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Methamphetamine-induced decrease in tryptophan hydroxylase activity: role of 5-hydroxytryptaminergic transporters

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Abstract

Methamphetamine-induced 5-hydroxytryptaminergic neuronal damage purportedly involves transport of newly released dopamine from extracellular spaces into 5-hydroxytryptaminergic terminals. This hypothesis is based primarily on findings that dopamine is required for, whereas 5-hydroxytryptamine (5-HT) uptake inhibitors prevent, methamphetamine-induced deficits in 5-hydroxytryptaminergic neuronal function. This hypothesis is not, however, supported by findings presented in this study that 5-hydroxytryptaminergic neuronal damage, induced by *p*-chloroamphetamine, does not decrease [^{3}H]dopamine uptake into rat brain synaptosomes prepared from 5-HT-transporter-containing tissue. Moreover, despite having greater affinity for the 5-HT transporter, citalopram has an IC_{50} for [^{3}H]dopamine transport into these synaptosomal preparations that is considerably greater than that of fluoxetine. These data suggest that 5-HT transporters may not effect dopamine uptake and thereby methamphetamine-induced 5-hydroxytryptaminergic neuronal damage. Other possible mechanisms related to 5-HT uptake inhibitor attenuation of methamphetamine-induced deficits were investigated. Fluoxetine pretreatment prevented the methamphetamine-induced decrease in tryptophan hydroxylase activity: this effect cannot be attributed to altered body temperatures or brain concentrations of methamphetamine which suggests that neither, per se, is sufficient to impair 5-hydroxytryptaminergic neuronal function.

Keywords: Methamphetamine; Hyperthermia; Fluoxetine; Dopamine transport; 5-HT (5-hydroxytryptamine, serotonin)

1. Introduction

Abuse of psychoactive phenylethylamines, including the amphetamine analog methamphetamine, is a major world-wide health problem. Administration of high doses of methamphetamine effects toxicity to central aminergic neurons in rodents (for review, see Gibb et al., 1994) and primates (Woolverton et al., 1989). In rats, reversible, short-term decreases in the activity of tryptophan hydroxylase, the rate-limiting enzyme in the synthesis of 5-hydroxytryptamine (5-HT), occur after a single high dose administration of methamphetamine (Bakhit and Gibb, 1981). In contrast, repeated methamphetamine administrations cause long-lasting damage to 5-hydroxytryptaminergic neurons as assessed immunocytochemically (Axt and Molliver, 1991), and by decreases in activity of tryptophan hydroxylase and concentrations of 5-HT and its metabolite 5-hydroxyindoleacetic acid (Bakhit et al., 1981; Ricaurte et al., 1980; Schmidt et al., 1985). Although in vitro experiments suggest a role for reactive oxidative species in mediating the damaging effects of methamphetamine on 5-hydroxytryptaminergic neurons (Stone et al., 1989), mechanisms whereby methamphetamine effects acute and long-term changes in tryptophan hydroxylase activity in vivo remain unclear.

Attenuation of methamphetamine-induced hyperthermia prevents methamphetamine-induced neurotoxicity in rats (Bowyer et al., 1992). Moreover, several pharmacological manipulations attenuate both the acute decreases in tryptophan hydroxylase activity and the long-term toxicity to

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5-hydroxytryptaminergic neurons resulting from methamphetamine administration. Inhibition of dopamine synthesis by α-methyltyrosine or destruction of dopaminergic neurons by 6-hydroxydopamine attenuates methamphetamine-induced 5-hydroxytryptaminergic impairment (Johnson et al., 1987; Schmidt et al., 1985): the effect of α-methyltyrosine is reversed if central dopamine is replenished by administering L-dihydroxyphenylalanine (L-DOPA; Schmidt et al., 1985). Administration of dopamine D_1 receptor antagonists likewise attenuates methamphetamine-induced effects on 5-hydroxytryptaminergic neurons (Sonsalla et al., 1986). These pharmacological data demonstrate the importance of dopamine to methamphetamine-induced 5-hydroxytryptaminergic neuronal toxicity. Consistent with a mediating role for dopamine in this toxicity, both methamphetamine-induced acute decreases in tryptophan hydroxylase activity and long-term damage to 5-hydroxytryptaminergic neurons occur in the striatum and cerebral cortex (Bakhit and Gibb, 1981; Bakhit et al., 1981; Schmidt et al., 1985); regions demonstrated to contain dopaminergic nerve terminals.

Not only dopamine, but also the 5-HT uptake system appears to be important in causing methamphetamine-induced 5-hydroxytryptaminergic neuronal toxicity: thus, 5-HT uptake inhibitors attenuate methamphetamine-induced damage to 5-hydroxytryptaminergic neurons (Hotchkiss and Gibb, 1980; Schmidt and Gibb, 1985). The finding that both dopamine depletion and 5-HT uptake inhibitors prevent methamphetamine-induced toxicity to 5-hydroxytryptaminergic neurons has led to the suggestion that dopamine, newly released following administration of methamphetamine or other amphetamine analogs, may compete with 5-HT for entry into 5-hydroxytryptaminergic nerve terminals, and, once inside, it or a related toxic species causes damage (Schmidt and Lovenberg, 1985; Schmidt et al., 1985; Sprague and Nichols, 1995). Transport of dopamine into 5-hydroxytryptaminergic nerve terminals in vitro has been suggested (Schmidt and Lovenberg, 1985; Berger and Glowinski, 1978). The purpose of this investigation was to explore this hypothesis by examining possible mechanisms whereby the 5-HT uptake inhibitor fluoxetine might attenuate methamphetamine-induced damage to 5-hydroxytryptaminergic neurons. To elucidate further the protective mechanism of fluoxetine, the importance of hyperthermia and central methamphetamine concentrations in causing toxicity were also considered. The results of these experiments suggest that: (1) methamphetamine-mediated damage to 5-hydroxytryptaminergic neurons may be due to effects unrelated to dopamine uptake by 5-HT transporters; (2) the ability of fluoxetine to prevent methamphetamine damage is not associated with a prevention of methamphetamine-induced hyperthermia; and (3) the ability of fluoxetine to alter methamphetamine-metabolism does not explain its attenuating effect on methamphetamine-induced 5-hydroxytryptaminergic damage.

2. Materials and methods

2.1. Animals

Male Sprague Dawley rats (200–300 g; Simonsen Laboratories, Gilroy, CA, USA) were maintained under conditions of controlled temperature and lighting, with food and water provided ad libitum. Rats were sacrificed by decapitation. All procedures were conducted in accordance with approved National Institutes of Health guidelines.

2.2. Drugs and chemicals

(±)-Methamphetamine hydrochloride and (−)-cocaine hydrochloride were supplied generously by the National Institute on Drug Abuse (Rockville, MD, USA). Fluoxetine hydrochloride, pargyline hydrochloride, and citalopram hydrobromide were supplied kindly by Eli Lilly (Indianapolis, IN, USA), Abbott Laboratories (North Chicago, IL, USA) and H. Lundbeck (Copenhagen, Denmark), respectively. *p*-Chloroamphetamine hydrochloride and desipramine hydrochloride were obtained from Sigma (St. Louis, MO, USA) and USV Laboratories (Tukahoe, NY, USA), respectively. 7,8-[^{3}H]Dopamine (43 Ci/mmol) was purchased from Amersham Life Sciences (Arlington Heights, IL, USA). Drugs were administered as indicated in the legends of appropriate figures; doses were calculated as the respective free bases.

2.3. Tryptophan hydroxylase activity

Tryptophan hydroxylase activity was determined in tissue homogenates by measuring 5-hydroxytryptophan formation resulting from the hydroxylation in vitro of tryptophan according to a modification of the method described by Johnson et al. (1992). Briefly, frozen tissue samples were sonicated in 100–200 μl of ice cold 50 mM *N*-(2-hydroxylethyl)piperazine-*N*′-(2-ethanesulfonic acid) (HEPES) buffer (pH 7.4) containing 0.2% Triton X-100 and 6 mM dithiothreitol, and the resulting suspension centrifuged ($22\,000 \times g$ for 15 min; 4°C). Duplicate aliquots (15 μl) of supernatant were then incubated for 10 min (37°C) with 10 μl reaction mixture (52.8 mM HEPES, 50 mM β-mercaptoethanol, 8 mM tryptophan, 1.25 mM *m*-hydroxybenzylhydrazine (NSD 1015) and 3.38 mM DL-6-methyl-5,6,7,8-tetrahydrobiopterin dihydrochloride ($6MPH_4$)). Boiled supernatant was used for blanks. The reaction was terminated by placing the tubes on ice and adding 500 μl of mobile phase (see below). The resulting mixture was centrifuged ($2500 \times g$ for 10 min; 4°C), and the supernatant retained for 5-HTP quantification using high performance liquid chromatography (HPLC) coupled with electrochemical detection (C_{18} reversed-phase analytical column (BAS); glassy carbon electrode set at +0.6 V relative to a Ag/AgCl reference electrode). The HPLC mobile phase (pH 3.1) consisted of 0.15 M monochloroacetic acid, 0.2 mM EDTA and 12.5% methanol.

2.4. Synaptosomal [^{3}H]dopamine uptake

Uptake of [^{3}H]dopamine was determined according to a modification of the method of Boja et al. (1992). Fresh tissue was homogenized in ice-cold 0.32 M sucrose and centrifuged ($800 \times g$ for 12 min; 4°C). The supernatant was centrifuged ($22\,000 \times g$ for 10 min; 4°C), and the resulting pellet resuspended in ice cold 0.32 M sucrose. The tissue suspension was incubated at 37°C for 10 min in assay buffer (in mM: 126 NaCl, 4.8 KCl, 1.3 $CaCl_2$, 16 sodium phosphate, 1.4 $MgSO_4$, 11 dextrose, 1 ascorbic acid; pH 7.4) and 1 μM pargyline. Nonspecific values were determined in the presence of 100 μM cocaine HCl. Assays were initiated by addition of a single concentration of [^{3}H]dopamine (0.5 nM final concentration) and samples were incubated at 37°C for 3 min and then filtered through Whatman GF/B filters soaked previously in 0.05% polyethylenimine. Filters were washed rapidly 3 times with 5 ml ice-cold 0.32 M sucrose using a Brandel filtering manifold. Radioactivity trapped in filters was counted using a liquid scintillation counter.

2.5. Methamphetamine and amphetamine

Brain tissues (whole brains minus the cerebellum, hippocampi, striatum, and some frontal cortex) were each homogenized in 2 ml distilled water and frozen at −70°C. On the day of the assay, these homogenates were equilibrated to room temperature. 300 ng of deuterated amphetamine and methamphetamine (amphetamine-d5 and methamphetamine-d8, Radian, TX, USA) were added as internal standards to each sample. Samples were vortexed for 5 s and then made alkaline (pH > 12) with 100 μl of concentrated ammonium hydroxide. A 500 μl or 1 ml aliquot from each homogenate was extracted into 4 ml butyl chloride/chloroform (4:1, v/v) for 30 min. Samples were centrifuged for 15 min at $1200 \times g$ and the organic phase containing analytes of interest were transferred to a clean tube. This step was repeated twice. Trifluoroacetic acid anhydride (200 μl) was added to each organic phase and heated for 30 min at 70°C. The extracts were cooled to room temperature and then evaporated to dryness at 40°C. A 14-point standard curve ranging from 10 to 5000 ng/ml was prepared with human plasma and extracted as described above. Analytical accuracy and intra-assay precision were verified by concurrent analysis of quality control samples that were prepared in drug-free rat brain homogenate (100, 500 and 2000 ng/ml). Extracts were reconstituted in 100 μl of chloroform prior to analysis by gas chromatography/mass spectrometry.

Concentrations of amphetamine and methamphetamine were determined with a Finnigan 4500 MAT mass spectrometer operating in positive chemical ionization mode (methane/ammonia reagent gas) coupled to a DB5MS-30 M–0.25 μm capillary column. Ions monitored were 249 m/z and 254 m/z (amphetamine and amphetamine-d5, respectively); 263 m/z and 271 m/z (methamphetamine and methamphetamine-d8, respectively). Accuracy was within 6–17% of spiked amphetamine and methamphetamine target values form brain homogenate quality control samples. The limit of quantitation in these experiments was 10 ng/ml.

2.6. Data analysis

IC_{50} values were determined using EBDA (McPherson, 1986) computer software. Statistical analyses between 2 groups were conducted using a 2-tailed Student's *t*-test. Analyses among three or more groups were conducted using analysis of variance followed by Scheffé's test. Differences among groups were considered significant if the probability of error was less than 5%.

3. Results

Results presented in Fig. 1 demonstrate that a single high dose (15 mg/kg) of methamphetamine decreased tryptophan hydroxylase activity in the striatum, hippocampus and frontal cortex 1 h after administration; an effect attenuated by pretreatment with fluoxetine. Since methamphetamine administration induces hyperthermia and prevention of this hyperthermia blocks methamphetamine-in-

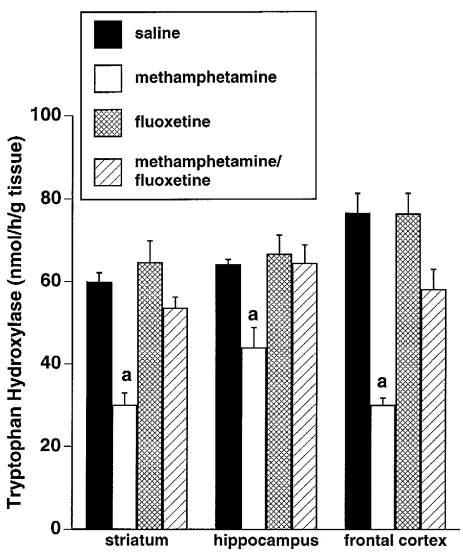

Fig. 1. Effects of methamphetamine on tryptophan hydroxylase activity in the striatum, hippocampus, and frontal cortex of saline- and fluoxetine-treated male rats. Fluoxetine hydrochloride (10 mg/kg, s.c.) or saline vehicle (1 ml/kg, s.c.) was administered 90 min, and methamphetamine hydrochloride (15 mg/kg, s.c.) or saline vehicle (1 ml/kg, s.c.) was administered 60 min prior to decapitation. Columns represent means and vertical lines 1 S.E.M. of determinations in 7–8 rats treated with saline (solid columns), methamphetamine (open columns), fluoxetine (cross-hatched columns) or methamphetamine and fluoxetine (striped columns). [a]Values for methamphetamine-treated rats that are significantly different from vehicle-treated controls ($P < 0.05$).

duced neurotoxicity (see Discussion below), the effect on rectal temperature was also examined. Fluoxetine alone did not affect rat body temperature, and only attenuated slightly methamphetamine-induced hyperthermia (Fig. 2). The effect of fluoxetine on brain concentrations of methamphetamine and its metabolite amphetamine was likewise determined. Fluoxetine increased brain methamphetamine and amphetamine concentrations in these animals by 27 and 21%, respectively (Table 1).

Table 1
Effects of fluoxetine on brain concentrations of methamphetamine and amphetamine

Drug treatment	Drug concentration	
	Methamphetamine	Amphetamine
Methamphetamine	9.5 ± 0.7	1.4 ± 0.1
Fluoxetine + methamphetamine	13.0 ± 0.5 [a]	1.7 ± 0.1 [a]

Data were obtained from the same experiment described in the legend of Fig. 1. Drug concentrations represent means expressed as ng/mg tissue (wet weight) ± 1 S.E.M. of determinations in 8 vehicle- or fluoxetine-treated rats. [a] Values for fluoxetine/methamphetamine-treated rats that are significantly different from methamphetamine-alone-treated rats ($P < 0.05$).

Data presented in Table 2 demonstrate that application of 500 nM fluoxetine prevents the uptake of [^{3}H]dopamine into synaptosomes prepared from rat hippocampal tissue. In this experiment, a single concentration (0.5 nM final concentration) of [^{3}H]dopamine was employed, a concentration considerably less than the K_m of [^{3}H]dopamine in rat striatum (105 nM; Hyttel, 1982). Although K_m and V_{max} were not determined, the data confirm previous findings (Sprague and Nichols, 1995) that fluoxetine application can affect [^{3}H]dopamine transport in this preparation. Fluoxetine attenuated similarly [^{3}H]dopamine uptake into synaptosomes prepared from frontal cortical tissue, but was without effect on uptake into synaptosomes prepared from rat striatum (Table 2).

5-HT neurons were severely impaired as demonstrated by large decreases in tryptophan hydroxylase activity in rat striatum, hippocampus and frontal cortex 14 days after administration of *p*-chloroamphetamine (Fig. 3, left panel).

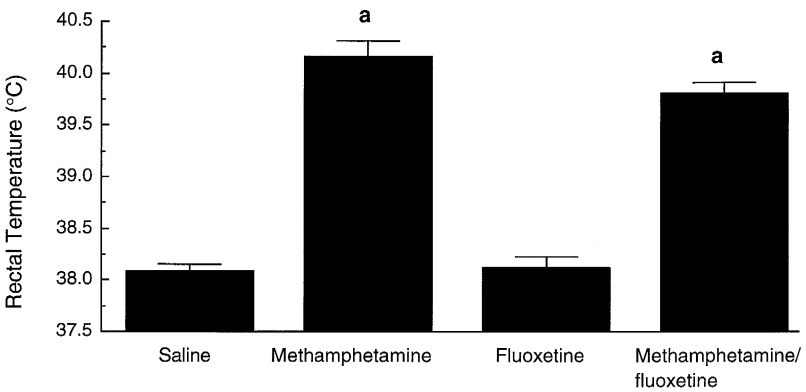

Fig. 2. Effects of methamphetamine on rectal temperature of saline- and fluoxetine-treated male rats. Data were obtained in the same experiment described in the legend of Fig. 1. Rectal temperatures were measured prior to each injection. Mean temperatures among the groups prior to the first (fluoxetine or saline) and second (methamphetamine or saline) injections ranged from 38.1–38.6°C, and 38.6–38.7°C, respectively, and did not differ significantly. Columns represent means and vertical lines 1 S.E.M. of determinations in 7–8 vehicle- or methamphetamine-treated rats. [a]Values for methamphetamine-treated rats that are significantly different from vehicle-treated controls ($P < 0.05$).

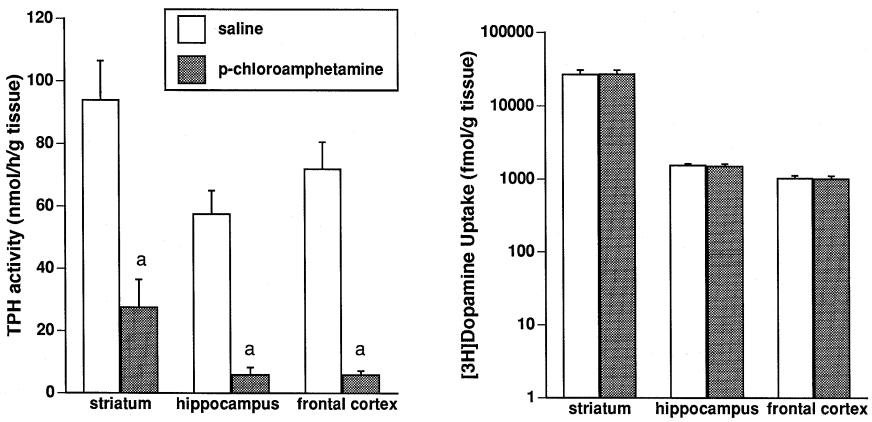

Fig. 3. Effects of *p*-chloroamphetamine on tryptophan hydroxylase activity and synaptosomal [^{3}H]dopamine uptake in striatal, hippocampal and cortical tissue. *p*-Chloroamphetamine (10 mg/kg, i.p.) or saline vehicle (1 ml/kg, i.p.) was administered 14 day prior to decapitation. Columns represent means and vertical lines 1 S.E.M. of determinations in 4–6 rats treated with saline vehicle (open columns) or *p*-chloroamphetamine (shaded columns). [a]Values for *p*-chloroamphetamine-treated rats that are significantly different from vehicle-treated controls ($P < 0.05$).

Table 3
Inhibition of [^{3}H]dopamine (0.5 nM) uptake in synaptosomes prepared from striatal, hippocampal and cortical tissue

Treatment	IC_{50} (nM)		
	Striatum	Hippocampus	Frontal cortex
Fluoxetine	3278 ± 319	258 ± 79	779 ± 149
Citalopram	32965 ± 3550	4633 ± 650	10486 ± 669
Cocaine	202 ± 24	118 ± 16	209 ± 34
Desipramine	7409 ± 631	1.7 ± 0.7	4.5 ± 2.5

Values were calculated as described in Section 2 from raw data presented in Fig. 4.

Table 2
Effects of fluoxetine (500 nM) on [^{3}H]dopamine (0.5 nM) uptake into synaptosomes prepared from striatal, hippocampal, and cortical tissue

	Striatum	Hippocampus	Frontal cortex
Control	23981 ± 8075	1854 ± 343	1331 ± 195
Fluoxetine	21234 ± 8579	427 ± 87 [a]	434 ± 75 [a]

Values represent mean fmol/g tissue (wet weight) ± 1 S.E.M. of 3 determinations run in quadruplicate in non-treated and fluoxetine-treated preparations. [a] Values for fluoxetine-treated synaptosomes that are significantly different from non-treated synaptosomes ($P < 0.05$).

In this experiment, *p*-chloroamphetamine was administered at a dose shown to be relatively selective for and toxic to 5-hydroxytryptaminergic neurons (10 mg/kg, i.p.; Sanders-Bush et al., 1975). Despite this destruction of approximately 70–90% of the 5-hydroxytryptaminergic neurons and presumably of the associated 5-HT transporters, no difference in uptake of [^{3}H]dopamine was observed between striatal, hippocampal or cortical synaptosomes prepared from *p*-chloroamphetamine- and saline-treated rats (Fig. 3, right panel).

Concentration-response curves for inhibition of [^{3}H]dopamine uptake into synaptosomes by several uptake inhibitors are presented in Fig. 4. IC_{50} values for these data are presented in Table 3. Results reveal that in the striatum, a brain region with a high concentration of dopamine transporters, cocaine was the most potent of the uptake inhibitors tested. The rank order of potency for dopamine uptake inhibition in the striatum was cocaine > fluoxetine > desipramine > citalopram. In the hippocampus and frontal cortex, brain regions containing relatively large concentrations of 5-HT and norepinephrine tranporters, a very different pattern emerged: desipramine was the most potent inhibitor of [^{3}H]dopamine uptake. The rank order of potency in both the hippocampus and cortex was desipramine > cocaine > fluoxetine > citalopram. For all tissues tested, citalopram had a much greater (10–18-fold) IC_{50} for [^{3}H]dopamine uptake than did fluoxetine.

4. Discussion

Repeated high-dose methamphetamine administration causes long-lasting damage to 5-hydroxytryptaminergic neurons (Axt and Molliver, 1991; Bakhit et al., 1981; Ricaurte et al., 1980; Schmidt et al., 1985). Even a single administration of this phenylethylamine alters 5-hydroxytryptaminergic neuronal function, as evidenced in the present study by a 30–60% methamphetamine-induced decrease in tryptophan hydroxylase activity (Fig. 1). It has been suggested by several investigators that these methamphetamine effects may be initiated by the transport of newly released dopamine from extracellular spaces into 5-hydroxytryptaminergic neurons. This hypothesis is based on observations that: (1) dopamine is required for methamphetamine-induced 5-hydroxytryptaminergic neuronal damage (Johnson et al., 1987; Schmidt et al., 1985); (2) 5-HT uptake inhibitors, including citalopram, chlorim-

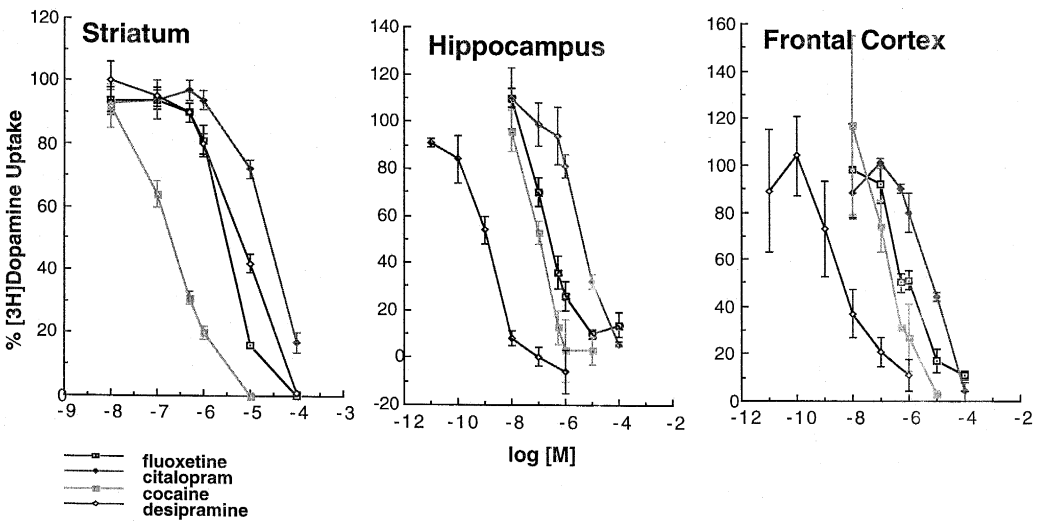

Fig. 4. Concentration-response effects of fluoxetine, citalopram, cocaine and desipramine on [^{3}H]dopamine uptake in synaptosomes prepared from striatal, hippocampal and cortical tissue. Values represent mean determinations of 3–6 individual experiments with samples at each concentration run in triplicate.

ipramine and fluoxetine, attenuate methamphetamine-induced decreases in 5-hydroxytryptaminergic parameters such as brain tryptophan hydroxylase activity (Hotchkiss and Gibb, 1980; Schmidt and Gibb, 1985); (3) fluoxetine blocks [^{3}H]dopamine uptake into synaptosomes prepared from hippocampal tissue (Sprague and Nichols, 1995): a tissue which is both susceptible to methamphetamine-induced 5-hydroxytryptaminergic neuronal damage (Hotchkiss and Gibb, 1980; see also Fig. 1) and is populated with 5-HT uptake sites (De Souza and Kuyatt, 1987). Transport of dopamine into 5-hydroxytryptaminergic nerve terminals in vitro has been observed (Schmidt and Lovenberg, 1985; Berger and Glowinski, 1978).

Results from the present study confirmed that 5-HT uptake inhibitors block methamphetamine-induced damage to 5-hydroxytryptaminergic neurons. Specifically, pretreatment with fluoxetine prevented methamphetamine-induced decreases in tryptophan hydroxylase activity in rat striatum, hippocampus and frontal cortex (Fig. 1). Moreover, fluoxetine application (500 nM) partially blocked [^{3}H]dopamine uptake into synaptosomes prepared from hippocampal and cortical tissues (Table 2, Fig. 4). These data are consistent with the hypothesis that dopamine enters 5-hydroxytryptaminergic terminals and causes toxicity. This assertion is not, however, supported by the present finding that destruction of 5-HT neurons with *p*-chloroamphetamine (i.e., as reflected in the present study by a 70–90% decrease in tryptophan hydroxylase activity 14 days after administration) did not affect [^{3}H]dopamine uptake into striatal, hippocampal or cortical synaptosomes. Even though this *p*-chloroamphetamine treatment regimen apparently eliminates the majority of 5-HT transporters, others have similarly observed a lack of long-term effect on [^{3}H]dopamine uptake into synaptosomal preparations; in this case prepared from rat striata (Sanders-Bush et al., 1975). These data suggest that dopamine is not taken up significantly via 5-HT transporters in these tissues.

Citalopram is a 5-HT uptake inhibitor with affinity for the 5-HT transporter slightly greater than that of fluoxetine (i.e., IC_{50} for 2.5 nM [^{3}H]imipramine binding of 30 versus 50 nM (Sette et al., 1983), IC_{50} for [^{3}H]5-HT uptake of 1.8 versus 6.9 nM (Hyttel, 1982), and K_i for 0.2 nM [^{3}H]paroxetine binding of 1 versus 13.5 nM (Habert et al., 1986) for citalopram and fluoxetine, respectively). Nevertheless, results from the present investigation demonstrate that citalopram has an IC_{50} for [^{3}H]dopamine uptake into synaptosomes prepared from striatal, hippocampal and cortical tissue that is considerably greater than that of fluoxetine (Table 3). This apparent inconsistency suggests that the ability of fluoxetine to prevent [^{3}H]dopamine uptake into these tissues may be unrelated to its blocking effect on the 5-HT transporter. Hence, like the results with the *p*-chloroamphetamine treatment, these data do not support the hypothesis that 5-HT uptake inhibitors attenuate toxicity by preventing dopamine uptake into 5-hydroxytryptaminergic terminals.

Fluoxetine, like other aminergic uptake inhibitors, is not entirely selective for 5-HT transporters (Hyttel, 1982) and its affinity for other transporters must be considered when interpreting in vitro experimental transporter data. Inhibition of [^{3}H]dopamine uptake into hippocampal synaptosomes by 500 nM fluoxetine, a concentration far less than that required for blockade of dopamine transporters (Wong et al., 1974; Hyttel, 1982), has been interpreted as evidence of blockade of dopamine uptake into 5-hydroxytryptaminergic terminals. As mentioned above, results from the present study confirm that this concentration of fluoxetine does inhibit [^{3}H]dopamine uptake into not only hippocampal, but also frontal cortical synaptosomal preparations. Besides 5-HT transporters, these tissues also contain significant concentrations of norepinephrine uptake sites (De Souza and Kuyatt, 1987; Tejani-Butt, 1992). Since norepinephrine transporter function can be inhibited by 500 nM fluoxetine (i.e., IC_{50} for norepinephrine uptake via norepinephrine transporters in rat cortex of 380 nM; Hyttel, 1982), it is possible that fluoxetine prevents [^{3}H]dopamine synaptosomal uptake via norepinephrine transporters in these preparations. This possibility is supported by several findings: (1) The norepinephrine-selective transporter inhibitor desipramine (IC_{50} for norepinephrine uptake via norepinephrine transporters of 1 nM; Hyttel, 1982) is considerably more potent (approximately 200- to 2000-fold) than the 5-HT uptake inhibitors, fluoxetine and citalopram, in inhibiting [^{3}H]dopamine uptake in hippocampal and frontal cortical synaptosomes (Fig. 4, Table 3). Desipramine-induced blockade of 5-HT transporters apparently contributes little to this [^{3}H]dopamine uptake inhibition since its IC_{50} observed in this study (1.7–4.5 nM) is far less than its IC_{50} for 5-HT uptake via 5-HT transporters (210 nM; Hyttel, 1982). (2) Fluoxetine (500 nM) had little effect on [^{3}H]dopamine uptake into synaptosomes prepared from rat striatum – a brain region wherein few norepinephrine transporters (as defined by [^{3}H]nisoxetine binding) are found (Bäckström et al., 1989). (3) The *p*-chloroamphetamine treatment described above which does not affect norepinephrine uptake capacity (Sanders-Bush et al., 1975), did not affect [^{3}H]dopamine uptake in the present study. Our observations that dopamine is likely transported by norepinephrine transporters confirms an earlier report in rat brain homogenates (Snyder and Coyle, 1969).

Since it appears that the protective effects of fluoxetine may not be due to its ability to inhibit dopamine transport into 5-HT neurons, other mechanisms whereby fluoxetine might prevent methamphetamine-induced 5-hydroxytryptaminergic neuronal damage were assessed. A role for hyperthermia in effecting methamphetamine-induced neurotoxicity has been suggested (Bowyer et al., 1992, 1994; Brownstein and Hong, 1995; Farfel and Seiden, 1995; Albers and Sonsalla, 1995; Fleckenstein et al., 1996). Consequently, the hypothesis that fluoxetine might interfere with methamphetamine-induced decreases in trypto-

phan hydroxylase activity by preventing methamphetamine-induced hyperthermia was assessed. Results from the present study reveal, however, that fluoxetine pretreatment prevented methamphetamine-induced decreases in tryptophan hydroxylase activity without preventing methamphetamine-induced hyperthermia (Fig. 2), and suggest that the neuroprotection afforded by fluoxetine is not related to preventing methamphetamine-induced elevation of body temperature. In agreement with the conclusion of others (Axt and Molliver, 1991; Albers and Sonsalla, 1995), these data demonstrate that hyperthermia is not solely responsible for methamphetamine-induced neurotoxicity. Interestingly, other investigators (Farfel and Seiden, 1995) have demonstrated recently that hyperthermia appears not to be prerequisite, although it may potentiate, the neurotoxicity associated with a related amphetamine analog, methylenedioxymethamphetamine. Clearly, factors and mechanisms responsible for effecting methamphetamine neurotoxicity remain to be elucidated.

The effects of fluoxetine on central methamphetamine concentrations were also assessed, since methamphetamine-induced effects on tryptophan hydroxylase activity are dose-related (Hotchkiss and Gibb, 1980; Bakhit and Gibb, 1981) and fluoxetine-induced decreases in methamphetamine concentrations in the brain could conceivably result in decreased methamphetamine-induced 5-hydroxytryptaminergic neuronal damage. Changes in methamphetamine-induced pharmacokinetics appear not, however, to be the explanation for the protective effect of fluoxetine since pretreatment with the uptake blocker increased brain concentrations of methamphetamine and its metabolite amphetamine: similar results have been reported previously (Ricaurte et al., 1983). The ability of fluoxetine, like other transporter blockers, to inhibit potently hepatic mixed function oxidases and thereby amphetamine metabolism may explain these data (for discussion, see Ricaurte et al., 1983). Hence, the protective effect of fluoxetine does not result from a reduction in central methamphetamine concentrations.

In conclusion, the results from the present studies indicate that 5-HT transporters do not contribute significantly to [^{3}H]dopamine synaptosomal uptake: hence, methamphetamine-induced 5-hydroxytryptaminergic neuronal damage may be unrelated to the uptake of dopamine into 5-HT terminals. Because the present uptake studies were conducted in vitro, these findings do not preclude the possibility that dopamine might be taken up by 5-HT transporters in vivo, nor that a dopamine metabolite or dopamine-related reactive species might be taken up by 5-HT transporters and once inside, effect damage. A role for catecholamine-derived or -related reactive oxidative species in causing methamphetamine-induced 5-hydroxytryptaminergic damage has been suggested (Stone et al., 1989; Giovanni et al., 1995; Fleckenstein et al., 1996). These data do not preclude the possibility that methamphetamine itself may ride the carrier and once within the 5-HT nerve terminal, cause toxicity. These data do demonstrate, however, that the protective effect of fluoxetine is not related to altered body temperature nor methamphetamine metabolism. Further study as to the nature of the attenuating effects of fluoxetine are warranted since elucidation of this mechanism likely will provide insight to the causes of the 5-HT toxicity caused by methamphetamine.

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