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Effects of Fenfluramine and Norfenfluramine on Brain Serotonin Metabolism in Rats (40021)

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Fenfluramine [(*m*-trifluoromethyl)-*N*-ethyl-amphetamine] is an anorectic drug that depletes brain serotonin in rats (1). A major metabolite of fenfluramine is the *N*-dealkylated product, norfenfluramine (2). Norfenfluramine is structurally similar to *m*-chloroamphetamine and *p*-chloroamphetamine, halogenated amphetamines with well-characterized actions on brain serotonin neurons. We did the experiments described here to characterize further some of the effects of fenfluramine and norfenfluramine on parameters of serotonin neuronal function previously measured with *p*-chloroamphetamine.

Methods. Male Wistar rats (Harlan Industries, Cumberland, Ind.) weighing 130–150 g were given ip injections of fenfluramine hydrochloride or norfenfluramine hydrochloride (A. H. Robins), probenecid (Merck), or fluoxetine hydrochloride (Lilly). The rats were killed by decapitation, and whole brains were rapidly removed, frozen on dry ice, and stored at −15° prior to analysis of serotonin and 5-hydroxyindoleacetic acid (5-HIAA) (3) or tryptophan hydroxylase (4). Monoamine oxidase in brain mitochondria was determined radiometrically with 100 μM [^{14}C]serotonin (New England Nuclear) as substrate (5).

Results. Acute effects. Within 30 min, both fenfluramine and norfenfluramine caused a significant depletion of brain serotonin, the levels of which continued to decline throughout the 4-hr period of study (Table I). Though norfenfluramine depleted serotonin more than did fenfluramine, the differences between the two drugs were small. Brain 5-HIAA levels did not change significantly at 30 min or at 1 hr, but were decreased at 2 and 4 hr.

Since monoamine oxidase inhibition by *p*-chloroamphetamine may contribute to its lowering of 5-HIAA in brain (5), we compared the effects of these compounds as inhibitors of monoamine oxidase *in vitro* (Fig. 1). Fenfluramine and norfenfluramine were virtually identical in their ability to inhibit monoamine oxidase and were less than one-tenth as active as *p*-chloroamphetamine.

The lowering of brain serotonin by *p*-chloroamphetamine is probably largely attributable to a decrease in tryptophan hydroxylase (6). Table II shows that both fenfluramine and norfenfluramine decreased tryptophan hydroxylase activity as well as serotonin concentration acutely in rat brain.

Long-term effects. Table III shows that serotonin concentration in brain was decreased significantly still at 1 week after the administration of fenfluramine or norfenfluramine. Serotonin concentrations were not changed by fenfluramine and norfenfluramine in rats pretreated with fluoxetine, an inhibitor of uptake into serotonin neurons.

The turnover of serotonin, measured by the rate of accumulation of 5-HIAA after probenecid injection, was significantly decreased in rat brain 1 week after fenfluramine treatment (Fig. 2).

Discussion. Our findings with fenfluramine and norfenfluramine generally agree with previously published data in areas of overlap and extend the observations that have been made with these two serotonin-depleting drugs. Our results (Table I) showed a slightly greater and more rapid depletion of serotonin by norfenfluramine than by fenfluramine, in agreement with data of Duhault *et al.* (2). Norfenfluramine may contribute substantially to the effects observed after fenfluramine injection, especially at longer times (2).

We have previously suggested that *p*-chloramphetamine exerts multiple actions on brain serotonin neurons—inhibition of tryptophan hydroxylation, inhibition of ser-

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TABLE I. INITIAL EFFECTS OF FENFLURAMINE AND NORFENFLURAMINE ON BRAIN SEROTONIN AND 5-HIAA IN RATS.[a]

Treatment	Time (hr)	Brain 5-hydroxyindoles (μg/g)	
		Serotonin	5-HIAA
Control	–	0.55 ± 0.02	0.56 ± 0.02
Fenfluramine	0.5	0.47 ± 0.02*	0.54 ± 0.02
	1	0.37 ± 0.02*	0.59 ± 0.02
	2	0.30 ± 0.01*	0.47 ± 0.01*
	4	0.24 ± 0.01*	0.38 ± 0.01*
Norfenfluramine	0.5	0.44 ± 0.02*	0.58 ± 0.01
	1	0.31 ± 0.02*	0.52 ± 0.02
	2	0.21 ± 0.005*	0.47 ± 0.01*
	4	0.19 ± 0.01*	0.37 ± 0.01*

[a] Fenfluramine hydrochloride and norfenfluramine hydrochloride were injected at 15 mg/kg. Mean values ± standard errors for five rats per group are shown.

* Significantly different from control group, $P < 0.05$.

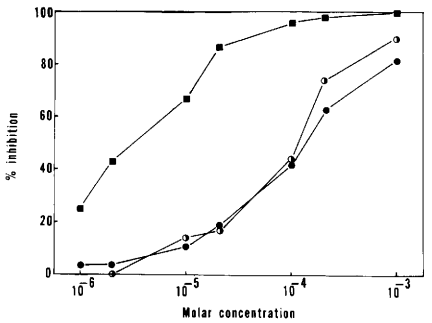

FIG. 1. *In vitro* inhibition of monoamine oxidase by fenfluramine (●), norfenfluramine (◑), and *p*-chloroamphetamine (■). All determinations were made in triplicate.

TABLE II. EFFECT OF FENFLURAMINE AND NORFENFLURAMINE ON SEROTONIN CONCENTRATION AND TRYPTOPHAN HYDROXYLASE ACTIVITY IN RAT BRAIN.[a]

Treatment group	Serotonin (μg/g)	Tryptophan hydroxylase (μg/g/hr)
Saline	0.63 ± 0.01	24.6 ± 0.5
Fenfluramine	0.27 ± 0.02*	16.2 ± 0.2*
Norfenfluramine	0.22 ± 0.01*	17.3 ± 0.7*

[a] Fenfluramine hydrochloride and norfenfluramine hydrochloride were injected at 15 mg/kg 6 hr before groups of five rats were killed.

* Different from saline group, $P < 0.001$.

otonin uptake, release of serotonin, and inhibition of monoamine oxidase (7). Fenfluramine may also exert multiple actions, but inhibition of monoamine oxidase is probably of no importance in fenfluramine's action based on the comparative data in Fig. 2. That may explain why 5-HIAA is lowered less rapidly than serotonin after fenfluramine (Table I). Though inhibition of tryptophan hydroxylation (Table II) (8) may contribute importantly to the *depletion* of serotonin stores by fenfluramine, the initial result of fenfluramine injection apparently is to *increase* the concentration of serotonin in the neural synapse by release and/or inhibition of reuptake (9, 10).

TABLE III. PREVENTION OF THE LONG-TERM DEPLETING EFFECTS OF FENFLURAMINE AND NORFENFLURAMINE ON BRAIN SEROTONIN IN RATS BY FLUOXETINE, AN INHIBITOR OF UPTAKE INTO SEROTONIN NEURONS.[a]

Treatment group	Brain serotonin (μg/g)	
	Control	Fluoxetine-pretreated
Saline	0.61 ± 0.01	0.62 ± 0.01
Fenfluramine	0.44 ± 0.02*	0.61 ± 0.02
Norfenfluramine	0.44 ± 0.05*	0.64 ± 0.01

* Different from saline group, $P < 0.025$.

[a] Fenfluramine hydrochloride and norfenfluramine hydrochloride were injected at 15 mg/kg 1 hr after fluoxetine hydrochloride (10 mg/kg) and 1 week before groups of five rats were killed.

Our data showing decreased serotonin turnover 1 week after fenfluramine complement the observations of Clineschmidt *et al.* (11) showing decreased serotonin turnover, measured by the rate of 5-hydroxytryptophan accumulation following decarboxylase inhibition, 8 days after fenfluramine.

The actions of fenfluramine resemble closely those of *p*-chloroamphetamine on brain serotonin neurons in rats (see Table IV). Both drugs are actively transported into the serotonin neuron and then exert

multiple effects including release of serotonin, inhibition of serotonin synthesis, and depletion of serotonin content. The depletion of serotonin persists after single ip doses of both drugs for weeks or months. Duhault and Boulanger (27) recently reported that serotonin concentration in rat brain returned to normal within 48 hr after discontinuing long-term oral administration of fenfluramine. They interpreted their results as conflicting with those of Clineschmidt *et al.* (24) and of Harvey and McMaster (25), who reported long-lasting depletion of serotonin, and considered that differences in strains of rats might have been important. Strain differences seem unlikely to be involved, for long-lasting depletion of serotonin by fenfluramine has now been observed in Wistar rats (this study), Sprague–Dawley rats from two different breeders (8, 26), Holtzman rats (25), CFE Carworth rats (24), and Charles River rats (11). A more likely basis of the differences in their results is the route of administration of the drug. We have found that daily oral administration of *p*-chloroamphetamine in the diet of rats produces reversible depletion of serotonin (R. W. Fuller and others, unpublished data), whereas ip injection leads to irreversible effects (20).

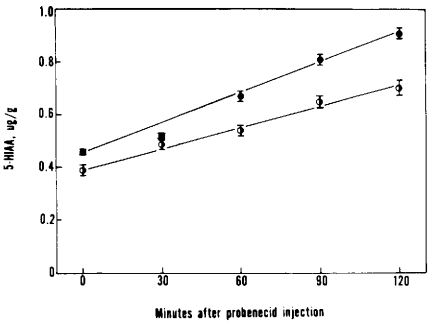

FIG. 2. Reduced turnover of brain serotonin measured by rate of 5-HIAA accumulation after probenecid in rats treated 1 week earlier with fenfluramine. Probenecid (200 mg/kg) was injected at zero time; fenfluramine hydrochloride (15 mg/kg) 1 week earlier. Solid dots indicate controls; half-filled circles indicate fenfluramine-pretreated rats. Mean values ± standard errors for five rats per group are shown.

Summary. Fenfluramine and its *N*-demethyl metabolite, norfenfluramine (3-trifluoromethyl-amphetamine), lowered brain serotonin, 5-hydroxyindoleacetic acid, and tryptophan hydroxylase in rats acutely and the effect persisted for more than 1 week after a single ip dose of the drugs. Serotonin depletion by fenfluramine and norfenfluramine was blocked by pretreatment with fluoxetine, an inhibitor of uptake into serotonin neurons. Serotonin turnover, as measured by the rate of 5-hydroxyindoleacetic

TABLE IV. SIMILARITIES BETWEEN ip DOSES OF *p*-CHLOROAMPHETAMINE AND FENFLURAMINE IN TERMS OF THEIR EFFECTS ON BRAIN SEROTONIN NEURONS IN RATS.

Characteristic	*p*-Chloroamphetamine	Fenfluramine
Requires active uptake into the serotonin neuron, inasmuch as the depletion of serotonin is prevented by pretreatment with an uptake inhibitor	Meek *et al.* (12) Fuller *et al.* (13) Harvey *et al.* (14) Fuller and Molloy (7)	Ghezzi *et al.* (22) Garattini *et al.* (23) Clineschmidt *et al.* (11) This paper
Decreases not only serotonin but also other parameters associated with serotonin neuronal function – tryptophan hydroxylase activity, 5-HIAA concentration, and high-affinity uptake of serotonin	Sanders-Bush *et al.* (6) Sanders-Bush *et al.* (8) Pletscher *et al.* (15) Wong *et al.* (16) Miller *et al.* (17)	Garattini *et al.* (23) Clineschmidt *et al.* (11) Sanders-Bush *et al.* (8) This paper
Depletion of brain serotonin persists for weeks or months after a single drug dose	Sanders-Bush *et al.* (18) Sanders-Bush *et al.* (8) Fuller and Snoddy (19) Fuller *et al.* (20)	Clineschmidt *et al.* (24) Clineschmidt *et al.* (11) Sanders-Bush *et al.* (8) Harvey and McMaster (25) Sherman *et al.* (26) This paper
Causes histologic changes in the midbrain raphe region indicative on cell body degeneration	Harvey *et al.* (21) Harvey *et al.* (14)	Harvey and McMaster (25) Harvey *et al.* (14)

acid accumulation after probenecid administration, was decreased significantly still at 1 week after fenfluramine administration. The effects of fenfluramine and norfenfluramine on brain serotonin neurons in rats resemble those of *p*-chloroamphetamine and probably occur via similar mechanisms.

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