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Psychostimulant-like effects of *p*-fluoroamphetamine in the rat

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Abstract

The present study was undertaken to compare the pharmacological properties of *p*-fluoroamphetamine with those of amphetamine and of other halogenated amphetamines, using several in vivo and in vitro tests. These included substitution testing in (+)-amphetamine (1 mg/kg, 5.4 μmol/kg, i.p.)-, (+)-*N*-methyl-1-(1,3-benzodioxol-5-yl)-2-butanamine [(+)-MBDB] (1.75 mg/kg, 7.18 μmol/kg, i.p.)-, and 5-methoxy-6-methyl-2-aminoindan (MMAI) (1.71 mg/kg, 8 μmol/kg, i.p.)-trained rats, [^{3}H]5-HT and [^{3}H]dopamine uptake inhibition in whole brain synaptosomes, and changes in striatal extracellular levels of dopamine, 3,4-dihydroxyphenylacetic acid (DOPAC), and homovanillic acid (HVA) as measured by in vivo microdialysis in freely moving rats. In drug discrimination substitution tests, *p*-fluoroamphetamine fully mimicked (+)-amphetamine (ED_{50} 0.43 mg/kg, 2.11 μmol/kg), whereas 'no substitution' was observed in rats trained to discriminate the serotonin (5-hydroxytryptamine, 5-HT)-releasing agents (+)-MBDB or MMAI from saline. *p*-Chloroamphetamine did not substitute for amphetamine but fully substituted for the (+)-MBDB and MMAI cues (ED_{50} 0.17 mg/kg, 0.82 μmol/kg, and 0.14 mg/kg, 0.69 μmol/kg, respectively). *p*-Fluoroamphetamine, in comparison with *p*-chloroamphetamine and *p*-iodoamphetamine, showed much stronger inhibition of [^{3}H]dopamine than [^{3}H]5-HT uptake into rat brain synaptosomes but was less selective than amphetamine. *p*-Fluoroamphetamine (7.0 mg/kg, i.p.), 1 h after administration, strongly elevated (849% of baseline) extracellular dopamine in rat striatum measured using in vivo microdialysis. Amphetamine (2 mg/kg, i.p.) increased extracellular dopamine in rat striatum with a maximum at the same time as did *p*-fluoroamphetamine, but the latter gave a smaller increase. The data presented suggest that *p*-fluoroamphetamine resembles amphetamine more than it does the 5-HT-releasing type amphetamines.

Keywords: *p*-Fluoroamphetamine; Drug discrimination; ^{3}H-Monoamine uptake inhibition; Microdialysis, in vivo, in freely moving rat

1. Introduction

Structure-activity relationship studies carried out in the early 1960s revealed that *para* substitution of phenylalkylamines led to compounds having different pharmacological properties than the parent drug (Van der Schoot et al., 1962; Pletscher et al., 1963; Fuller et al., 1965). It was well established in the 1970s that *p*-chloroamphetamine produced a reversible short-term and an irreversible long-term depletion of rat brain serotonin (5-hydroxytryptamine, 5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) (Fuller et al., 1975b,c; Harvey et al., 1975). Fuller et al. (1975a) presented comparative studies of three different haloamphetamines having chlorine, bromine, or fluorine in the *para*-position of the aromatic ring. They reported however, that *p*-fluoroamphetamine showed an interesting difference from the other two. It lowered 5-HT levels initially to a lesser degree than did *p*-bromo- or *p*-chloroamphetamine but the effect was transient, contrasting with the long term deficit caused by the other two halogenated amphetamine derivatives. This finding indicated that *p*-fluoroamphetamine might share with *p*-chloroamphetamine the action that is responsible for short-term 5-HT depletion but lack the property of *p*-chloroamphetamine required for long-term effects. A later study confirmed that *p*-fluoroamphetamine was less potent than *p*-chloroamphetamine in depleting brain 5-HT (Harvey et al., 1977; Fuller, 1978). More

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recent evidence suggests that *p*-chloroamphetamine results in a selective degeneration of the fine axon serotonergic projections ascending from the dorsal raphe nucleus (Mamounas and Molliver, 1988).

Fuller et al. (1975a) also compared the monoamine oxidase (MAO) inhibition by the *p*-haloamphetamines; *p*-fluoroamphetamine was distinctly less potent than *p*-bromo- or *p*-chloroamphetamine as an inhibitor of monoamine oxidase. Those workers proposed that the ability of the amphetamines to inhibit monoamine oxidase was related to the lipophilic character of the *para*-substituent. More recently we have also studied *p*-chloro- and *p*-iodoamphetamine, which proved to be relatively selective 5-HT-releasing agents, but the iodo compound had much weaker serotonergic neurotoxic properties than did *p*-chloroamphetamine (Johnson et al., 1991a; Nichols et al., 1991).

p-Fluoroamphetamine is also less potent than *p*-chloroamphetamine in stimulating corticosterone release (McElroy et al., 1984). However, the inability of *p*-chlorophenylalanine (PCPA), 5,7-dihydroxytryptamine (5,7-DHT), methysergide or fluoxetine to antagonize *p*-fluoroamphetamine-induced secretion of corticosterone strongly suggested that *p*-fluoroamphetamine affected hormone release through a non-serotonergic mechanism (McElroy et al., 1984). The plausibility that *p*-chloroamphetamine and *p*-fluoroamphetamine may elevate corticosterone via different mechanisms is further supported by the demonstration that *p*-chlorophenethylamine produces the 5-HT behavioral syndrome through a presynaptic action, whereas the *p*-fluoro derivative produces the same action by a direct postsynaptic effect (Sloviter et al., 1980). In addition, *p*-chloroamphetamine and *p*-fluoroamphetamine appeared to have different modes of action in stimulating pituitary-adrenocortical activity (McElroy et al., 1984).

While it is well known that *p*-chloroamphetamine can also release dopamine, which may be responsible for hyperlocomotion induced by this drug (Sharp et al., 1986; Sugita et al., 1994), no studies have been reported of dopamine-releasing activity or dopamine uptake inhibition by *p*-fluoroamphetamine. However, Sloviter et al. (1980) noted that *p*-fluoroamphetamine induced not only a 'serotonin syndrome' but also caused hyperlocomotion. A direct agonistic action of *p*-fluoroamphetamine at postsynaptic 5-HT receptors seems to be inadequate however, to explain this behavior. Only Bergi et al. (1970) reported that *p*-fluoro derivatives of phenethylamine display considerable amphetamine-like activity.

In a drug discrimination study, it was reported that *p*-fluoroamphetamine fully mimicked fenfluramine, a serotonergic agent (McElroy and Feldman, 1984), but no comparison has been made between the effects of *p*-fluoroamphetamine in the drug discrimination paradigm using dopamine or other 5-HT-releasing agents as training drugs.

All these studies suggested that *p*-fluoroamphetamine had pharmacological properties that were distinct from the other *p*-halogenated amphetamines. The present studies were therefore undertaken to compare the pharmacological properties of *p*-fluoroamphetamine with those of amphetamine and of two other halogenated amphetamines, *p*-chloroamphetamine and *p*-iodoamphetamine, using several in vivo and in vitro tests. These included substitution testing using drug discrimination methodology. Much evidence now exists for the dopaminergic mediation of the discriminative stimulus (DS) properties of (+)-amphetamine (Ho and Huang, 1975; Glennon et al., 1984; Young and Glennon, 1986) and for 5-HT release as responsible for the discriminative stimulus (DS) properties of (+)-*N*-methyl-1-(1,3-benzodioxol-5-yl)-2-butanamine [(+)-MBDB] (Oberlender and Nichols, 1990), and 5-methoxy-6-methyl-2-aminoindan (MMAI) (Marona-Lewicka and Nichols, 1994). In the present study we therefore used these latter three compounds as training drugs. We also characterized the abilities of the *p*-haloamphetamines to inhibit [^{3}H]5-HT, [^{3}H]norepinephrine, and [^{3}H]dopamine uptake into whole brain synaptosomes, and compared changes in extracellular levels of striatal dopamine, 3,4-dihydroxyphenylacetic acid (DOPAC), and homovanillic acid (HVA) in response to (+)-amphetamine and *p*-fluoroamphetamine administration, as measured by in vivo microdialysis in freely moving rats.

2. Material and methods

2.1. Animals

Male Sprague-Dawley rats (Harlan Laboratories, Indianapolis, IN) weighing 200–220 g at the beginning of the drug discrimination study were divided into three groups ($n = 8$–15 per group), trained to discriminate (+)-MBDB · HCl, (+)-amphetamine sulfate, or MMAI · HCl from saline. None of the rats had previously received drugs or behavioral training. Water was freely available in the individual home cages and a rationed amount of supplemental feed (Purina Lab Blox) was made available after experimental sessions so as to maintain approximately 80% of free-feeding weight. Lights were on from 07:00 to 19:00. The laboratory and animal facility temperature was 22–24°C and the relative humidity was 40–50%. Experiments were performed between 08:30 and 17:00 each day, Monday-Friday.

For acute microdialysis and neurochemical studies male Sprague-Dawley rats (Harlan Laboratories, Indianapolis, IN) weighing 200 ± 25 g were used. The ani-

mals were kept in groups of five rats per cage, under the same conditions as described above, but with free access to food and water. In neurochemical studies, rats were killed by decapitation, the brains were removed and rapidly dissected on ice according to the procedure of Glowinski and Iversen (1966).

2.2. Drug discrimination

The procedure and equipment employed have been described in detail (Oberlender and Nichols, 1990; Marona-Lewicka and Nichols, 1994). Briefly, rats were trained to discriminate either (+)-amphetamine sulfate (1 mg/kg), (+)-MBDB hydrochloride (1.75 mg/kg), or MMAI hydrochloride (1.71 mg/kg) from saline using a fixed ratio (FR50) 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 maintenance session. Test sessions ended after 5 min or when 50 responses were made on either lever, whichever came first. If 5 min passed without the rat emitting 50 responses, the animal was scored as disrupted and was not used in the calculation of the ED_{50}. Animals were not tested if, in the preceding two maintenance sessions, the rat gave less than 85% responding on the correct lever prior to the first reinforcement. Following the procedure of Colpaert et al. (1982), test data were discarded and the condition later retested if the rat responded incorrectly in either of the following two maintenance sessions. At least eight to fourteen rats were tested at each dose. All animals responded exclusively on the saline lever when administered saline.

2.3. Microdialysis procedure

Microdialysis probes with a 2 mm long dialysis membrane attached to a stainless steel shaft were constructed as described by Yamamoto and Pehek (1990). Recoveries for these probes were measured in the range of 15–20%. Rats were then anesthetized with ketamine (90 mg/kg) and xylazine (10 mg/kg), and the probes were stereotaxically placed into the striatum [A +0.7, L +3.0, V −6.5 from bregma (Paxinos and Watson, 1986)]. The probes were then fixed in place with dental acrylic supported by a stainless steel screw threaded into the skull and glued into place. The animals were given a 24 h recovery period before experimental procedures were begun.

After the recovery period, rats were placed into plastic cages. The dialysis probe was connected to a microinfusion pump (Carnegie Medicin, Stockholm, Sweden) calibrated to deliver Ringer's solution at a rate of 2.0 μl/min. The probe was perfused for at least 60 min to allow equilibration. Dialysate samples were collected every 30 min. After the equilibration period, two baseline samples were collected, followed by a saline injection. Three more samples were then collected before treatments were given. The treatments consisted of i.p. injections of *p*-fluoroamphetamine · HCl in doses of 1.75, 3.5, and 7.0 mg/kg, or (+)-amphetamine sulfate (2 mg/kg, i.p.). Five more samples were collected after drug treatments.

After completion of the experiments, rats were decapitated. The brains were removed and post-fixed in 10% formalin for 24 h. The brains were then sliced and probe placement verified under a dissecting microscope.

2.4. Biochemical analysis

The concentrations of dopamine, DOPAC, and HVA were determined in dialysis samples using high performance liquid chromatography (HPLC) with electrochemical detection. A 50 μl aliquot from each sample was injected onto the HPLC column (Brownlee C18, Anspec; Ann Arbor, MI). The electrochemical detector utilized was a model 400 EG and G Princeton electrochemical detector (Princeton, NJ) with series dual electrodes set at $E_1 = -260$ mV (2 nA) and $E_2 = 500$ mV (0.5 nA) versus the Ag/AgCl reference electrode. This setup yielded a detection limit of approximately 0.2 pg/μl. The basal levels of dopamine in these studies ranged from 0.8 to 1 μg/μl; basal DOPAC levels ranged from 195 to 250 pg/μl; and basal HVA ranged from 180 to 230 pg/μl. The mobile phase consisted of 50 mM NaH_2PO_4, 30 mM citric acid, 0.1 mM Na_2EDTA, 0.034% sodium octyl sulfate and 25% methanol. Peaks were integrated with the Dynamax Methods Manager software (Rainin, Woburn, MA) implemented on an Apple Macintosh SE computer.

2.5. In vitro ^{3}H-monoamine uptake inhibition

A modified procedure of Steele et al. (1987) was employed. Briefly, whole brain minus cerebellum was homogenized in 15 volumes of ice-cold 0.32 M sucrose using a glass mortar with a motor-driven pestle. The homogenate was centrifuged at 1086 × *g* for 10 min at 4°C. The supernatant was then recentrifuged at 17800 × *g* for 10 min and the resulting pellet was resuspended in the same volume of sucrose solution. Incubations were carried out in a shaking incubator under an atmosphere of 95% O_2-5% CO_2 at 37 or 0°C to measure total tissue uptake and non-specific uptake, respectively. A 5 min preincubation was begun by adding 0.2 ml of the synaptosomal preparation to test tubes containing 1.65 ml of O_2-saturated Krebs-Henseleit buffer (mM: 118 NaCl, 4.8 KCl, 1.3 $CaCl_2$, 1.2 KH_2PO_4, 25 $MgSO_4$, 25 $NaHCO_3$, 10 glucose, 0.06 ascorbic acid and 0.03 Na_2EDTA) with 50 μl of test

drug solution or 50 μl of pargyline HCl solution (final concentration 100 μM). Then [^{3}H]5-HT, [^{3}H]norepinephrine, or [^{3}H]dopamine was added in a 50 μl aliquot (final concentration of 10 nM), and incubations were continued for 5 min. Experiments were terminated by cooling in ice and rapid filtration through Whatman GF/B filters with a Brandel Cell Harvester (Brandel, Gaithersburg, MD). The filters were washed with ice-cold buffer and allowed to air dry before placing them in plastic scintillation vials. Scintillation cocktail (10 ml) was added and the vials were sealed and allowed to sit overnight before counting at an efficiency of 54%.

2.6. Drugs

Training drugs and dosages used in drug discrimination studies were as follows: (+)-amphetamine sulfate (5.4 μmol/kg, 1.0 mg/kg) purchased from Smith Kline and French Laboratories (Philadelphia, PA), (+)-*N*-methyl-1-(1,3-benzodioxol-5-yl)-2-butanamine hydrochloride [(+)-MBDB; 7.18 μmol/kg, 1.75 mg/kg], and 5-methoxy-6-methyl-2-aminoindan hydrochloride (MMAI; 8.0 μmol/kg, 1.71 mg/kg), both synthesized in our laboratory (Nichols et al., 1973,1986; Johnson et al., 1991a,b). Halogenated amphetamine derivatives: *p*-chloroamphetamine hydrochloride, *p*-iodoamphetamine hydrochloride, and *p*-fluoroamphetamine sulfate (drug discrimination), or hydrochloride (all other studies) were synthesized in our laboratory using standard methods. Drugs used in drug discrimination studies were dissolved in 0.9% saline and were injected intraperitoneally (i.p.) in a volume of 1 ml/kg, 30 min before sessions.

HPLC standards were purchased from Sigma Chemical Co. (St. Louis, MO). [^{3}H]5-HT, [^{3}H]norepinephrine, and [^{3}H]dopamine were purchased from Amersham (Arlington Heights, IL) at specific activities of 12.3, 15.7, and 5 Ci/mmol, respectively.

2.7. Statistical analysis

Data from drug discrimination studies were scored in a quantal fashion, with the lever on which the rat first emitted 50 presses in a test session scored as the 'selected' lever. The percentage of rats selecting the drug lever (%SDL) for each dose of the test compound was determined. The degree of substitution was determined by the maximum %SDL for all doses of the test drug. 'No substitution' (N.S.) is defined as 59% SDL or less, and 'partial' substitution 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 then determined from quantal dose-response curves according to the procedure of Litchfield and Wilcoxon (1949). If the percentage of rats disrupted (%D) was 50% or higher, the ED_{50} value was not determined, even if the %SDL of non-disrupted animals was higher than 80%.

For high performance liquid chromatography with electrochemical detection (HPLC-EC) assays, the concentrations of dopamine, DOPAC, and HVA were determined using the Dynamax Methods Manager software (Rainin, Woburn, MA) implemented on an Apple Macintosh SE computer. All comparisons utilized an analysis of variance followed by a post-hoc comparison as embodied in the computer program EPISTAT (EPISTAT Services, Richardson, TX).

Percent uptake inhibition was defined as the difference between specific tritium uptake in control and drug test tubes divided by control uptake, times 100% as described in Steele et al. (1987). The IC_{50} values reported are the average of three experiments as determined from graded dose-response curves, according to the procedure of Tallarida and Murray (1981). To compare IC_{50} values for ^{3}H-monoamine uptake inhibition between (+)-amphetamine and the *para*-halogenated derivatives, a Student's *t*-test was employed.

3. Results

3.1. Substitution tests using rats trained to discriminate (+)-amphetamine, (+)-MBDB, or MMAI from saline

Results from the drug discrimination studies shown in Fig. 1 indicate that *p*-fluoroamphetamine fully substituted only in (+)-amphetamine-trained rats (Fig. 1A). In rats trained to discriminate (+)-MBDB from saline (Fig. 1B), some rats selected the drug appropriate lever but the total percentage was below our criterion for partial substitution (less than 59%, see Materials and methods for details). *p*-Fluoroamphetamine did not mimic the training drug in MMAI-trained rats (Fig. 1C). However, test doses chosen for *p*-fluoroamphetamine were clearly centrally active, as a large percentage of rats was disrupted at the highest doses administered. Opposite results were obtained for the prototypical *p*-halogenated amphetamine derivative, *p*-chloroamphetamine. *p*-Chloroamphetamine did not mimic amphetamine at any of the tested doses but fully substituted for the 5-HT-releasing agents used as training drugs: (+)-MBDB or MMAI.

3.2. Inhibition of ^{3}H-monoamine uptake in rat synaptosomes

In synaptosome uptake inhibition experiments, *p*-fluoroamphetamine was more than 2 times more potent than *p*-chloroamphetamine in the inhibition of

[^{3}H]dopamine uptake, but approximately 13 times less potent in inhibiting [^{3}H]5-HT uptake. ^{3}H-Monoamine uptake inhibiting effects of *p*-fluoroamphetamine were not significantly different from the results obtained for (+)-amphetamine (Table 1). The order of potency for uptake inhibition of dopamine for the *para*-substituent was H > F > Cl > I and for inhibition of 5-HT uptake was I > Cl > F > H. Clearly, the uptake carrier for dopamine cannot easily tolerate *para*-substituents larger than fluorine, while affinity for the 5-HT uptake carrier is enhanced by larger, more lipophilic halogens. This latter result also parallels the earlier finding by Fuller et al. (1975a) and Fuller and Hemrick-Luecke (1982) with respect to potency for inhibition of monoamine oxidase. *para*-Halogenated amphetamines are significantly less potent than amphetamine itself in inhibition of the norepinephrine uptake carrier. No difference was observed in [^{3}H]norepinephrine uptake inhibition between the different *para*-substituents.

3.3. Microdialysis study in awake, freely moving rats

The effects of *p*-fluoroamphetamine and (+)-amphetamine on extracellular concentrations of dopamine, DOPAC, and HVA were monitored in the striatum of conscious, freely moving rats using microdialysis procedures. Fig. 2 shows percent baseline of dopamine (upper panel), DOPAC (middle panel), and HVA (lower panel) in dialysates collected over a period starting 1.5 h before and extended 3 h after injection of *p*-fluoroamphetamine or (+)-amphetamine. Injection of 7 mg/kg i.p. of *p*-fluoroamphetamine was followed by a rapid rinse in extracellular dopamine levels reaching a maximum (849% of baseline) 1 h after administration. At the same time a slight, non-significant decrease in DOPAC concentration was observed. However, when the dopamine level returned to baseline 3 h later, the DOPAC and HVA concentrations were still significantly decreased. The 1.75 mg/kg dose of *p*-fluoroamphetamine had no significant effect on any of the three parameters measured. On the other hand, the 3.5 mg/kg dose of *p*-fluoroamphetamine resulted in a significant (at 90

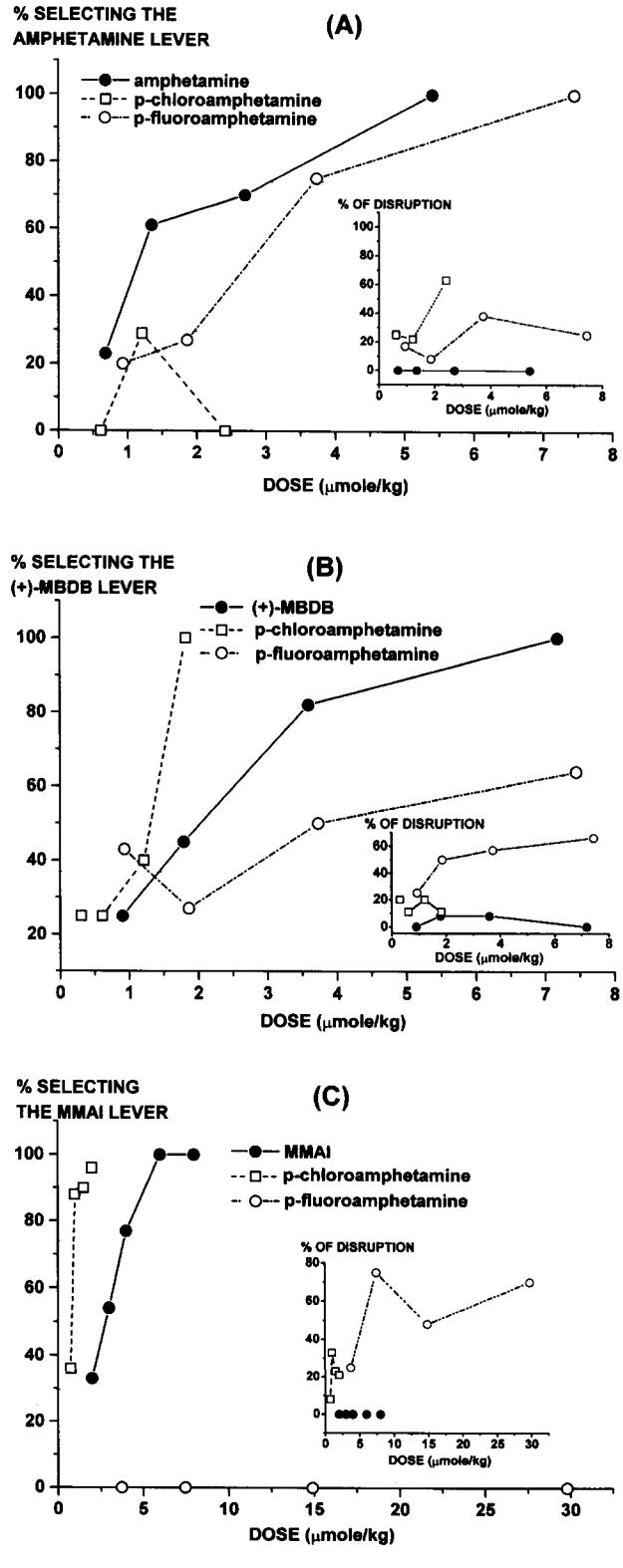

Fig. 1. Effects of *p*-fluoroamphetamine (○) and *p*-chloroamphetamine (□) in rats trained with (+)-amphetamine (1 mg/kg, 5.4 μmol/kg, i.p., panel A), (+)-MBDB (1.75 mg/kg, 7.18 μmol/kg, i.p., panel B), or MMAI (1.71 mg/kg, 8 μmol/kg, i.p., panel C) as a discriminative stimulus. Ordinate: the percentage of rats selecting the drug lever. Abscissa: dose in μmol/kg. Each data point reflects the results obtained using 8–15 rats. The ED_{50} values (with 95% confidence limits) were as follows: in (+)-amphetamine-trained rats 0.23 (0.16–0.35) mg/kg, 1.25 (0.84–1.86) μmol/kg for the training drug, and 0.43 (0.25–0.7) mg/kg, 2.11 (1.29–3.46) μmol/kg for *p*-fluoroamphetamine; in (+)-MBDB-trained rats 0.4 (0.25–0.62) mg/kg, 1.65 (1.07–2.55) μmol/kg for the training drug and 0.17 (0.1–0.28) mg/kg, 0.82 (0.5–1.36) μmol/kg for *p*-chloroamphetamine; and in MMAI-trained rats 0.56 (0.46–0.7) mg/kg, 2.64 (2.14–3.26) μmol/kg for the training drug and 0.14 (0.09–0.22) mg/kg, 0.69 (0.44–1.08) μmol/kg for *p*-chloroamphetamine. For saline the percentage of rats selecting the drug lever was zero in all groups. The inset in each graph illustrates the dose-related percentage of behavior disruption produced by the tested compounds.

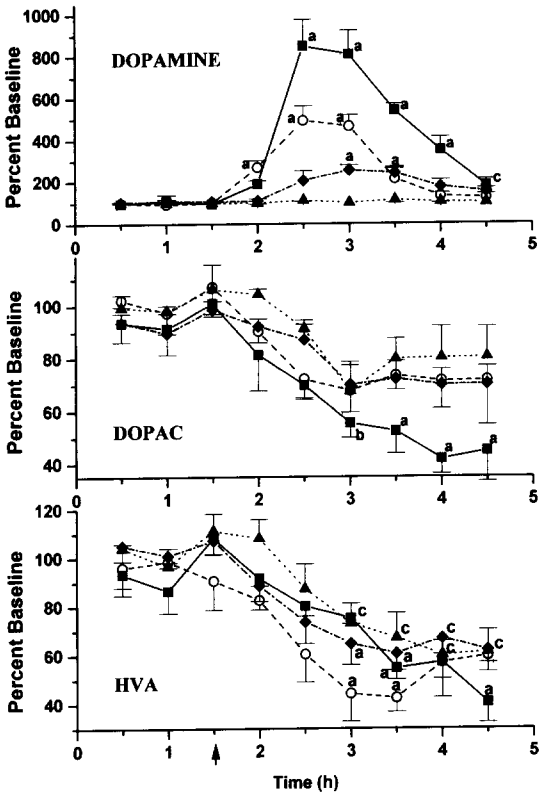

Fig. 2. Effects of 1.75, 3.5, and 7.0 mg/kg of *p*-fluoroamphetamine and 2.0 mg/kg of (+)-amphetamine on the extracellular concentration of dopamine (upper panel), DOPAC (middle panel), and HVA (lower panel) in dialysates collected from rat striatum as measured by in vivo microdialysis. Each value represents the mean ± S.E. of five rats, expressed as the percent of basal concentrations for each rat. The following symbols were used: ○ (+)-amphetamine (2.0 mg/kg), ▲ *p*-fluoroamphetamine (1.75 mg/kg), ◆ *p*-fluoroamphetamine (3.5 mg/kg), and ■ *p*-fluoroamphetamine (7.0 mg/kg). The arrow on the abscissa is used to show the time when treatments were given. [a] $P < 0.001$ different from all time measurements for saline; [b] $P < 0.001$ different from saline at 1.5 h; [c] $P < 0.001$ different from saline at 0.5 and 1.5 h.

and 120 min post-injection) elevation of dopamine concentration over the baseline (Fig. 2, upper panel). (+)-Amphetamine (2.0 mg/kg, i.p.) significantly increased the dopamine level in dialysates from 30 to 120 min after injection. DOPAC concentration after this dose of amphetamine was not altered, although HVA was significantly decreased in dialysates at 1.5 and 2 h after amphetamine administration.

4. Discussion

The results of all three series of experiments demonstrate a significant amphetamine-like effect of *p*-fluoroamphetamine. In previous drug discrimination studies we demonstrated that the *p*-halogenated amphetamines *p*-chloroamphetamine and *p*-iodoamphetamine potently mimicked 5-HT-releasing agents used as training drugs (Nichols et al., 1991; Marona-Lewicka and Nichols, 1994). By contrast, the (+)-amphetamine stimulus failed to generalize to *p*-chloroamphetamine. Indeed, *p*-chloroamphetamine never engendered more than 27% drug appropriate responding in rats trained to discriminate (+)-amphetamine from saline. *p*-Fluoroamphetamine fully mimicked (+)-amphetamine with an ED_{50} of 2.11 μmol/kg, not significantly different from the ED_{50} for the training drug of 1.24 μmol/kg. However, *p*-fluoroamphetamine failed to substitute in (+)-MBDB- or MMAI-trained rats. Thus, in our drug discrimination study *p*-fluoroamphetamine clearly shows psychostimulant-like properties. Nevertheless, these results are at variance with data reported earlier by McElroy and Feldman (1984), who reported full substitution of *p*-fluoroamphetamine in rats trained to discriminate a (relatively high) 3 mg/kg dose of racemic fenfluramine from saline. Fenfluramine is a substituted amphetamine derivative with a trifluoromethyl group on the *meta*-position of the phenyl ring and an ethyl group on its terminal nitrogen (Bergi et al., 1970). Racemic fenfluramine has been used clinically for the treatment of obesity. Its anorectic actions are believed to be mediated through central release and reuptake inhibition of 5-HT from nerve terminals (Garattini et al., 1979). Symmetrical substitu-

Table 1
The inhibition of [^{3}H]5-HT, [^{3}H]dopamine, and [^{3}H]norepinephrine uptake was examined in rat whole brain synaptosomes

	IC_{50} (nM) to inhibit monoamine uptake			Ratio of $1/IC_{50}$ values	
	[^{3}H]5-HT	[^{3}H]Dopamine	[^{3}H]Norepinephrine	Dopamine/5-HT	Norepinephrine/5-HT
Amphetamine	3769 ± 346	172 ± 23	148 ± 16	21.91	25.47
p-Fluoroamphetamine	2352 ± 290	270 ± 33	356 ± 15 [a]	8.71	6.61
p-Chloroamphetamine	187 ± 25 [a]	551 ± 73 [a]	257 ± 8 [a]	0.34	0.73
p-Iodoamphetamine	46 ± 3 [a]	1055 ± 135 [a]	490 ± 7 [a]	0.04	0.09
(+)-MBDB	784 [a,b]	7825 [a,b]	1233 [a,b]	0.10	0.63
MMAI	212 [a,b]	19793 [a,b]	11618 [a,b]	0.01	0.02

The IC_{50} values represent the means ± S.E.M. of three separate experiments. Each experiment utilized five concentrations, run in triplicate. The IC_{50} values were determined from the linear portion of graded dose-response curves, according to the procedure of Tallarida and Murray (1981). [a] Significantly different from (+)-amphetamine IC_{50} ($P < 0.001$, Student's *t*-test). [b] Taken from reference Nichols et al. (1991).

tion has been shown to occur between fenfluramine and MBDB (Oberlender and Nichols, 1990) and between fenfluramine and MMAI (Marona-Lewicka and Nichols, 1994). From those data we concluded that all these agents have a similar interoceptive cue, in which release of neuronal 5-HT plays a primary role. It is not clear however, why *p*-fluoroamphetamine substitutes for fenfluramine, but fails to substitute in rats trained to discriminate different 5-HT releasers from saline.

However, in spite of the fact that the behavioral effects in laboratory animals and human psychopharmacology of amphetamine and fenfluramine are quite different (Bergi et al., 1970; Griffith et al., 1975), fenfluramine partially substituted for amphetamine at 2–5 times the amphetamine training dose when tested in humans, pigeons, rats, or gerbils (for review see: Kamien et al., 1993). In contrast to those data, the 5-HT-releasing agents used as training drugs in the present study never even partially mimicked (+)-amphetamine in drug discrimination substitution tests carried out in our laboratory (Oberlender and Nichols, 1990; Marona-Lewicka and Nichols, 1994). From the point of view of development of a model for the central action of substituted amphetamines, disparities in effects from drug discrimination studies with data from other pharmacological tests are a troubling aspect and require further study.

The neuronal process responsible for the transduction of (+)-amphetamine's actions into stimulus properties is centrally mediated through a mechanism that presumably involves dopamine and, perhaps to a lesser extent, norepinephrine (Young and Glennon, 1986; Dworkin and Bimle, 1989). For example, the (+)-amphetamine stimulus can be mimicked by dopamine agonists (Furmidge et al., 1991) and by drugs which increase the release or block the uptake of this transmitter (D'Mello and Stolerman, 1977). More recent evidence suggests that the discriminable effects of (+)-amphetamine are due, at least in part to inhibition of dopamine uptake (Van Groll and Appel, 1992). The relative potency for *p*-halogenated amphetamines and for training drugs (shown in Table 1) to inhibit dopamine uptake further supports our data from the drug discrimination experiments. For example, the highest ratio of IC_{50} values for dopamine/5-HT, except for (+)-amphetamine, was obtained for *p*-fluoroamphetamine and only this drug mimicked amphetamine in the drug discrimination paradigm.

Thus, the stimulant-like effects of substituted amphetamine derivatives in the drug discrimination assay appear to be correlated not only with the dopamine-releasing/uptake-blocking properties of the compound, but also with the ratio of values describing releasing and/or uptake-blocking potencies between dopamine and 5-HT. High selectivity for dopamine will lead to the expectation that the compound will have stimulant-like properties. On the other hand, if the molecule has greater selectivity for 5-HT, even though it is still a relatively potent dopamine-releasing agent/uptake inhibitor (e.g. *p*-chloroamphetamine), the compound will be characterized in drug discrimination as one that substitutes for 5-HT-releasing agents.

The role of norepinephrine in the stimulus properties of amphetamine remains unclear. The norepinephrine uptake inhibitor nisoxetine, however, has been shown to produce an amphetamine-like discriminative stimulus in mice (Snoody and Tessel, 1983) and rhesus monkey (Woolverton, 1984). It should be noted that in rats, the discriminative stimulus effects of amphetamine were not blocked by noradrenergic antagonists (Ho and Silverman, 1978). The ability of *p*-fluoroamphetamine to block [^{3}H]norepinephrine uptake into brain synaptosomes is significantly weaker than that of amphetamine but similar to the effects produced by the other *p*-halogenated amphetamines (Table 1). Thus, norepinephrine uptake inhibition properties of *p*-fluoroamphetamine do not appear to play a significant role in the stimulus properties of this drug.

The pronounced excitatory behavioral effects of amphetamine in both animals and man are generally thought to be closely associated with an increased release of dopamine (for review, see Kuczenski, 1983). Low doses of the drug lead to increased locomotor activity in rats and stimulant effects in man, while high doses lead to stereotyped behaviors in animals and stereotypy, psychosis, and violent behaviors in man. The 1.75, 3.5, and 7.0 mg/kg doses of *p*-fluoroamphetamine are very potent in an elevation of spontaneous locomotor activity in mice (our preliminary, unpublished results) and induced a hyperactivity in rats (unpublished observation). It is widely believed that mesolimbic dopamine neurons are involved in expression of this behavior (Sharp et al., 1987) and discriminative stimulus properties of amphetamine (Woolverton and Cervo, 1986). Although Sugita et al. (1994) reported strong correlation between *p*-chloroamphetamine-induced hyperlocomotion and levels of dopamine in dialysates from the rat nucleus accumbens, the effect of (+)-amphetamine on dopamine release in the striatum is also quite robust (e.g., Zaczek et al., 1991a). There are also many similarities between the dopamine uptake complex present in both the accumbens and the striatum (Boja and Kuhar, 1989). Further, amphetamine increases extracellular dopamine levels also in both regions (Zetterström et al., 1983). Thus, we have chosen the striatum for microdialysis experiments as a structure in which elevation of extracellular levels of dopamine closely corresponds to hyperactivity in laboratory animals. Our results for *p*-fluoroamphetamine from the microdialysis study in freely moving rats parallel data presented for (+)-amphetamine and *p*-chloroamphetamine (Sharp et al.,

1986,1987; Sugita et al., 1994). The increase of extracellular dopamine levels after systemic administration of *p*-fluoroamphetamine is dose-dependent, with a maximum reached in the second dialysate sample collected between 30 and 60 min post-injection. Nevertheless, the effect of *p*-fluoroamphetamine on extracellular levels of dopamine in rat striatum is less apparent than that of amphetamine. The 2 mg/kg dose of amphetamine produced a significant elevation of extracellular dopamine, whereas 3.5 mg/kg of *p*-fluoroamphetamine elevated dopamine levels only about 2.5-fold above baseline. Systemic administration of *p*-fluoroamphetamine led to reduction of the monoamine metabolites DOPAC and HVA but it had a much weaker effect on HVA levels than did amphetamine. A possible explanation for the differences in potency between *p*-fluoroamphetamine and amphetamine on extracellular levels of dopamine and its metabolites may be that *p*-fluoroamphetamine is less potent than amphetamine as an inhibitor of monoamine oxidase (Fuller and Hemrick-Luecke, 1982) and it is significantly weaker as a norepinephrine uptake inhibitor (present results).

In conclusion, the data presented suggest that *p*-fluoroamphetamine resembles amphetamine more than it does the 5-HT-releasing type amphetamines. Clearly, the monoamine uptake carriers are sensitive to the nature of the *para*-substituent. Even fluorine, a small halogen sometimes considered to be a bioisostere for hydrogen on aromatic rings, can change the relative importance of the different monoamine uptake proteins as targets, albeit to a much smaller extent than do the other halogens. Larger, more lipophilic halogens such as iodine primarily target the 5-HT uptake carrier, being relatively more excluded from the dopamine carrier. One might speculate that such groups lead the phenethylamines to have a greater structural resemblance to the bicyclic indole nucleus of serotonin.

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