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Effect of D-fenfluramine on serotonin release in brains of anaesthetized rats

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Using in vivo microdialysis of brains of anaesthetized rats, we have examined the acute and chronic effects of D-fenfluramine on the release of serotonin (5-HT) and 5-HIAA within the frontal cortex, the lateral hypothalamus and the nucleus accumbens. A single dose of the drug (10 mg/kg) stimulated 5-HT release by 331–810% and decreased 5-hydroxyindoleacetic acid (5-HIAA) release by 30%, within all 3 brain areas. These changes were maximal 30 min after drug administration, and values returned to baseline after 120 min. Among animals receiving D-fenfluramine (3 or 10 mg/kg, i.p.) daily for 8 days and examined 24 h after the last dose, the basal release of 5-HT from frontal cortex was unaffected. However, the levels of 5-HT in this region, and its evoked release after a subsequent dose of D-fenfluramine (10 mg/kg), were significantly reduced in animals that had received the larger chronic dose. 5-HT release was restored to normal if such rats were given tryptophan (100 mg/kg, i.p.) 1 h prior to the acute D-fenfluramine dose; moreover, 5-HT release from, and levels in, frontal cortex also returned to normal without additional treatment after a 28-day washout period. These observations suggest that the chronic administration of D-fenfluramine fails to affect spontaneous 5-HT release in rat brain, and reduces the release evoked by acute D-fenfluramine only when very high doses are given. Moreover, this reduction is reversible with time or with administration of 5-HT's circulating precursor, tryptophan.

INTRODUCTION

D-Fenfluramine, an anorectic agent in rats[30], is used in humans to reduce appetite and decrease body weight[26], particularly by suppressing the intake of carbohydrate-rich snacks[35]. Its ED_{50} in rats is 1.3 mg/kg[11], and its recommended dose for humans is 30 mg/day[9], or approximately 0.3–0.4 mg/kg, depending on the patient's weight. Although the drug is, like D-amphetamine, a substituted phenethylamine, its neurochemical effects are markedly different from D-amphetamine's: D-fenfluramine and its principal metabolite D-norfenfluramine act specifically on serotoninergic terminals, enhancing 5-HT's (serotonin) release and suppressing its reuptake[11], while D-amphetamine acts on catecholaminergic terminals[4].

The administration to rats of very high doses of D-fenfluramine[10] or D,L-fenfluramine[20] can cause prolonged decreases in brain 5-HT levels. Garattini and colleagues found that brain 5-HT levels were still reduced by 33–35% 2–4 weeks after a single dose of D-fenfluramine (7.5 mg/kg, i.p.) of D,L-fenfluramine (15 mg/kg)[10]; Kleven et al. observed significant reductions in cortical but not hypothalamic 5-HT 8 weeks after rats received 8 doses of D,L-fenfluramine (6.25 mg/kg) at 12 h intervals[20].

The mechanism of this depletion is unknown. It could result from functional changes secondary to chronic re-uptake blockade, as may underlie the reduction in brain serotonin produced by chronic treatment with fluoxetine[15]; or perhaps from the depletion of 5-HT's precursor tryptophan, analogous to the tyrosine depletion that occurs when the release of dopamine from superfused rat striatal slices is stimulated for prolonged periods[23]; or perhaps from damage to the raphe neurons[13]. The possibility that the decrease represents neurotoxicity is contradicted by the apparent failure of any dose of fenfluramine to cause the loss of raphe neurons[2], as well as by the failure of the therapeutic dose of D,L-fenfluramine to increase even among patients taking the drug for many months[6,12].

We have applied the technique of in vivo microdialysis to examine the effects of a very high D-fenfluramine dose (10 mg/kg, equivalent in its actions on serotoninergic neurons to 20 mg of D,L-fenfluramine) administered daily for 8 days, on spontaneous or D-fenfluramine-evoked 5-HT release within the rat's brain. Single systemic doses of D-fenfluramine[32] or D,L-fenfluramine[24] have previously been shown by this technique to enhance serotonin release.

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MATERIALS AND METHODS

Animals

Male Sprague–Dawley rats (250–300 g) were housed in pairs at 22 °C; given free access to food and water; and exposed to light from 08.00 to 20.00 h. Between 08.00 and 09.00 h on the day of an experiment rats were anaesthetized with α-chloralose-urethane (60/600 mg/kg, i.p., 4 ml/kg) and placed in a Kopf stereotaxic frame. The skull was exposed and a hole drilled through the cranium above: (a) the right frontal cortex (from Bregma: A +3.5, R 2.5, V −4.5); (b) the right nucleus accumbens (A +1.7, R −1.2, V −7.5); or, (c) the right lateral caudal hypothalamus (A −3.5, R −1.6, V −9)[28]. The position of the probe was verified by post-mortem sectioning and macroscopic examination or, for experiments performed on the hypothalamus, by microscopic examination after histological fixation using the Nissl method[27]. Microdialysis probes were perfused with an artificial cerebrospinal fluid (Na^+ 147 mM; K^+ 3.5 mM; Ca^{2+} 1.0 mM; Mg^{2+} 1.2 mM; Cl^- 129 mM; phosphate 1 mM; HCO_3^- 25 mM; CO_2/O_2 to pH 7.4)[8], at 1.3 μl/min, using a CMA microperfusion pump[34] (Carnegie Medicine, Solna, Sweden). During the course of an experiment each animal was maintained at a stable level of anaesthesia by providing additional doses of anaesthetic as required. Body temperature was monitored using a colonic probe and maintained at 27 °C using a heating pad.

Samples were collected during 15-min intervals into 0.3 μl of 0.5 M perchloric acid to minimize degradation of neurotransmitters. Following probe implantation (between 10.00 and 11.00 h) the first 8 samples, representing 120 min of collections, were discarded, inasmuch as preliminary studies had shown that such samples contained high serotonin concentrations (probably reflecting 'injury' release resulting from neuronal damage during probe placement, and the release of serotonin from activated platelets)[17]. Samples collected subsequently were analysed for 5-HT, 5-hydroxyindoleacetic acid (5-HIAA) and the dopamine metabolites dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA). Once serotonin levels attained a baseline, defined as 3 consecutive samples in which concentrations of the indoleamine varied by less than 15%, D-fenfluramine (10 mg/kg) or saline were administered intraperitoneally to assess the evoked release of 5-HT.

Experimental protocol

The spontaneous release of serotonin was assessed using dialysates from untreated control rats or from animals receiving 0.9% saline (2 ml/kg); samples were collected for 2 h after the baseline had been established and/or saline had been administered. Values from the two types of animals did not differ significantly (data not shown).

The evoked release of 5-HT was assessed by giving rats a single dose (10 mg/kg, i.p.) of D-fenfluramine (Servier Laboratories; Neuilly-sur-Seine, France) dissolved in 0.9% NaCl (2 ml/kg), or its vehicle. Samples also were collected for 2 h thereafter.

In studies that examined the effects of chronic D-fenfluramine administration, groups of rats received a single daily i.p. injection of 3 or 10 mg/kg of the drug, dissolved in 0.9% NaCl (2 ml/kg), between 09.00 and 10.00 h; control animals received the saline. The basal release of 5-HT and the release evoked by an additional acute dose of D-fenfluramine (10 mg/kg. i.p.) were then examined after 1, 7 or 28 days.

Assays

Serotonin and various neurotransmitter metabolites were assayed by HPLC. The liquid chromatographic system consisted of an Altex pump with dual SSI suppressors in series; a Rheodyne switching valve with a 20 μl loop; a 3 μM HR-80 C18 column (ESA Inc. Bedford, MA); and an ESA coulometric detector (oxidation voltage +0.35 V)[8]. In-line filters were placed post-Rheodyne and post-column. The mobile phase consisted of 70 mM sodium phosphate, 0.63 mM heptane sulfonic acid, and 0.22 mM EDTA, pH at 4.56, methanol 8% v/v. The flow rate was 1.5 ml/min. Chromatograms displaying DOPAC, 5-HIAA, HVA and 5-HT were completed within 10 min, thereby allowing immediate on-line analysis. The limits of sensitivity for all four compounds were approximately 10 fmol.

Characteristics of the microdialysis probe: recoveries

Daily recoveries for each probe were measured before and after completion of the experiment. Probes were calibrated (at a flow rate of 1.3 μl/min) in vitro, by placing them in 1 μm standards, at room temperature[8]. After several minutes (to allow development, for each compound to be measured, of a steady state across the probe membrane), concentrations of the standards were measured in 2 or 3 samples. Under these conditions (i.e., at the temperature and flow rate indicated), the Carnegie Medicin probes exhibited little between-probe variability in recoveries, before and at the end of the experiment. Hence, recoveries were used in our calculations only to allow between-animals comparisons, and not to give 'calculated' extracellular fluid concentrations. Such calculations could be misleading, since the diffusion kinetics in vivo may differ from those in vitro[16].

Tissue levels

At the end of the experiment, brains were removed and washed in ice-cold saline; both frontal cortices were then dissected and immediately frozen at −70 °C. On the day of the assay, each frozen tissue sample was sonicated in 1 ml of perchloric acid, 0.1 M, containing *N*-methyl serotonin (1 μM) as an internal standard. The homogenate was centrifuged, and the supernatant fluid was filtered (nylon filter; 0.2 μm pore) under centrifugation (3000 *g* for 15 min) to remove any debris. Tissue levels of serotonin and of various monoamine metabolites were measured by HPLC as described above. Results are reported as pmol/mg protein. Protein was determined by the method of Lowry[21].

Statistics

The statistical significance of differences between treated groups was determined using one-way Analysis of Variance (ANOVA), followed by Newman–Keuls test for pair-wise comparisons, or by Dunnett's test. Possible relationships between the release of 5-HT or 5-HIAA and their tissue contents were analysed by correlation analysis. Paired *t*-tests were used to assess the effects of acute D-fenfluramine on the release of 5-HIAA, DOPAC and HVA.

RESULTS

Effect of an acute dose of D-fenfluramine on 5-HT and 5-HIAA release

Basal serotonin levels in dialysates of frontal cortex, lateral hypothalamus and nucleus accumbens were similar to each other (Table I) and to levels previously described elsewhere[14,24,32]. The acute administration of saline had no effect on 5-HT release within frontal cortex during the 120 min following its i.p. administration (Fig. 1).

An acute dose of D-fenfluramine (10 mg/kg) increased 5-HT release to 810 ± 380% of baseline release in frontal cortex, to 315 ± 60% in lateral hypothalamus, and to 370 ± 60% in nucleus accumbens (Fig. 1). In all 3 regions, 5-HT release peaked 30 min after drug administration and returned to baseline values after 60-120 min (Fig. 1).

The basal release of 5-HIAA was lower in frontal cortex than in hypothalamus. D-Fenfluramine (10 mg/kg) decreased 5-HIAA levels in the dialysates by 27–30% (Table I). This effect was maximal at 120 min after

TABLE I

Effect of a single dose of D-fenfluramine (10 mg/kg) on 5-HT and 5-HIAA release in lateral hypothalamus, nucleus accumbens and frontal cortex

Rats (n = 5 for each region) were treated as described in Fig. 1; dialysate samples were assayed for 5-HT and 5-HIAA. Basal 5-HT release did not differ significantly among the 3 regions; 5-HIAA release was significantly lower ($P < 0.05$, ANOVA) in frontal cortex than in hypothalamus. In each region, the release of 5-HT evoked by D-fenfluramine (10 mg/kg), differed significantly ([a]$P < 0.05$, t-test) from basal release. 5-HIAA release after D-fenfluramine (10 mg/kg), was significantly reduced (by 27–30%; [a]$P < 0.05$, t-test). (Data, given as means ±S.E.M. are for the 15-min intervals during which maximal effects of D-fenfluramine administration were observed i.e., after 30 min for 5-HT and 120 min for 5-HIAA).

Brain region	*5-HT release (fmol/15 min)*		*5-HIAA release (pmol/15 min)*	
	Basal	*Evoked*	*Basal*	*Evoked*
Frontal cortex	19.8 ± 2.4	160.0 ± 75.2	0.82 ± 0.05	0.58 ± 0.01[a]
Hypothalamus	17.2 ± 3.1	54.2 ± 8.6[a]	1.46 ± 0.17[b]	1.06 ± 0.24[a]
Nucleus accumbens	16.4 ± 3.3	60.7 ± 9.8[a]	1.09 ± 0.16	0.76 ± 0.21[a]

[a]differs from basal release in that region. [b]differs from frontal cortex basal release, $P < 0.05$.

D-fenfluramine administration, and was similar across the three brain areas.

The acute dose of D-fenfluramine (10 mg/kg) decreased 5-HT levels in frontal cortex by 54% ($P < 0.05$) but failed to affect 5-HIAA levels (Table II).

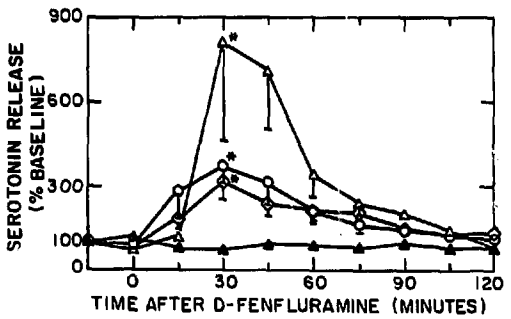

Fig. 1. Effect of a single large dose of D-fenfluramine (10 mg/kg) on 5-HT release within rat frontal cortex, lateral hypothalamus and nucleus accumbens. Microdialysis probes were placed in the indicated brain regions of anaesthetized rats, and perfused with artificial CSF. Starting 2 h after probe implantation, samples were collected for a 15-min period and assayed for 5-HT. As described in Methods, the first 3 consecutive samples whose serotonin contents varied by less than 10% were used to determine mean baseline release, taken as 100%. Animals (n = 5) then received D-fenfluramine (10 mg/kg i.p.; open symbols) or saline (closed symbols) at time 0, and additional samples were collected during the next 2 h; their 5-HT contents were expressed as percents of baseline release ± S.E.M. In frontal cortex (△), lateral hypothalamus (◇), as well as in nucleus accumbens (○), significant increases in 5-HT release were observed 30 and 45 min after D-fenfluramine (*$P <$ 0.05, by ANOVA), and release returned to baseline levels after 120 min.

TABLE II

Effect of D-fenfluramine and tryptophan on 5-HT and 5-HIAA levels in frontal cortex

Rats (n = 5) received no treatment, saline, or D-fenfluramine (10 mg/kg/day) for 8 days, followed the next day by an acute dose of saline; D-fenfluramine (10 mg/kg); or tryptophan (100 mg/kg) + D-fenfluramine (10 mg/kg). In vivo microdialysis was performed in frontal cortex, as described in Fig. 1. Two hours after the last dose, animals were killed, and frontal cortex levels of 5-HT and 5-HIAA were measured.

Treatment		*5-HT pmol/mg*	*5-HIAA pmol/mg*
chronic	*acute*		
0	SALINE	29.7 ± 6.5	10.8 ± 1.9
SALINE	D-F	13.6 ± 2.9[a]	10.8 ± 1.1
D-F	SALINE	5.2 ± 0.6[a,b]	1.4 ±0.4[a,b]
D-F	D-F	4.2 ± 0.4[a,b]	2.2 ± 0.7[a,b]
D-F	TRYPTOPHAN	6.5 ± 0.6[a,b]	4.4 ± 0.7

[a]$P < 0.05$ (ANOVA) differs from the group receiving no chronic treatment and saline acutely.
[b]$P < 0.05$ (ANOVA) differs from the group receiving saline chronically and D-fenfluramine acutely.

Effect of chronic treatment with D-fenfluramine on 5-HT release

Chronic treatment with either 3 or 10 mg/kg/day of D-fenfluramine for 8 days failed to modify basal 5-HT release in the frontal cortex (Table III). The release of 5-HT evoked by an additional single dose of D-fenfluramine (10 mg/kg, i,p.) was significantly decreased in the group receiving 10 mg/kg chronically ($P < 0.005$, ANOVA) in terms of both peak values and areas under the curve relating dialysate levels to minutes, but not in the group receiving 3 mg/kg (Table II). Chronic treat-

TABLE III

Effect of D-fenfluramine and tryptophan on 5-HT release in frontal cortex of D-fenfluramine pretreated rats

Rats (n = 5) received saline or D-fenfluramine (3 to 10 mg/kg) i.p., daily, for 8 days. A probe for in vivo microdialysis was implanted in the frontal cortex on the following day, and 5-HT release was measured under basal conditions (as described in Fig. 1), and also during the 2 h after an additional dose of D-fenfluramine (10 mg/kg). Some animals also received tryptophan (100 mg/kg) i.p., 1 h before the additional dose of D-fenfluramine.

Treatment		*5-HT release*		
chronic	*acute*	*Basal (fmol/15 min)*	*Peak (% basal)*	*Evoked AUC*
0	D-F	19.8 ± 2.4	810 ± 380	2.60 ± 0.90
3	D-F	23.3 ± 3.1	317 ± 25	1.18 ± 2.18
10	D-F	18.3 ± 1.1	146 ± 9[a]	0.18 ± 0.10[a]
10	TRYPT. + D-F	16.0 ± 1.4	730 ± 220	1.61 ± 0.42

[a]$P < 0.05$ (ANOVA) differs from the group receiving saline chronically.

ment with D-fenfluramine (10 mg/kg) decreased the basal release of 5-HIAA in frontal cortex by 69%, i.e. from 0.82 to 0.26 pmol/15 min collection ($P < 0.05$) (Table IV).

Effect of chronic treatment with D-fenfluramine on 5-HT and 5-HIAA levels

Chronic treatment with 10 mg/kg of D-fenfluramine decreased frontal cortex 5-HT levels from 27.9 to 5.2 pmol/mg protein ($P < 0.05$) and 5-HIAA levels from 10.8 to 1.4 pmol/mg protein ($P < 0.05$) (Table II). Among animals receiving an acute dose of D-fenfluramine after chronic treatment with the drugs or with saline, frontal cortex serotonin levels were similary reduced from 13.8 to 4.2 pmol/mg protein ($P < 0.05$), and 5-HIAA levels from 10.8 to 2.2 pmol/mg protein ($P < 0.05$).

The basal release of 5-HIAA was positively correlated with frontal cortex 5-HIAA content ($r = 0.648$, $P = 0.003$, basal release of 5-HIAA = $130.34 + 26.16 \times$ tissue content of 5-HIAA). No similar correlation was observed between 5-HT levels and basal release, nor between the basal and the D-fenfluramine-evoked release of 5-HT. Although brains with markedly reduced 5-HT levels (i.e., of animals receiving 10 mg/kg of D-fenfluramine chronically) released less neurotransmitter after acute D-fenfluramine than controls, the relation between this 5-HT release and tissue 5-HT content was nonlinear.

Effect of tryptophan on 5-HT release evoked by D-fenfluramine

The addition of tyrosine to the superfusion medium has been shown to sustain the release of dopamine from electrically-stimulated striatal slices[23], by restoring tissue tyrosine levels. To determine whether tryptophan administration could similarly sustain 5-HT release among rats in which frontal cortex serotonin levels had been depleted by chronic D-fenfluramine (10 mg/kg), we administered a single dose of tryptophan (100 mg/kg, i.p.) to such animals. The tryptophan was given 24 h after the eighth D-fenfluramine dose, and followed after 1 h by an acute D-fenfluramine dose (10 mg/kg). Among rats so treated, the evoked release of 5-HT was restored to normal (Table III), even though tissue 5-HT levels remained depressed (Table II).

Effect of a washout period on frontal cortex 5-HT release among rats chronically treated with D-fenfluramine

Among animals killed 24 h after the last of 8 daily doses of D-fenfluramine, tissue 5-HT levels were reduced by 72% ($P < 0.05$), and the release of 5-HT evoked by a ninth dose of D-fenfluramine was diminished by 82% ($P < 0.05$) (Table IV). If animals were studied 7 days after the eighth D-fenfluramine dose, frontal cortex 5-HT levels were still reduced, but to a lesser extent (by 38%, $P < 0.05$); the evoked release of 5-HT remained depressed. Among animals examined 28 days after the eighth D-fenfluramine dose, both tissue 5-HT levels and the evoked release of the transmitter no longer differed from those of control animals. The basal release of 5-HT was normal in all of the experimental groups. Tissue levels of 5-HIAA and the basal release of this metabolite were significantly reduced in animals examined 1 or 7 days after the eighth D-fenfluramine dose. 5-HIAA levels were still depressed after 28 days but less so; basal 5-HIAA release returned to normal.

Effect of D-fenfluramine on DOPAC, HVA release and tissue levels

We also measured the levels in artificial CSF of two brain dopamine metabolites, DOPAC and HVA, after D-fenfluramine treatment in order to obtain indirect information about possible effects of the drug on dopamine release. Basal DOPAC and HVA levels in dialysate samples from nucleus accumbens were higher than in

TABLE IV

Effect of washout period on 5-HT and 5-HIAA levels in, and release from frontal cortex of, rats chronically treated with D-fenfluramine

Groups of rats ($n = 5$) received saline (control) or D-fenfluramine (10 mg/kg) i.p., daily for 8 days; animals were subjected to in vivo microdialysis on the following day or after 7 or 28 days. The release of serotonin and 5-HIAA within the frontal cortex was measured before and after an acute dose of D-fenfluramine (10 mg/kg i.p.), as described in Fig. 1. Rats were then killed, and frontal cortex serotonin and 5-HIAA levels were measured.

	Serotonin			*5-HIAA*	
	Basal release (fmol/15 min)	*Evoked release (% basal)*	*Tissue levels (pmol/mg)*	*Basal release (pmol/15 min)*	*Tissue levels (pmol/mg)*
Control	19.8 ± 2.4	810 ± 380	13.6 ± 2.9	0.82 ± 0.05	10.8 ± 1.9
Washout 1 day	18.3 ± 1.1	146 ± 9[a]	3.8 ± 0.7[a]	0.26 ± 0.02[a]	2.2 ± 0.7
Washout 7 days	16.3 ± 1.6	138 ± 19[a]	8.5 ± 0.4[a]	0.27 ± 0.01[a]	3.5 ± 0.2[a]
Washout 28 days	14.3 ± 2.7	340 ± 152	10.8 ± 0.7	0.60 ± 0.18	6.0 ± 0.8[a]

[a] $P < 0.05$ differs from control.

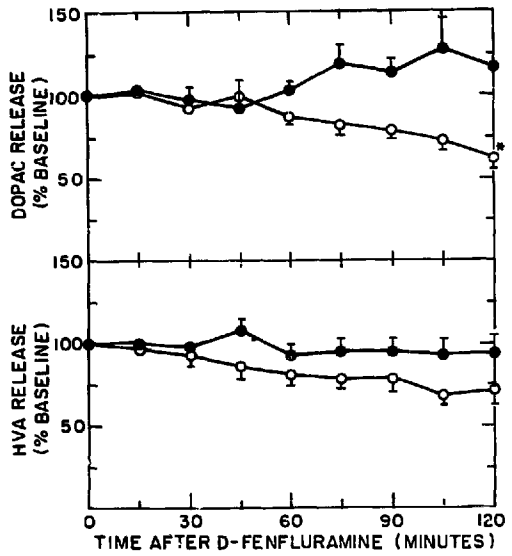

Fig. 2. Effect of a single dose of D-fenfluramine (10 mg/kg) on DOPAC and HVA release with frontal cortex. Rats ($n = 5$) treated as described in Fig. 1, received D-fenfluramine (10 mg/kg; open symbols), or saline (dark symbols), i.p. DOPAC and HVA release were measured by in vivo microdialysis. $^*P < 0.01$ (t-test) differs from control value.

those from frontal cortex: i.e., 10.17 ± 1.46 vs 0.37 ± 0.07 pmol/sample for DOPAC; 3.48 ± 0.45 vs 0.42 ± 0.06 pmol/sample for HVA ($P < 0.005$, t-test, for both comparisons). An acute dose of D-fenfluramine (10 mg/kg) significantly decreased DOPAC release by 38.6 ± 7% at 120 min (Fig. 2) ($P < 0.05$, t-test). The small decrease in HVA release after D-fenfluramine (10 mg/kg) was not significant.

DISCUSSION

Serotonin levels vary among rat brain regions, with relatively highest concentrations reported in hypothalamus, and lower levels in cerebral cortex[1,19]. We observed no regional differences in the basal release of 5-HT within the 3 regions examined (Table I), and, paradoxically, we found evoked release to be greatest within frontal cortex (Table I). This high degree of responsiveness of cortical serotoninergic neurons to D-fenfluramine could be related to their firing frequencies or perhaps the density of their autoreceptors. The failure of chronic D-fenfluramine to diminish basal 5-HT release in frontal cortex (Table III) even though 5-HT levels were depressed (Table II), is compatible with the dissociations observed by Mennini and his colleagues[22] in studies on cortical synaptosomes, and Schaechter and Wurtman in studies on hypothalamic slices[31]. The evoked release of 5-HT was markedly diminished within brains that contained very low 5-HT levels after chronic D-fenfluramine treatment (Table III); however it was not possible to demonstrate a linear relationship between release and levels among D-fenfluramine-treated rats, perhaps because too few D-fenfluramine doses were examined. A linear relationship between 5-HT levels and release has been demonstrated in electrically stimulated rat brain slices exposed to various tryptophan concentrations[31a].

The mechanism responsible for the prolonged decrease in brain serotonin levels among rats receiving very high doses of D-fenfluramine or D,L-fenfluramine awaits discovery[29,33]. That this mechanism may reflect a tardive response to a prolonged blockade of serotonin uptake is suggested by the similar reduction in brain serotonin observed in rats chronically treated with fluoxetine[15]: this drug shares D-fenfluramine's ability to inhibit 5-HT reuptake but apparently not its ability to release 5-HT directly[3]. Our observations that the 5-HT release evoked by a subsequent dose of D-fenfluramine can be restored acutely by tryptophan (Table III) and recovers spontaneously with time (Table IV) support the view that the prolonged reduction in rat brain serotonin after D-fenfluramine treatment is not evidence of neuronal damage. Tryptophan administration was previously shown to restore 5-HT levels in brains of fenfluramine-treated rats[7], but it remains to be determined whether prolonged treatment with D-fenfluramine actually affects tryptophan concentrations within serotoninergic terminals. That the prolonged reduction in 5-HT levels is not related to neuronal damage is further suggested by the failure of the drug to affect the number of raphe neurons[2].

The 10 mg/kg dose needed to diminish evoked 5-HT release (Table III) probably increased the area under the curve (AUC) (of fenfluramine's plasma concentration) by a 30-fold or greater amount than the increase that would have occurred after an ED_{50} dose[5]. Hence it is highly unlikely that the changes in 5-HT release observed here would take place in patients receiving the drug. D-Fenfluramine is a phenylethylamine, like D-amphetamine and its analogues[25]; however, its neurochemical and psychopharmacologic effects are very different from those of D-amphetamine, reflecting actions on serotoninergic and not dopaminergic neurons. The decrease in dialysate levels of the brain dopamine metabolite DOPAC after acute D-fenfluramine treatment (Fig. 2) suggests that the drug actually diminishes dopaminergic function in the frontal cortex. Our data do not allow conclusions as to the mechanism of this decrease; however, fenfluramine is not known to inhibit monoamine oxidase, nor are it and its metabolite norfenfluramine effective inhibitors of dopamine's reuptake[18]. Hence, the most likely explanation for the decline in DOPAC is that the 5-HT released by the D-fenfluramine suppressed the firing of dopaminergic neurons terminating in the cortex.

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REFERENCES

1 Adell, A., Garcia-Marques, C., Armario, A. and Gelpi, E., Chronic stress increases serotonin and noradrenaline in rat brain and sensitizes their responses to a further acute stress, *J. Neurochem.*, 50 (1988) 6:1678–1681.

2 Appel, N.M. and DeSouza, E.B., Fenfluramine selectively destroys serotoninergic terminals in brain: Immunocytochemical evidence, *Soc. Neurosci. Abstr.*, 14 (1988) 556.

3 Benfield, P., Heel, R.C. and Lewis, S.P., Fluoxetine: A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic efficacity in depressive illness, *Drugs*, Suppl. 32 (1986) 481–508.

4 Butcher, S.P., Fairbrother, I.S., Kelly, J.S. and Arbuthnott, G.W., Amphetamine-induced dopamine release in the rat striatum: an in vivo microdialysis study, *J. Neurochem.*, 50 (1988) 346–355.

5 Caccia, S., Dagnino, G., Garattini, S., Guiso, G., Madonna, R. and Zanini, M.G., Kinetics of fenfluramine isomers in rats, *Eur. J. Drug Metab. Pharmacokin.*, 6 (1981) 297–301.

6 Craighead, L.W., Stunkard, A.J. and Obrien R., Behavior therapy and pharmacotherapy of obesity, *Arch. Gen. Psych.*, 38 (1981) 763–768.

7 Duhault, J. and Verdavainne, C., Modifications du tax de serotonine cerebral chez le rat par le trifluoromethyl-phenyl-s-aminopropane-fenfluramine 768-S, *Arch. Inter. Pharmacodyn.*, 170 (1967) 2:276–286.

8 During, M.J., Acworth, I.N. and Wurtman, R.J., Effects of systemic l-tyrosine on dopamine release from rat corpus striatum and nucleus accumbens, *Brain Research*, 452 (1988) 378–380.

9 Finer, N., Cradock, D., Lavielle, R. and Keen, N., Dextrofenfluramine in the treatment of refractory obesity, *Curr. Ther. Res.*, 38 (1985) 847–854.

10 Garattini, S., Mennini, T., Bendotti, C., Invernizzi, R. and Samanin, R., Neurochemical mechanism of action of drugs which modify feeding via the serotoninergic system, *Appetite*, 7, Suppl. (1986) 15–38.

11 Garattini, S., Mechanisms of anorectic activity of dextrofenfluramine. In A.E. Bender and L.J. Brookes (Eds.), *Body Weight Control*, Churchill Livingstone, 1987 pp. 261–270.

12 Guy-Grand, B., Apfelbaum, M., Crepaldi, G., Gries, A., LeFebure, P. and Turner, P., Dexfenfluramine international study in obesity (ISIS): six month report. (Members of the ISIS Scientific Committee) Université Paris 6, Paris, France., *1st European Congress on Obesity*, Stockholm, June 29, 1989.

13 Harvey, J.A. and McMaster, S.E., Fenfluramine cumulative neurotoxicity after chronic treatment with low doses in the rat, *Communic. Psychopharmacol.*, 1 (1977) 3–17.

14 Hernandez, L., Auerbach, S. and Hoebel, B.G., Phencyclidine (PCP) injected in the nucleus accumbens increases extracellular dopamine and serotonin as measured by microdialysis, *Life Sci.*, 42 (1988) 1713–1723.

15 Hrdina, P.D., Regulation of high- and low-affinity [^{3}H]imipramine recognition sites in rat brain by chronic treatment with antidepressants, *Eur. J. Pharmacol.*, 138 (1987) 159–168.

16 Jacobson, I., Sandberg, M. and Hamberger, A., Mass transfer in brain dialysis devices — a new method for the estimation of extracellular amino acids concentration, *J. Neurosci. Methods*, 15 (1985) 263–268.

17 Kalen, P., Strecker, R.E., Rosengren, E. and Bjorklund, A., Endogenous release of neuronal serotonin and 5-hydroxyindoleacetic acid in the caudate putamen of rat as revealed by intracerebral dialysis coupled to high-performance liquid chromatography with fluorimetric detection, *J. Neurochem.*, 51 (1988) 1422–1435.

18 Kannengiesser, M.H., Hunt, P.F. and Raynaud, J.P., Comparative action of fenfluramine on the uptake and release of serotonin and dopamine, *Eur. J. Pharmacol.*, 35 (1976) 35–43.

19 Kennet, G.A., Chaouloff, F., Marcou, M. and Curzon, G., Female rats are more vulnerable than males in an animal model of depression: the possible role of serotonin, *Brain Research*, 382 (1986) 416–421.

20 Kleven, M.S., Schuster, C.R. and Seiden, L.S., Effect of depletion of brain serotonin by repeated fenfluramine on neurochemical and anorectic effects of acute fenfluramine, *J. Pharmacol. Exp. Ther.*, 246 (3) (1988) 822–828.

21 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J., Protein measurement with the folin phenol reagent, *J. Biol. Chem.*, 193 (1951) 265–275.

22 Mennini, T. and Garattini, S., Personal communication.

23 Milner, J. and Wurtman, R.J., Tyrosine availability determines stimulus-evoked dopamine release from rat striatal slices, *Neurosci. Lett.*, 59 (1985) 215–220.

24 Mizenberg, M. and Auerback, S., Measurements of serotonin release by in vivo dialysis in hypothalamus of freely moving rats, *Soc. Neurosci. Abstr.*, 13 (1987) 831.

25 Morgan, D., Lofstrandh, S. and Costa, E., Amphetamine analogues and brain amines, *Life Sci.*, 11 (1972) 83–96.

26 Nathan, C., Pharmacological treatments that affect CNS activity: Serotonin. In R.J. Wurtman and J.J. Wurtman, (Eds.), *Human Obesity, Vol. 1*, New York Academy of Sciences, 1987, pp. 277–296.

27 Nissl, F., Ueber Rosin's neue Faerbemethode des gesammten Nervensystems und dessen Bemerkungen ueber Ganglienzellen, *Neurol. Zentralblatt*, 13 (1894) 141–144.

28 Paxinos, G. and Watson, C., *The Rat Brain*, Academic Press, 1986.

29 Rowland, N.E. and Carlton, J., Neurobiology of an anorectic drug: fenfluramine, *Prog. Neurobiol.*, 27 (1986) 133–162.

30 Rowland, N.E. and Carlton, J., Dexfenfluramine: Effect on food intake in various animal models, *Clin. Neuropharmacol.*, 11 Suppl. 1 (1988) S33–50.

31 Schaechter, J.D. and Wurtman, R.J., Effect of chronic D-fenfluramine administration on rat hypothalamic serotonin levels and release, *Life Sci.*, 44 (1989) 265–271.

31a Schaechter, J.D. and Wurtman, R.J., Tryptophan availability modulates serotonin release from rat hypothalamic slices, *J. Neurochem.*, in press.

32 Schwartz, D.H., Kloecker, J.B., Hernandez, L. and Hoebel, B.G., Fenfluramine increases extracellular serotonin measured by microdialysis in freely moving rats, *Soc. Neurosci. Abstr.*, (1987) 336.

33 Steranka, L.R. and Sanders-Bush, E., Long-term effects of fenfluramine on central serotoninergic mechanisms, *Neuropharmacology*, 18 (1979) 895–903.

34 Ungerstedt, U. and Hallstrom, A., In vivo microdialysis —A new approach to the analysis of neurotransmitters in the brain, *Life Sci.*, 41 (1987) 861–864.

35 Wurtman, J.J., Wurtman, R.J., Mark, S., Tsay, T., Gilbert, W. and Growdon, J., D-fenfluramine selectively suppresses carbohydrate snacking by obese subjects, *Int. J. Eating Disorders*, 4 (1985) 89–99.