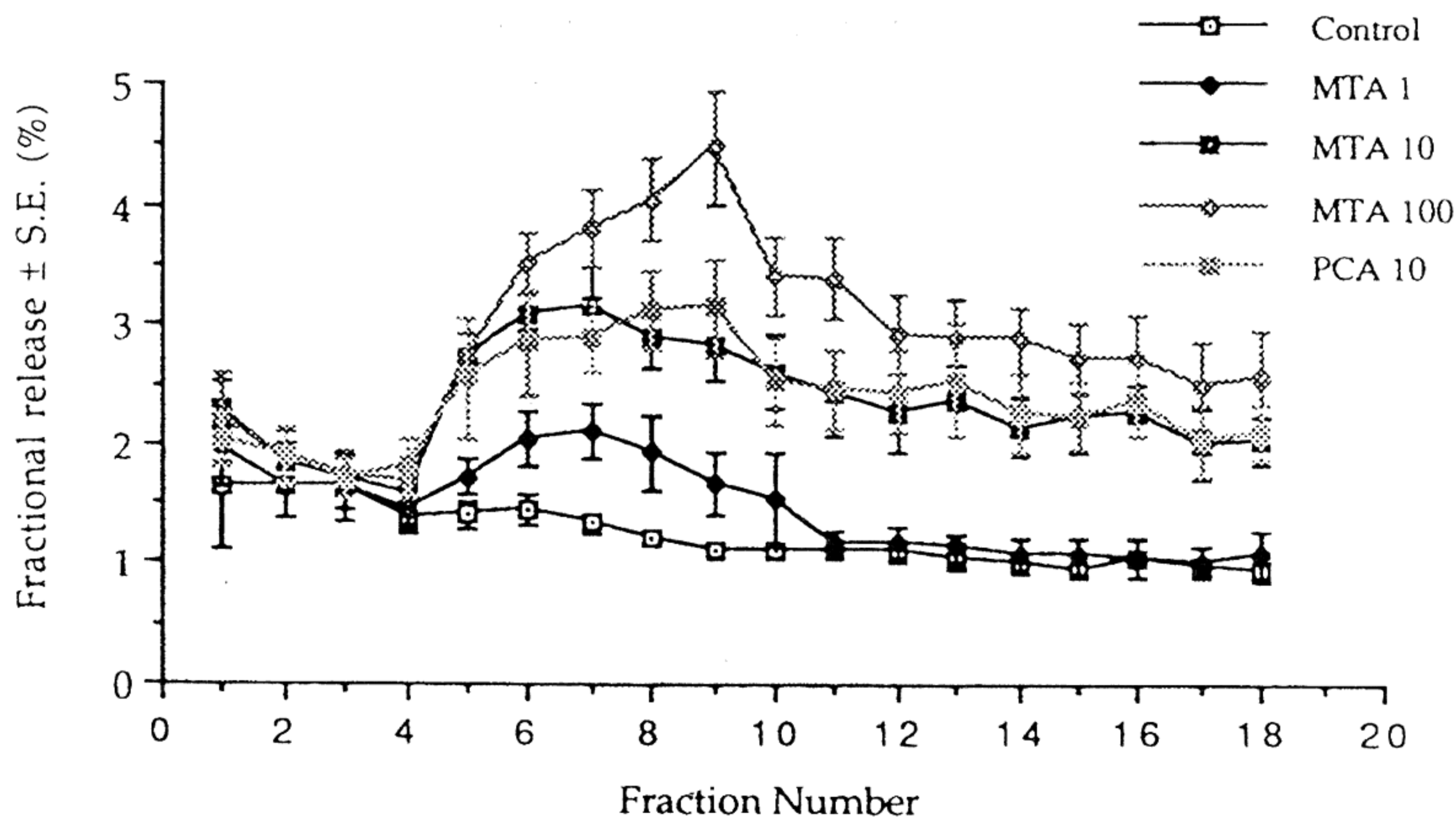

FIGURE 1 The fractional release of tritium from rat frontal cortex slices preloaded with 100 nM [^{3}H]-serotonin induced by 1, 10, and 100 micromolar p-methylthioamphetamine (MTA) and 10 micromolar PCA.

had been a good deal of preceding work, particularly by Gibb and coworkers, and by Schmidt and coworkers, indicating that the serotonin neurotoxicity induced by MDMA could be attenuated or blocked by pretreatments that depleted endogenous dopamine stores.[33,34] Conversely, MDMA neurotoxicity was exacerbated by treatments that enhanced endogenous dopamine synthesis.[35,36] These experiments, and others, suggested that catecholamines, probably dopamine, played a key role in the induction of neurotoxicity.

As we gathered data for the ability of the compounds we had prepared to inhibit monoamine uptake, or induce release either from synaptosomes or from superfused brain slices, it became clear that serotonin neurotoxicity was not correlated with ability to release serotonin, but rather seemed correlated with ability to interact with catecholamine uptake carriers. For example, in Table I are presented uptake inhibition data for the compounds that we have so far characterized.[37] Entries are ranked in approximate order of decreasing neurotoxic potential. The most potent serotonergic agent, methylthioamphetamine (MTA) lacks MDMA-like neurotoxicity, at least following a single high dose, yet it is equipotent to PCA as an inhibitor of serotonin uptake.

Similarly, MMA and MMAI are potent releasers of serotonin, are almost completely inert at the catecholamine uptake carriers, and completely lack serotonin neurotoxic effects. The hypothesis that the release of non-vesicular dopamine played a critical role in the expression of serotonin neurotoxicity led us to treat rats with non-neurotoxic doses of (+)-amphetamine combined with the non-neurotoxic analogues 7 or 12.[38,39] In both cases, the combined amphetamine-nontoxic analogue regimen led to severe deficits in central serotonin markers upon sacrifice at one week

TABLE I

Approximate potency to inhibit [^{3}H]-monoamines into rat whole brain synaptosomes. Values are arranged in order of decreasing ability to inhibit the uptake of [^{3}H]-dopamine. Values are taken from Johnson *et al.*[37]

Drug	IC$_{50}$ values (nM)			
	[^{3}H]-5-HT	[^{3}H]-DA	[^{3}H]-NE	Neurotoxicity*
PCA	182	424	207	+ +
PIA	82	589	993	+ +
MDA	478	890	266	+ +
IAI	241	992	612	+
MDMA	425	1442	405	+ +
CAB	330	2343	4885	+ +
MDE	313	2031	1674	+ / + +
MTA	74	3073	2375	?
6-CAT	120	3137	1262	?
MDAI	512	5920	1426	+
MBDB	784	7825	1233	+
MMA	136	16317	16293	—
MMAI	212	19793	11618	—
Fluoxetine[a]	52	2343	4885	—

*Neurotoxicity is indicated as follows: + + causes long term depletion of serotonin markers following a single, acute injection, + indicates long term depletion after subacute administration, but not after a single dose, ? indicates that no neurotoxicity occurs after a single dose, but subacute dosing has not been examined, and — indicates no serotonin deficits even after subacute administration.
[a]The selective serotonin uptake inhibitor Fluoxetine was included for comparison.

following drug administration. While not completely definitive proof, these studies certainly suggest that the serotonin neurotoxicity induced by PCA and MDMA is a consequence not only of serotonin release and depletion, but also of concomitant release of endogenous catecholamines. Of course, this implies that efforts to develop selective serotonin releasing agents free of effects on catecholamine neurons will ultimately lead to compounds also lacking serotonin neurotoxicity.

Mechanism of action

As can be deduced from the foregoing discussion, the mechanism of the behavioral effects of MDMA is complex.[40,41] Not only does MDMA induce the release of neuronal serotonin, but it also releases dopamine[7,9,42,43] and inhibits the reuptake of norepinephrine and/or induces neuronal norepinephrine release. However, as this discussion has proceeded, it should now also be clear that some of the structures, particularly MTA and MMAI, are relatively specific serotonin releasing agents. Rudnick and Wall[44] have recently reported that the serotonin releasing action of MDMA, at least in membrane vesicles from bovine adrenal chromaffin granules, can probably be attributed to combined effects of dissipation of the transmembrane pH difference generated by ATP hydrolysis and to a direct interaction with the vesicular amine transporter. The latter was suggested to possibly involve serotonin-MDMA exchange. Recently, Rudnick (personal communication) has carried out a similar study with MMAI and has concluded that it releases serotonin by a mechanism identical to that described for MDMA. Thus, MMAI retains the serotonin releasing component of action displayed by MDMA or PCA, but lacks the range of other pharmacological interactions that characterize the latter.

In rat brain slice superfusion studies, the serotonin uptake inhibitor Fluoxetine antagonized the ability of MMAI to induce the release of [³H]-serotonin from prelabeled slices (Figure 2). This is consistent with the proposal of Rudnick. However, in contrast to fluoxetine, paroxetine, or the tricyclics, which act by inhibiting the reuptake of synaptic serotonin, the compounds developed in our laboratory probably displace intraneuronal serotonin, resulting in reversal of the uptake process and leading to increased intrasynaptic serotonin concentrations. The consequences of both types of actions in the synapse would seem to be similar—an increase in serotonergic transmission.

The conclusion that the behavioral effects of these drugs are mediated by serotonin release is consistent with the data presented in Table II. In contrast to the wide range of activities of these compounds to interact with the dopamine or norepinephrine carriers (Table I) their potencies to interact with the serotonin carrier lie within one order of magnitude and are generally correlated with potencies obtained in the drug discrimination assay, shown as ED_{50} values.

Having established that these compounds were potent and selective serotonin releasing agents, it seemed essential to determine whether they might interact with other neurotransmitter or neuromodulator receptors. Table III illustrates that a prototype compound, MMAI (**12**) had low affinity for a variety of other receptor sites. Affinity at the dopamine D_2 receptor labeled by sulpiride in rat striatal tissue was highest, with the 10 micromolar concentration of MMAI giving a mean displacement of 59% of the radioligand in two experiments with duplicate determinations. Nevertheless, this must be considered relatively low affinity. We are still awaiting the results of data for binding to the alpha and beta adrenergic receptors, so it is still possible that this compound may exert some effect at these sites.
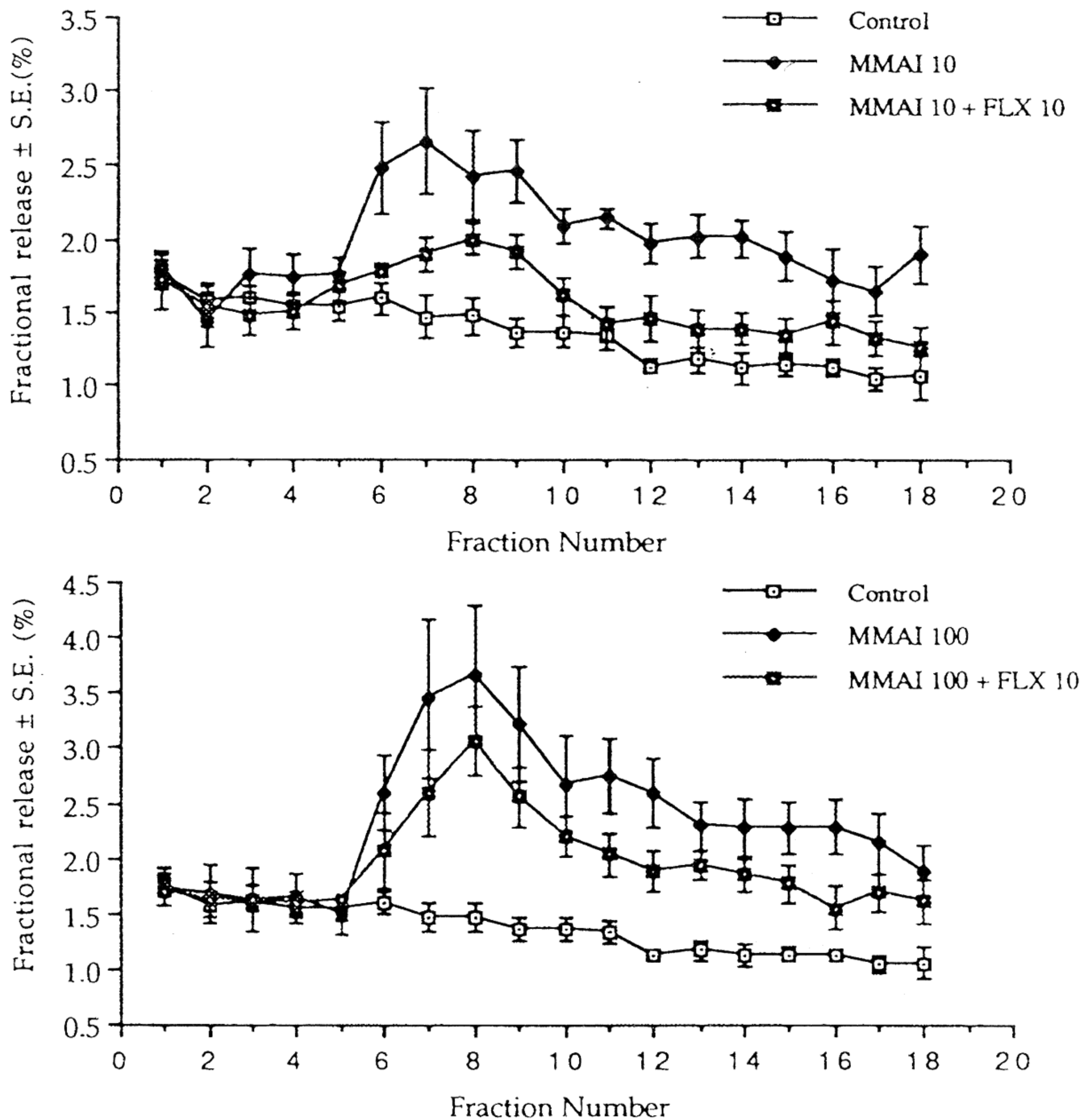
FIGURE 2   The fractional release of tritium from rat frontal cortex slices preloaded with 100 nM [^{3}H]-serotonin induced by 10, and 100 micromolar 5-methoxy-6-methyl-2-aminoindan (MMAI) in the absence or presence of 10 micromolar fluoxetine, a serotonin uptake inhibitor.

METHODS

Affinity for other neurotransmitter receptors

The radioligand receptor binding assays were performed by the Nova Pharmaceutical Corporation using their PROFILE Novascreen program, as generously provided by a contract with the National Institute of Mental Health. MMAI was screened at a concentration of 10 micromolar against the ligands listed in Table III. With several ligands, some competition ($<25\%$) was seen, but in only one case (the D_2 receptor) did competition reach 50%. It was therefore estimated that the IC_{50} was greater than 10 micromolar for all but this ligand.

TABLE II
Comparison of potency in drug discrimination, in rats trained to discriminate saline from (+)-MBDB, and ability to inhibit the uptake to [^{3}H]--serotonin into rat whole brain synaptosome

Drug	IC$_{50}$ (nM)	ED$_{50}$ (μM/kg)
PCA	184	0.82
MTA	74	0.83
6-CAT	120	1.40
PIA	82	1.82
MDAI	512	2.04
MDA	478	2.07
MMA	136	2.16
MMAI	212	2.63
IAI	241	2.67
MBDB	784	3.25
MDMA	425	3.38

Serotonin release experiments

Rat frontal cortex was chopped coronally into 0.3 mm slices. The slices were immediately transferred into O$_2$-saturated Krebs-Henseleit buffer (pH 7.4) containing 100 μM pargyline. After incubation with [^{3}H]-serotonin (100 nM) for 30 min at 37°C, one slice was placed into each of 12 superfusion chambers (Brandel, Gaithersburg, MD). The slices were superfused at a rate of 0.5 ml/min with fresh oxygenated modified Krebs-Henseleit buffer (mM: 118 NaCl, 4.8 KCl, 1.2 MgSO$_4$, 25 NaHCO$_3$, 1.2 KH$_2$PO$_4$, 12 glucose, 0.01 ascorbic acid, 0.03 Na$_2$EDTA, and 0.1 pargyline). After a 40 min wash, serial 2 min fractions were collected. Buffer solution containing test compound was introduced into the superfusion buffer for 4 min, during the collection of fractions 5 and 6. At the end of the experiment, 5 ml of Ecolite Scintillation Cocktail (ICN, Cleveland, OH) was added to all fractions of superfusate. Slices were recovered and transferred to vials containing 5 ml of scintillation cocktail. After vigorous shaking, radioactivity was counted with a Packard Scintillation Counter.

Tritium efflux into the superfusate was calculated as the percentage of the tritium content of the slice at the beginning of the collection of that fraction. Each point in the tritium efflux curve is the average of five experiments, performed on five different days.

CONCLUSIONS

We believe that these novel agents will eventually find an appropriate therapeutic niche. Based on the indications for the use of serotonin uptake inhibitors, one might anticipate utility for serotonin releasing agents in depression, panic and anxiety disorders, obsessive-compulsive disorders, and perhaps as appetite suppressants.

TABLE III
Affinity of MMAI for other receptor sites[a]

Receptor	Selectivity	Radioligand	IC_{50} (μM)
Adenosine	Adenosine	$[^3H]$-NECA	>10
Dopaminergic	Dopamine	$[^3H]$-SCH23390	>10
	Dopamine 2	$[^3H]$-sulphide	=10
Excitatory amino acids	Glycine	$[^3H]$-glycine	>10
	Kainate	$[^3H]$--kainic acid	>10
	MK-801	$[^3H]$-MK-801	>10
	NMDA	$[^3H]$-CGS 19755	>10
	PCP	$[^3H]$-TCP	>10
	Quisqualate	$[^3H]$-AMPA	>10
Inhibitory amino acids	Benzodiazepine	$[^3H]$-flunitrazepam	>10
	GABA$_A$	$[^3H]$--GABA	>10
	GABA$_B$	$[^3H]$--GABA + isoguavacine	>10
	Glycine	$[^3H]$-strychnine	>10
Serotonergic	Serotonin 1	$[^3H]$-serotonin	>10
	Serotonin 2	$[^3H]$-ketanserin	>10
Channel proteins	Calcium	$[^3H]$-nitrendipine	>10
	Calcium	$[^{125}I]$-ω-conotoxin	>10
	Chloride	$[^3H]$-TBOB	>10
	Potassium	$[^{125}I]$-apamin	>10
Peptide factors	ANF1	$[^{125}I]$-ANP	>10
	EGF	$[^{125}I]$-EGF	>10
	NGF	$[^{125}I]$-NGF	>10
Peptides	Angiotensin	$[^{125}I]$-angiotensin II	>10
	Arg-vasopressin V$_1$	$[^{125}I]$-manning cmpd	>10
	Bombesin	$[^{125}I]$-GRP	>10
	CCK central	$[^{125}I]$-CCK	>10
	CCK peripheral	$[^{125}I]$-CCD	>10
	Neurotensin	$[^3H]$--neurotensin	>10
	NPY	$[^{125}I]$-NPY	>10
	Somatostatin	$[^{125}I]$-somatostatin	>10
	Substance K	$[^{125}I]$-neurokinin A	>10
	Substance P	$[^3H]$--substance P	>10
	VIP	$[^{125}I]$-VIP	>10
Second messenger systems:			
Adenylate cyclase	Forskolin	$[^3H]$--forskolin	>10
Protein kinase C	Phorbol ester	$[^3H]$--PDBU	>10
	Inositol triphosphate	$[^3H]$--IP$_3$	>10

[a] See Methods for details of the screening of MMAI in these assays.

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