Pharmacology Biochemistry and Behavior, Vol. 59, No. 3, pp. 709–715, 1998

0091-3057/98 $19.00 + .00

ELSEVIER

PII S0091-3057(97)00557-1

Indan Analogs of Fenfluramine and Norfenfluramine Have Reduced Neurotoxic Potential

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Received 21 March 1997; Revised 25 July 1997; Accepted 20 August 1997

COZZI, N. V., S. FRESCAS, D. MARONA-LEWICKA, X. HUANG AND D. E. NICHOLS. *Indan analogs of fenfluramine and norfenfluramine have reduced neurotoxic potential.* PHARMACOL BIOCHEM BEHAV **59**(3) 709–715, 1998.—*N*-Ethyl-5-trifluoromethyl-2-aminoindan (ETAI) and 5-trifluoromethyl-2-aminoindan (TAI) were synthesized to examine the effects of side-chain cyclization on the pharmacology of the anorectic drugs fenfluramine (FEN) and norfenfluramine (norFEN), respectively. ETAI and TAI inhibited synaptosomal accumulation of 5-HT but were less effective at inhibiting catecholamine uptake than FEN or norFEN, respectively. In vivo, ETAI and TAI were less neurotoxic than FEN or norFEN; decreases in the number of [^{3}H]paroxetine-labeled 5-HT uptake sites were 50% less than the decreases produced by FEN or norFEN. Rats treated with ETAI, TAI, FEN, and norFEN lost 10–15% of their pretreatment body weight over a 4-day period, while saline-treated control animals gained 8%. In two-lever drug discrimination (DD) assays in rats, TAI fully substituted for the 5-HT releaser/uptake inhibitor, (+)-MBDB [(+)-*N*-methyl-1-(1,3-benzodioxol-5-yl)-2-aminobutane]. ETAI produced only partial substitution in this test. Neither TAI nor ETAI mimicked (+)-amphetamine in the DD assay. These studies demonstrate that incorporation of the side-chain of phenylisopropylamines into the five-membered ring of a 2-aminoindan changes both the molecular pharmacology and the neurotoxic profile of FEN and norFEN, but does not diminish the drugs' ability to reduce body weight. © 1998 Elsevier Science Inc.

Fenfluramine Norfenfluramine Neurotoxic Serotonin Catecholamine Anorectic Drug discrimination Synaptosomes

FENFLURAMINE (FEN) is a ring-substituted phenylisopropylamine (Fig. 1) that has been used for many years in Europe as an adjunct to dietary restrictions for the clinical treatment of obesity (8,36,38). The dextro isomer of fenfluramine (Redux®) has recently been approved by the Food and Drug Administration for use in the United States. The actions of fenfluramine and its *N*-dealkylated metabolite, norfenfluramine (norFEN) (Fig. 1) appear to be mediated primarily through the release and inhibition of reuptake of serotonin (5-HT) from central neuron terminals (12–16,40). It has been suggested (15) that fenfluramine modifies feeding via the serotonergic system, and it is thought that the paraventricular nucleus is the site of action for the anorectic actions of 5-HT agonists (3). Nevertheless, Olyomi et al. (35) showed that the anorectic properties of fenfluramine and norfenfluramine are not dependent on increased 5-HT release in the hypothalamus.

Recently, considerable evidence has accumulated that fenfluramine produces long-lasting central serotoninergic deficits (1,5,9,20,23,37,39,41). The mechanism of fenfluramine-induced serotonergic neurotoxicity is unknown, but the pattern of degeneration is similar to that produced by other neurotoxic phenylisopropylamine derivatives such as *para*-chloroamphetamine, 3,4-methylenedioxyamphetamine, and *N*-methyl-3,4-methylenedioxyamphetamine (37). Nevertheless, several lines of evidence suggest that the neurotoxic mechanism of fenfluramine is different from that of *para*-chloroamphetamine and

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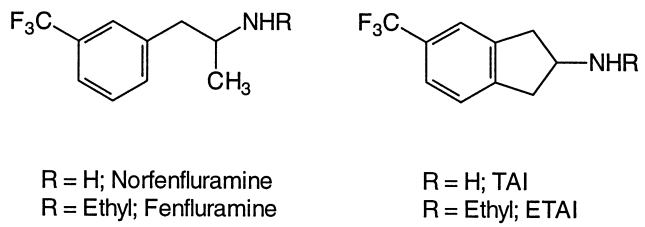

FIG. 1. Chemical structures of fenfluramine, norfenfluramine, and aminoindans.

3,4-methylenedioxymethamphetamine (6,9,28). The modest effects of fenfluramine on the dopaminergic system have always been difficult to reconcile with the suggestion that dopamine (DA) release is an obligatory component of the serotonergic neurotoxicity of amphetamines. However, De Deurwaerdere et al. (7) demonstrated recently that *d*-fenfluramine is able to enhance striatal DA outflow in vivo by a Ca^{2+}-dependent and tetrodotoxin-sensitive mechanism, which appears to be independent of striatal 5-HT terminals as well as from dopamine uptake sites.

Previous work by Fuller et al. (10,11) and Nichols et al. (30,31) demonstrated that indan and tetralin analogs of phenylisopropylamine-based neurotoxins lacked serotonergic neurotoxicity with no diminution in behavioral effects. These compounds are serotonin releasing agents, and the most selective nonneurotoxic compound reported to date is 5-methoxy-6-methyl-2-aminoindan (MMAI) (22). This compound produces behavioral effects (e.g., drug discrimination; conditioned place aversion; food intake) very similar to those of fenfluramine (25,26), but does not elevate extracellular levels of dopamine. In addition, subacute coadministration of amphetamine and MMAI to rats induced serotonergic neurotoxicity (21). This strongly suggests that increased DA function is somehow involved in the mechanism of serotonergic neurotoxicity, and that this effect can be selectively attenuated by appropriate chemical structure modifications.

By analogy, it was hypothesized that the cyclic homologue of fenfluramine, *N*-ethyl-5-trifluoromethyl-2-aminoindan (ETAI) and its *N*-dealkylated derivative, 5-trifluoromethyl-2-aminoindan (TAI) (Fig. 1), would retain the 5-HT–releasing, uptake blocking, and anorectic properties of fenfluramine and norfenfluramine. It was further hypothesized that the new indan compounds would be less effective than the respective phenylisopropylamines as inhibitors of DA uptake or as DA releasing agents, and would therefore, exhibit decreased neurotoxicity. Therefore, ETAI, TAI, FEN, and norFEN were synthesized and examined for their effects on monoamine neurotransmitter uptake and release in synaptosome preparations and for their discriminative cue properties in rats trained to discriminate various drugs from saline. The test drugs were also evaluated for their effects on [^{3}H]paroxetine binding site density as a marker for serotonergic neurotoxicity and for their effects on body weight as an indication of anorectic potential.

METHOD

Animals

Male Sprague–Dawley rats (175–200 g initial weight; 46–49 days old) were obtained from Harlan Labs, Indianapolis, IN. The animals for in vitro experiments were group-housed under a 12 L:12 D schedule and received food (Lab Blox, Purina) and water ad lib. Animals which were used in drug discrimination experiments were individually caged and received an amount of food sufficient to maintain them at approximately 80% of their free-feeding weight; water was available ad lib.

Drugs

ETAI, TAI, FEN, and norFEN were synthesized in our laboratory; all analytical data were consistent with the predicted structures. (+)-*N*-methyl-1-(1,3-benzodioxol-5-yl)-2-aminobutane hydrochloride [(+)-MBDB] and 5-methoxy-6-methyl-2-aminoindan hydrochloride (MMAI) were synthesized as previously described (22,29). D-lysergic acid diethylamide tartrate (LSD) was obtained from the National Institute on Drug Abuse. All other compounds were obtained from commercial sources.

Inhibition of [^{3}H]Neurotransmitter Uptake

Crude synaptosomes were prepared according to the method of Gray and Whittaker (19), with slight modifications. For neurotransmitter uptake experiments, three rats were decapitated and their brains rapidly removed and dissected over ice. The cerebellums were removed and discarded and the remaining brain tissue (ca. 4 g, wet weight) was pooled, diced, and homogenized in 20 ml of ice-cold 0.32 M sucrose. Homogenizations were done in a prechilled Potter-Elvehjem tissue grinder with a motor-driven Teflon pestle at 0°C, for two periods of 1 min each, six strokes/min, with a 15-s interval between periods. The tissue homogenate was subjected to centrifugation (Beckman J2-21 with JA-20 rotor; 4°C) at $1090 \times g$ for 10 min. The pellet was discarded and the supernatant was subjected to centrifugation at $17{,}400 \times g$ for 30 min. The resulting pellet was resuspended with a polytron (setting 5, 20 s; Kinematica) in 30–40 ml ice-cold, aerated (5% CO_2 in O_2) modified Krebs-Ringer bicarbonate (KR) buffer containing (mM): NaCl (124.3), KCl (2.95), $MgSO_4$ (1.30), KH_2PO_4 (1.25), $NaHCO_3$ (26.0), $CaCl_2$ (2.41), *d*-glucose (10.4), and Na ascorbate (0.06), pH 7.4–7.6. The synaptosomal suspension was stored on ice until use.

The ability of synaptosomes to accumulate tritiated serotonin ([^{3}H]5-HT), dopamine ([^{3}H]DA), and norepinephrine ([^{3}H]NE) was measured in the absence and presence of various concentrations of test drugs as follows: a 200-μl aliquot of the synaptosomal suspension was added to test tubes containing 1.65 ml ice-cold KR buffer, 50 μl test drugs (dissolved in deionized water) or deionized water (for total and nonspecific determinations), and 50 μl pargyline HCl solution (final concentration, 100 μM). The test tubes were preincubated in an aerated (5% CO_2 in O_2) 37°C shaking water bath for 5 min. The tubes were then returned to the ice bath and chilled for 10–15 min. Tritiated neurotransmitter (New England Nuclear) was added (50 μl of stock solution; final concentration, 10 nM), giving a final incubation volume of 2 ml. All tubes except nonspecific assays were returned to the aerated 37°C shaking water bath for 5 min to initiate neurotransmitter uptake. Uptake was terminated by chilling the test tubes in an ice bath, then rapidly filtering them through glass fiber filters (Whatman GF/C) pretreated with 0.05% polyethylenimine using a 24-well cell harvester (Brandel). Filters were washed with 2×3 ml ice-cold KR buffer, allowed to air dry for 10 min, and then placed in plastic liquid scintillation vials. Scintillation cocktail (10 ml Ecolite; ICN Biomedicals) was added, and the vials were sealed, vortexed, and allowed to stand

overnight. Radioactivity was measured using liquid scintillation spectroscopy (Packard model 4430). Specific uptake was defined as uptake at 37°C minus uptake at 0°C, in the absence of drugs. Under these conditions, specific uptake represented greater than 74% of the total uptake of [^{3}H]5-HT, greater than 91% of the total uptake of [^{3}H]DA, and greater than 85% of the total uptake of [^{3}H]NE. Drugs were screened for [^{3}H]5-HT, [^{3}H]DA and [^{3}H]NE uptake inhibition at 25 μM. If a drug displayed greater than 50% inhibition of neurotransmitter accumulation at 25 μM, the IC_{50} was determined from displacement curves using eight concentrations of the drug. IC_{50} values were calculated from at least three experiments, each done in triplicate.

Drug-Evoked [^{3}H]5-HT Release

Synaptosomes preloaded with [^{3}H]5-HT were superfused with KR buffer in the absence and presence of three concentrations of ETAI, TAI, FEN, and norFEN to determine whether the drugs stimulated the release of serotonin. Each experiment was performed in triplicate. Because the released neurotransmitter is continually swept away in a superfusion experiment, reuptake is minimized, and any increase in radioactivity in the superfusate can be ascribed to evoked release, not uptake inhibition. For superfusion experiments, synaptosomes were prepared as described above, except only one rat was used for each experiment and the synaptosomal pellet was resuspended in 10 ml aerated (5% CO_2 in O_2) 37°C KR buffer containing 100 μM pargyline HCl. [^{3}H]5-HT (1 μCi/ml, final concentration ca. 25 nM) was added to the synaptosomal suspension, which was then incubated and aerated at 37°C for 20 min to allow neurotransmitter uptake. After the labeling incubation, 250 μl aliquots of the synaptosomal suspension were transferred to each of 12 superfusion chambers of a superfusion apparatus (Brandel model SF12). The synaptosomes were superfused with KR buffer (0.5 ml/min) for a 20 min washout period to achieve a basal level of spontaneous neurotransmitter release. Following the washout period, 10 serial 2 min (1.0 ml) superfusate fractions were collected directly into plastic scintillation vials. At the end of the experiment the glass fiber filters containing the synaptosomes were also placed into scintillation vials containing 1.0 ml KR buffer. Scintillation cocktail (5 ml) was added to all the vials and the vials were sealed and vortexed. Radioactivity was measured using liquid scintillation spectroscopy.

To test the effect of drugs on spontaneous neurotransmitter release, various concentrations of drugs were introduced to some of the superfusion chambers during fractions 3 and 4. The amount of tritium released was compared to the amount released in the absence of drugs and is expressed as percent released. Percent released for any fraction is calculated by dividing the amount of tritium released during that fraction by the total synaptosomal tritium present at the start of that fraction collection period and multiplying by 100. The synaptosomal tritium present at the start of a collection period is the sum of the tritium released during that collection period, all subsequent collection periods, and the glass fiber filter tritium content at the end of the experiment.

Drug Effects on Body Weight

Some animals were treated with ETAI, TAI, FEN, and norFEN to determine the effects of these drugs on body weight. Rats were randomly assigned to one of five treatment groups, with eight animals per group. Rats were weighed on the first day of the experiment and were then injected intraperitoneally with 10 mg/kg of test drugs or saline twice a day for 4 days. On the fourth day the animals were weighed again. The difference between these two weighings was used to determine the change in body weight.

Drug Effects on [^{3}H]Paroxetine Binding

The same groups of animals that were tested for changes in body weight were also assessed for changes in the density of brain [^{3}H]paroxetine binding sites. Ten days after the last drug or saline injection, these animals were sacrificed and cortical, hippocampal, and neostriatal tissues were assayed for specific [^{3}H]paroxetine binding using a modified procedure of Battaglia et al. (2) with a single-point determination at a saturating concentration of [^{3}H]paroxetine. Nonspecific binding was defined with 1 μM fluoxetine. Briefly, brain tissues were thawed, weighed, and homogenized in 5 ml TNC buffer (Tris HCl 50 mM, NaCl 120 mM, KCl 5 mM) with a Brinkman polytron (setting 6, 2 × 2 s). The homogenates were subjected to centrifugation twice at 30,000 × *g* for 10 min with an intermittent buffer wash and were then resuspended in the same volume of assay buffer. Incubations were initiated by the addition of 150 μl of tissue suspension to 1.50 ml of the TNC buffer containing [^{3}H]paroxetine to give a total volume of 1.65 ml. The final [^{3}H]paroxetine concentration was 1 nM. The tubes were allowed to equilibrate at 24°C for 1 h before adding 4 ml of ice-cold buffer and vacuum filtration through Whatman GF/C filters presoaked in 0.05% polyethylenimine. The tubes and filters were then washed twice with 4 ml of ice-cold buffer and the filters were allowed to air dry before placing them into scintillation vials. Scintillation cocktail (10 ml) was added into each vial. The vials were sealed and allowed to sit overnight before counting at an efficiency of 37–55%.

Drug Discrimination Studies

The procedure and equipment employed have been described in detail (25,33). Briefly, rats (10–15 per group) were trained to discriminate either (+)-amphetamine sulfate (1 mg/kg), (+)-MBDB hydrochloride (1.75 mg/kg), MMAI hydrochloride (1.71 mg/kg), or *d*-LSD tartrate (0.08 mg/kg) from 0.9% saline using a fixed ratio 50 (FR 50) schedule of food reinforcement. Intraperitoneal injections were given 30 min prior to sessions. Test sessions were separated by at least one drug and one saline training session. To receive a test drug, animals were required to maintain the 85% correct response criterion on training days. Test sessions ended after 5 min or when 50 responses were made on either lever, whichever came first. If a rat did not emit 50 presses on either lever in 5 min, the test session was ended and the rat was scored as disrupted; those data were not used for subsequent calculations.

Data Analysis

Data for all experiments except drug discrimination studies are expressed as the mean ± standard error of the mean (SEM). Multiple comparisons of the drugs' ability to inhibit neurotransmitter uptake (IC_{50}) were made using one-way analysis of variance (ANOVA) and a post hoc Tukey–Kramer *t*-test. Data from the [^{3}H]paroxetine binding experiments were analyzed using one-way ANOVA followed by a Dunnett's *t*-test. For drug discrimination experiments, the lever upon which a rat first completed 50 responses during a test session was scored as the "selected" lever. The percentage of rats selecting the drug lever (%SDL) was determined for each dose of test compound, and the degree to which a test drug

TABLE 1

IC_{50} VALUES FOR INHIBITION OF MONOAMINE UPTAKE*

Compound	IC_{50} (μM)		
	[^{3}H]5-HT	[^{3}H]DA	[^{3}H]NE
FEN	0.490 ± 0.017	21.5 ± 0.85	8.28 ± 1.4
NorFEN	0.590 ± 0.062	5.41 ± 0.92‡	2.75 ± 0.22¶
ETAI	2.61 ± 0.34†	>25	>25
TAI	0.604 ± 0.066	17.8 ± 0.041§	7.41 ± 0.96

*The ability of the test compounds to inhibit accumulation of monoamines was examined in crude synaptosomes. For IC_{50} determination, compounds were examined at eight concentrations, each run in triplicate. The data from these experiments were combined and fitted to a four-parameter logistic curve from which IC_{50} values ± SEM were calculated. Multiple comparisons were made using ANOVA followed by a Tukey–Kramer *t*-test.

†$p < 0.001$ vs. FEN, norFEN, TAI.

‡$p < 0.001$ vs. FEN, TAI.

§$p < 0.05$ vs. FEN.

¶$p < 0.05$ vs. FEN, TAI.

substituted for a training drug was determined by the maximum percentage of rats selecting the drug lever over all doses of the test drug. Thus, "no substitution" (NS) is defined as 59% or less SDL, "partial substitution" (PS) is 60–79% SDL. If the drug was one that completely substituted for the training drug (at least one dose resulted in a %SDL = 80 or higher) the ED_{50} value and 95% confidence intervals (95% C.I.) were calculated from quantal dose–response curves according to the method of Litchfield and Wilcoxon (24). If 50% or more of the test animals were scored as disrupted, then no ED_{50} was calculated, regardless of the %SDL in non-disrupted animals.

RESULTS

The test compounds exhibited single-site displacement curves for monoamine uptake, with slope coefficients not different from unity (data not shown). All of the test drugs were potent inhibitors of [^{3}H]5-HT accumulation into synaptosomes in the uptake assay (Table 1). FEN, norFEN, and TAI inhibited [^{3}H]5-HT uptake with similar IC_{50}s, in the range of 500–600 nM. ETAI was four- to fivefold less potent, with an IC_{50} of 2.6 μM.

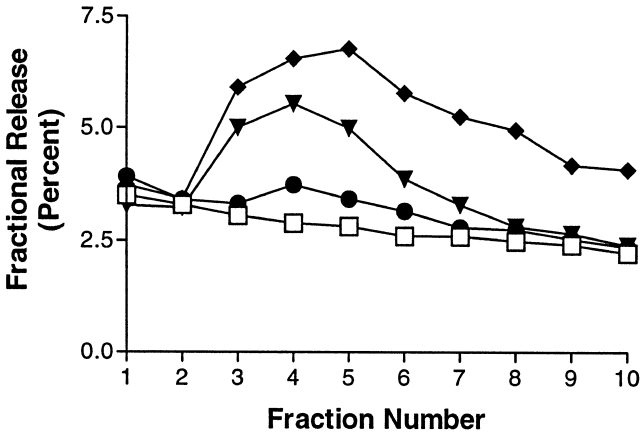

FIG. 2. Effect of FEN on synaptosomal [^{3}H]5-HT release. Crude synaptosomes were prepared and superfused with various concentrations of fenfluramine during fractions 3 and 4. Symbols represent: □ no drug control; ● 1 micromolar FEN; ▼ 10 micromolar FEN; ◆ 100 micromolar FEN. Data were transformed from dpm to percent tritium released; each point is the mean of three determinations, each run in triplicate. SEM not shown for clarity, 10%.

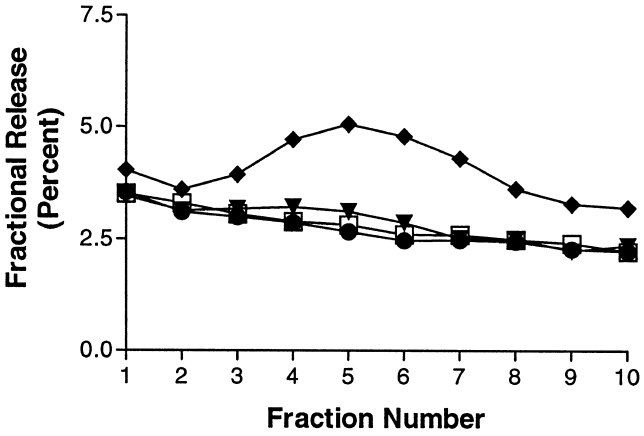

FIG. 3. Effect of ETAI on synaptosomal [^{3}H]5-HT release. Crude synaptosomes were prepared and superfused with various concentrations of ETAI during fractions 3 and 4. Symbols represent: □ no drug control; ● 1 micromolar ETAI; ▼ 10 micromolar ETAI; ◆ 100 micromolar ETAI. Data were transformed from dpm to percent tritium released; each point is the mean of three determinations, each run in triplicate. SEM not shown for clarity, 10%.

The indan compounds were about threefold less potent at inhibiting [^{3}H]DA and [^{3}H]NE uptake than the respective phenylisopropylamine homologues. The presence of the *N*-ethyl moiety decreased potency three- to fourfold relative to the primary amine compounds (Table 1).

The experiments using superfused synaptosomes revealed that TAI, FEN, and norFEN depressed 5-HT accumulation mainly by evoking the release of 5-HT (illustrated for FEN in Fig. 2), whereas ETAI was only active at the highest concentration (Fig. 3); at 100 mM ETAI also stimulated 5-HT release.

The results of the [^{3}H]paroxetine binding experiments revealed that all of the test drugs caused some reduction in the number of binding sites, but that FEN and norFEN were the most potent compounds. FEN and norFEN each re-

TABLE 2

DRUG EFFECTS ON SPECIFIC [^{3}H]PAROXETINE BINDING SITE DENSITY*

Treatment	Cortex	Hippocampus	Neostriatum
Saline	100.0 ± 2.8	100.0 ± 4.0	100.0 ± 10.8
FEN	39.7 ± 2.6†	30.8 ± 6.5†	43.7 ± 5.4†
norFEN	30.4 ± 4.4†	34.9 ± 4.3†	43.7 ± 2.2†
ETAI	69.4 ± 3.6†	63.5 ± 4.9†	79.7 ± 6.8
TAI	65.9 ± 3.2†	70.0 ± 6.5†	75.4 ± 7.5

*The density of specific [^{3}H]paroxetine binding sites in cortex, hippocampus, and neostriatum was assessed 10 days after a 4-day regimen of drug (2 × 10 mg/kg/day) or saline treatment. Densities are expressed as percent of saline-treated controls. $n = 8$ per treatment.

†$p < 0.01$ vs. saline.

TABLE 3

DRUG DISCRIMINATION DATA FOR ETAI AND TAI*

Training Drug	Test Drug	Dose, mg/kg	*n*	%SDL†	%Disrupted	Level of Substitution‡
MMAI	ETAI	0.25	9	0	0	NS
		0.5	8	38	0	
		1.0	12	18	8	
		2.0	9	17	33	
		4.0	9	33	66	
	TAI	0.5	10	11	10	NS
		1.0	11	22	18	
		2.0	11	25	64	
S-MBDB	ETAI	0.5	12	40	18	PS
		1.0	15	31	13	
		2.0	14	70	29	
		4.0	14	25	71	
	TAI	0.25	21	16	9	ED_{50} =
		0.5	18	50	11	0.56 mg/kg
		1.0	16	64	12.5	
		2.0	13	100	31	
AMP	ETAI	0.5	7	0	0	NS
		1.0	7	0	14	
		2.0	7	0	29	
		4.0	7	0	43	
	TAI	0.5	7	16	14	NS
		1.0	7	0	71	
		2.0	7	33	57	
LSD	ETAI	0.5	12	33	0	PS
		1.0	12	45	8	
		2.0	12	62.5	33	
	TAI	0.25	10	40	0	NS
		0.5	16	38	0	
		1.0	14	58	14	
		2.0	15	33	60	

*Rats were trained to discriminate training drugs from saline. ETAI and TAI were administered at the indicated doses to test whether they substituted for training drugs.

†Percent selecting drug lever.

‡See the Method section for level criteria.

duced the density of binding sites by 60–70% ($p < 0.01$) in cortex, hippocampus, and neostriatum (Table 2). ETAI and TAI were about half as effective at reducing the number of binding sites in cortex and hippocampus on a mg/kg basis than were FEN and norFEN, producing only 30–35% decreases ($p < 0.01$) in binding site density (Table 2). There were also regional differences observed in striatal tissue; although the density of [^{3}H]paroxetine binding appeared to be less than controls for ETAI and TAI, this decrease was not significant.

In drug discrimination studies, TAI fully substituted and ETAI partially substituted for (+)-MBDB (Table 3). TAI had an ED_{50} of 0.56 mg/kg (95% confidence interval 0.38–0.79 mg/kg) in this assay. ETAI partially substituted for *d*-LSD. Neither ETAI nor TAI substituted for MMAI or for (+)-amphetamine at any dose tested.

All of the test compounds were equally effective at reducing body weight over a 4-day period compared to saline-treated controls (Table 4). In animals treated with the test drugs, there was a 10–15% reduction in body weight over the 4-day period. In contrast, saline-treated control animals gained about 8% over their beginning weight in the same time.

DISCUSSION

These studies demonstrate that selectivity of drug interactions with serotonergic and catecholaminergic neurons can be modulated by constraining the geometry of the alkylamine side chain of phenylisopropylamine-based drugs. This confirms and extends previous work with various substituted aminoindans (30,31,34). As hypothesized, the new drugs were selective for 5-HT uptake carriers. Generally, when the α-methyl group of the amphetamine molecule is tethered back to the aromatic ring, as in 2-aminoindan, catecholamine releasing/uptake blocking properties are significantly decreased. Thus, TAI and norFEN possessed similar potency at the serotonergic uptake carrier, but TAI was only one-third as potent at inhibiting catecholamine uptake. The new indans were less effective at inducing serotonergic deficits than FEN and norFEN. While both FEN and norFEN reduced the number of [^{3}H]paroxetine binding sites by 60–70% in cortex, hippocampus, and neostriatum, ETAI and TAI reduced the number of binding sites in cortex and hippocampus by only 30–35% and did not significantly reduce the number of striatal binding sites (Table 2). These results are consistent with the hypothesis that increased synaptic catecholamine concentrations are necessary for serotonergic neurotoxicity.

The presence of the *N*-ethyl group in the molecule of FEN does not produce dramatic changes in its pharmacological properties compared to those of its metabolite norFEN. The major difference between FEN and norFEN seems to be in the mechanism by which they elevate extracellular levels of serotonin. NorFEN is primarily a 5-HT releaser, whereas FEN possesses a mixture of 5-HT release and 5-HT uptake blocking properties (4,27). Similarly, the substitution of an ethyl for a hydrogen in the aminoindan structure was accompanied by a reduction in its 5-HT–releasing property. The superfusion studies revealed that ETAI was ineffective as a

TABLE 4

CHANGES IN BODY WEIGHT (PERCENTAGE) FOLLOWING DRUG TREATMENT

Saline	FEN	norFEN	ETAI	TAI
8.0 ± 0.85	−10.38 ± 1.29	−12.25 ± 1.21	−9.38 ± 2.24	−10.25 ± 1.41

Rat body weights were taken after a 4-day regimen of drug (2 × 10 mg/kg/day) or saline treatment. Data are expressed as percent change from pretreatment weight, with $n = 8$ per treatment. Data were analyzed by a one-way ANOVA followed by Neuman–Keuls post hoc multiple comparisons. All drug treatments differed from saline ($p < 0.001$) and none of the treatments differed from each other ($p > 0.05$).

5-HT releasing agent, even at five times its IC_{50} in the uptake experiments (Fig. 3). ETAI did stimulate 5-HT release, but only at a high (100 μM) concentration. Results from superfusion experiments indicate that some of the 5-HT uptake-inhibiting properties of FEN, norFEN, and TAI observed in the uptake experiments are due to the ability of the drugs to evoke 5-HT release, superimposed on their ability to act as competitive substrates for 5-HT at the uptake carrier. This result is consistent with work reported by Gobbi et al. (18), showing that FEN at 500 nM induced synaptosomal 5-HT release through an exocytotic mechanism dependent upon extracellular Ca^2+, and with work by Garattini et al. (16), suggesting that FEN uses the 5-HT uptake carrier to enter synaptosomes.

Alkylation of the amino group in an aminoindan molecule decreases activity at all monoamine carriers. TAI was about fourfold more potent than ETAI at inhibiting 5-HT uptake and two- to fourfold more potent than its *N*-ethyl analogue at inhibiting catecholamine uptake. The 5-HT carrier mechanism may not tolerate a bulky amine substituent (ethyl group) in an aminoindan. This substituent is better tolerated, however, in FEN, a phenylisopropylamine. These differences may be attributable to the more flexible side chain of FEN, which is able to adopt a favorable binding conformation despite the presence of the *N*-ethyl substituent.

In general, ETAI and TAI were less effective than FEN and norFEN at inhibiting NE and DA accumulation in vitro (Table 1) and were less effective at decreasing the number of 5-HT uptake sites in vivo (Table 2). Interestingly, the patterns of [^{3}H]paroxetine binding decreases across the different brain regions were similar for drugs with *N*-ethyl substituents (FEN and ETAI: hippocampus < cortex < neostriatum) and for those with primary amines (norFEN and TAI: cortex < hippocampus < neostriatum) (Table 2).

All of the test compounds were equally effective in causing a reduction in body weight following a 4-day treatment. This supports the idea that brain serotonin is involved in body weight regulation, because the test drugs share the ability to increase synaptic 5-HT at low concentrations but are 10- to 30-fold less potent at increasing synaptic catecholamine concentrations (Table 1). However, the present experiments do not elucidate the mechanism of body weight reduction. Although FEN and norFEN decrease food intake (27), it is possible that ETAI and TAI decrease body weight through some other, nonanorectic mechanism. For example, these compounds may elevate basal metabolism through a nonserotonergic mechanism or may interact with serotonergic neurons in the gut to change bowel transit times or other parameters to interfere with nutrient absorption. Feeding studies in which the animals' food intake is monitored, metabolic studies, and experiments that assess the gut transit time in the absence and presence of these drugs would address such questions.

The drug discrimination experiments with the analogues exhibited various degrees of lowered serotonergic potency and diminished (+)-amphetamine-like activity. While 2-aminoindan fully (17), or partially (34) substituted for amphetamine, TAI produced only a saline-like response. Only one similarity existed between unsubstituted 2-aminoindan and TAI in drug discrimination experiments: they both caused significant numbers of rats to be disrupted. TAI mimicked the relatively selective serotonin releaser/uptake blocker, (+)-MBDB with an ED_{50} similar to that obtained for fenfluramine in the same assay (32). On the basis of our results from the in vitro pharmacology experiments, it is hard to explain why TAI did not substitute for the selective serotonin releaser, MMAI. FEN and its metabolite, norFEN, fully mimicked MMAI in the drug discrimination paradigm (25) and cross-substitution occurred between (+)-MBDB and FEN. In MMAI-trained rats, similar to our observations in (+)-amphetamine-trained rats, TAI evoked a significant dose-dependent disruption. It is possible that TAI has a FEN-like ability to enhance dopaminergic function by an unknown mechanism(s) that is independent of serotonergic or dopaminergic transporters.

The drug discrimination results for ETAI paralleled the results of the in vitro pharmacology. ETAI did not mimic (+)-amphetamine or MMAI, and did not produce a high percentage of disruption in tested animals. This compound evoked only partial substitution in (+)-MBDB–trained rats, perhaps suggesting weaker properties than TAI or purely 5-HT reuptake inhibiting effects.

In summary, these studies demonstrate that cyclization of the side chain of phenylisopropylamines to produce an indan congener produces minor effects on the pharmacological characteristics of amphetamines possessing an indirect serotonergic effect. The indan structure did change the mechanism of inhibition of 5-HT accumulation from one that is a combination of uptake-blocking and release-evoking properties (FEN) to one that is essentially all uptake blocking (ETAI). *N*-Alkylation also affected the pharmacology in that the primary aminoindan (TAI) is a better substrate for the 5-HT uptake carrier than is the *N*-ethyl compound (ETAI). On the other hand, the indan ring attenuates the interaction with catecholamine transporters: it decreased the ability to inhibit accumulation of dopamine and norepinephrine.

Together, these results suggest that ETAI and TAI may be useful drugs for the treatment of obesity with reduced neurotoxic liability compared to existing drugs. Further studies are indicated to assess this potential.

ACKNOWLEDGEMENTS

This work was supported by grant DA-04758 from the National Institute on Drug Abuse.

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