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Neuropharmacological Effects of Low and High Doses of Repeated Oral Dexfenfluramine in Rats: A Comparison with Fluoxetine

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CACCIA S., S. CONFALONIERI, A. BERGAMI, C. FRACASSO, M. ANELLI AND S. GARATTINI. *Neuropharmacological effects of low and high doses of repeated oral dexfenfluramine in rats. A comparison with fluoxetine.* PHARMACOL BIOCHEM BEHAV **57**(4) 851–856, 1997.—The neuropharmacological effects of repeated oral doses of dexfenfluramine (DF; 1.25–10 mg/kg, twice daily for 21 days) were examined in rats and related to the drug brain levels. Results were compared with fluoxetine (FL) given at similar doses relative to its anorectic ED_{50}. Both drugs dose-dependently slowed body weight gain and reduced brain serotonin (5-HT). However, at 1.25 mg/kg DF caused only a slight and transient decrease in cortical 5-HT. Comparable doses of FL (6.25–12.5 mg/kg) lowered 5-HT more than DF, besides slightly reducing striatal dopamine. At higher doses DF markedly reduced 5-HT in all regions, and to a lesser extent noradrenaline in hippocampus. There was a negative relationship between 5-HT and log total active drug levels and the indole was approximately halved at drug levels about 50 times lower with DF than FL. However, the ratio between drug levels causing marked 5-HT reductions and those considered anorectic was similar for DF and FL because brain levels at the anorectic ED_{50} were higher with FL than DF. Long-lasting reductions of 5-HT were also observed but recovery was only consistently slow beginning from 5 mg/kg DF. Comparable doses of FL could not be used because its general toxicity leads to the death of rats after only 2–4 multiples of its anorectic ED_{50}. © 1997 Elsevier Science Inc.

Dexfenfluramine Fluoxetine Repeated dosing Drug brain concentrations
Indole and catecholamine contents

DEXFENFLURAMINE (DF) is an anorectic agent developed from its racemic form fenfluramine because of its more specific activity on food intake. Its repeated administration in animals causes a dose-related reduction of brain serotonin (5-HT) and other markers thought to reflect the integrity of projections of serotoninergic neurons (7,14,16,23). In analogy with findings on neurotoxic amphetamine derivatives it has been argued that these effects may indicate a potential toxicity of the drug on 5-HT neurons (14,16). However, DF has been found to cause a transient decrease of 5-HT from neocortical serotoninergic axons (loss of immunoreactivity) in the rat without evidence of axon pathology after high oral doses (13). Unlike other amphetamine derivatives, its indole-depleting effect in mice was not accompanied by evidence of structural injury, suggesting that it does not damage brain serotoninergic pathways (20).

On the basis of studies in rats using *in vivo* microdialysis it has been postulated that DF may induce prolonged increases in intrasynaptic 5-HT content, causing persistent autoreceptor activation which may mediate long-term changes in the control of 5-HT synthesis and metabolism. This mechanism is shared by 5-HT uptake inhibitor anorectic agents such as fluoxetine (FL), sertraline and zimeldine (11) which also reduce brain 5-HT after repeated dosing in rat (2,5,26).

Controversy persists on whether the indole-depleting effects of DF reflect a dose-related extension of its acute neuropharmacological actions or are a characteristic of its toxic effects. An important issue, however, is the ratio between the

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dose causing anorexia and those inducing marked, persistent depletion of 5-HT-ergic markers. Earlier studies of the neurochemical effects of repeated regimens in animals have been contradictory since decreases (14), no change (6,22,25) or even increases (27) in brain 5-HT has been found after doses in the range of anorectic activity of DF as such or in the racemic form.

In the present study therefore we examined the initial and long-term neuropharmacological effects of a range of repeated oral doses of DF in the rat, which has been widely used in neurochemical studies of this drug. Results were compared with FL given at similar doses relative to its anorectic ED_{50} in this species. The relationships between the neurochemical effects and the brain concentrations of DF and FL and their active metabolites—dexnorfenfluramine (DNF) or norfluoxetine (NFL)—were also examined.

METHODS

Animals and Drug Treatment

Young adult male CD-COBS rats weighing about 150–175 g (Charles River, Italy) were housed five for cage and were used one week after their arrival. Procedures involving animals and their care were conducted in conformity with the institutional guidelines that are in compliance with national (D.L. n. 116, G.U., suppl 40, 18 Febbraio 1992) and international laws and policies (EEC Council Directive 86/609, OJ L 358,1, Dec. 12, 1987; NIH Guide for the Care and Use of laboratory animals, NIH Publication No 85–23, 1985).

DF hydrochloride (Les Laboratoires Servier, Gidy, France) and FL hydrochloride (Menarini, Florence, Italy) were dissolved in water and administered orally twice daily (at 0800 and 1800) for three weeks at doses of 1.25–10 mg/kg for DR and 6.25–50 mg/kg for FL. Control animals received the vehicle only and were either allowed food ad lib or their food intake was restricted to that of rats given the higher doses of drugs (pair-fed controls).

In a first study rats were killed by decapitation 4 h after the last dose of vehicle or drug. In a subsequent study rats were given DF (1.25–5 mg/kg) or FL (6.25–25 mg/kg) and were killed two or eight weeks after the last injection. Brain areas were dissected and stored at −80°C. Blood samples were collected in heparinized tubes, centrifuged and the plasma was stored at −20°C until assayed.

Assay Methods

Concentrations of 5-HT and 5-HIAA, noradrenaline (NA), dopamine (DA) and its acidic metabolites homovanillic acid (HVA) and dihydroxyphenylacetic acid (DOPAC) in selected brain regions were measured by high-performance liquid chromatography with electrochemical detection (2).

Plasma and brain concentrations of DF and DNF were analyzed by the gas chromatographic procedures previously described (3). FL and NFL were extracted from plasma and brain (previously homogenized in water and deproteinized with 100 μl of 20% TCA) with hexane/isoamyl alcohol (98/2 v/v), after adding imipramine as internal standard. Quantitation was by high-performance liquid chromatography, as described by Norman et al., (19), with minor modifications (i.e., the column was a μBondapack C18, 30 cm × 3.9 mm ID, particle size 10 μM and the mobile phase was 33:67 acetonitrile-KH_2PO_4 (0.016 M), buffered to pH 2.85 with H_3PO_4. In these experimental conditions, approximate retention times were 14 min for imipramine, 16 min for NFL and 18 min for FL. The coefficients of variation were generally less than 10% for plasma and brain samples containing 0.05–1 μg/ml of FL and NFL.

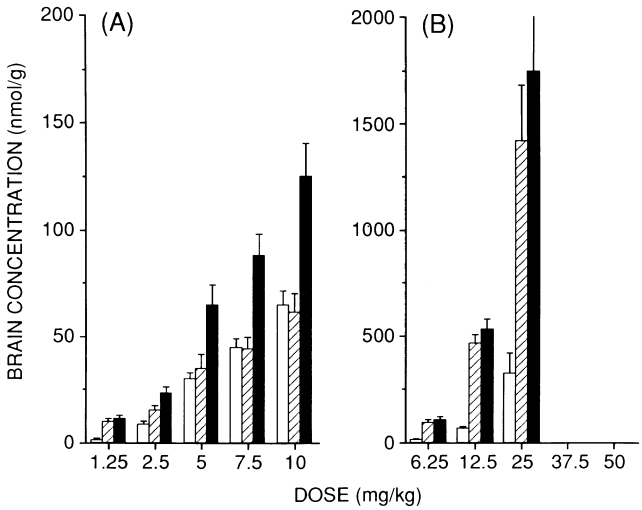

FIG. 1. Brain concentrations of the parent drug (□), normetabolite (▨) and total drug (■) after increasing oral doses of dexfenfluramine (A) and fluoxetine (B) in rats. Animals ($n = 5$) were given the drugs at the doses indicated for three weeks and killed 4 h after the last dose. At the higher fluoxetine doses all animals died.

Statistical Analysis

The effects of dose on body weight, monoamine and drug contents were assessed by one-way analysis of variance with post hoc comparisons, using Duncan's multiple *t*-test. Least-squares linear regression was used to analyze for significant correlations between pharmacological and biochemical parameters and brain drug (or log drug) concentrations. The criterion for statistical significance was $p < 0.05$.

RESULTS

Behavioural Changes and Mortality

Rats readily tolerated the DF dosing regimen and even the highest multiple of the acute anorectic ED_{50} (1.25 mg/kg) did not induce gross behavioural changes. Rats given FL were generally distinguished by restlessness and hyper-reactivity to handling. Doses up to 12.5 mg/kg were apparently tolerated but just above this the drug was markedly toxic. At 25 mg/kg (first study) one of the five rats died just after dosing on day 9 and two of the survivors showed slight convulsions on the last day of dosing. At 37.5 and 50 mg/kg all rats died on the 7th–9th day.

Plasma and Brain Levels of Drugs

The mean brain concentrations of DF and FL, their normetabolites and total drug, 4 h after the last dose of the three-week oral regimen, are presented in Fig. 1. The brain concentrations of unchanged compound rose faster than the dose. Compared to the lowest dose (1.25 mg/kg for DF and 6.25 mg/kg for FL) the increase in mean brain concentrations approximated 7, 22, 33 and 48 at 2.5, 5, 7.5 and 10 mg/kg DF and 5 and 15 at 12.5 and 25 mg/kg FL, this being consistent with the dose-dependent kinetic behaviour of DF and FL in rats (2,3). The metabolite-to-parent drug ratio also changed with dose, decreasing from about 7 after the lowest doses of both drugs to less than 1 for DF at the higher doses and about 4 for FL at the maximum tolerated dose.

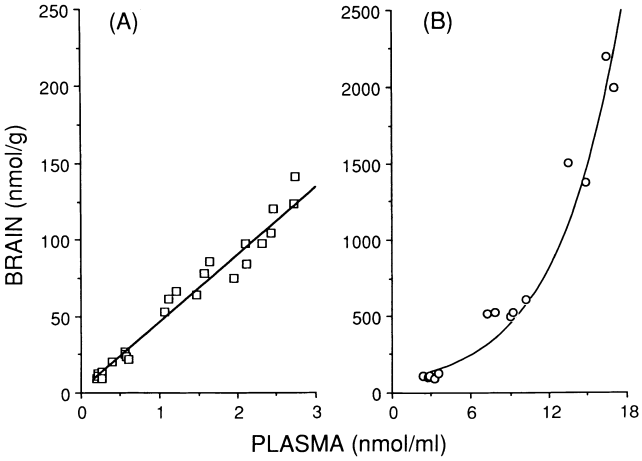

FIG. 2. Relationship between total drug plasma and brain levels after repeated oral doses of dexfenfluramine (A) and fluoxetine (B) in rats. Dexfenfluramine doses ranged from 1.25 to 10 mg/kg and fluoxetine from 6.25 to 25 mg/kg twice daily for three weeks and rats ($n = 5$) were killed 4 h after the last dose.

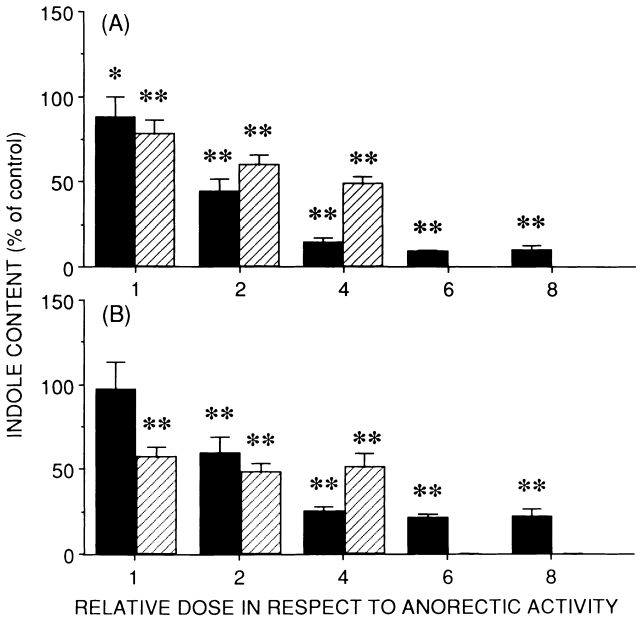

FIG. 3. Mean cortical contents of serotonin (A) and 5-hydroxyindoleacetic acid (B) 4 h after the last dose of three-week regimens of oral dexfenfluramine (1.25, 2.5, 5, 7.5 and 10 mg/kg twice daily; ■) and fluoxetine (6.25, 12.5 and 25 mg/kg twice-daily; ⊠). $^{**}p < 0.01$; $^{*}p < 0.05$ vs. vehicle.

Brain concentrations of DF and DNF and thus of total drug were linearly related to plasma concentrations ($r^2 = 0.96$, $p < 0.01$; Fig. 2A). By contrast, with FL and NFL the brain levels rose more than those in plasma on raising the FL dose (Fig. 2B). Consequently the brain-to-plasma total drug concentration ratio rose on increasing the FL dose, ranging from about 40 at 6.25 mg/kg to more than 100 at 25 mg/kg.

Effects on Body Weight

Both DF and FL dose-dependently slowed body weight gain compared to vehicle-treated rats. At the end of the three-week period the weight gain of the DF-treated groups ranged from about 96% (129 ± 11 g, at 1.25 mg/kg) to 67% (91 ± 12 g, at 10 mg/kg) of the control group (135 ± 18 g). The effect of 5–10 mg/kg DF (17 to 33% reduction compared to the gain of the vehicle-treated rats) was comparable to that of 6.25–12.5 mg/kg FL (17 to 38% of reduction). A dramatic reduction of body weight gain was seen with 25 mg/kg FL (≥ 50% reduction from the control value), but this is probably indicative of the compound's general toxicity.

Effect on Indole Contents

At 1.25 mg/kg, DF reduced cortical 5-HT by approximately 12% compared to control (0.25 ± 0.3 μg/g) (Fig. 3), with no significant effects in hippocampus (0.30 ± 0.05 vs 0.31 ± 0.03 μ/g) or striatum (0.33 ± 0.06 vs 0.34 ± 0.02 μg/g) 4 h after dosing. Comparable doses of FL (6.25–12.5 mg/kg) reduced 5-HT by approximately 20–40% and 5-HIAA by 40–50%, with no noteworthy regional differences.

The contents of indoles were reduced more in all regions after higher doses of DF, the effect again tending to be greater in cortex (Fig. 3) than hippocampus and striatum (data not shown). There was a negative correlation between cortical 5-HT (as % of control) and logarithm total drug level (%5-HT content = −32ln drug concentration + 152; $F(1, 23) = 254$; $p < 0.01$). The same was true with FL (%5-HT content = −11ln drug concentration + 127; $F(1, 12) = 54$; $p < 0.01$). The total drug levels causing a 50% reduction of cortical 5-HT, estimated from these equations, were about 20 nmol/g for DF and 1100 nmol/g for FL.

Effect on Catecholamine Contents

At doses higher than 2.5 mg/kg DF reduced hippocampal NA, by almost half at the highest dose (vehicle 0.35 ± 0.05 μg/g and pair-fed control 0.34 ± 0.02 μg/g) (Fig. 4A). This decrease was linearly related to the total drug concentration in brain (NA content = −0.40 drug concentration + 103; $F(1, 23) = 44.98$, $r^2 = 0.66$, $p < 0.01$). Cortical NA was only marginally affected by high doses of DF (≤ 20% of reduction). FL did not appreciably affect NA in any of the brain regions considered.

DA and its acidic metabolites DOPAC and HVA were not appreciably affected by DF in a typical dopaminergic area such as the striatum. In contrast FL slightly lowered striatal DA, from about 15% at 12.5 mg/kg to 30% at 25 mg/kg compared to the control value of 6.68 ± 0.67 μg/g (Fig. 4B).

Long-Term Studies

Two weeks after 1.25 mg/kg of DF no significant change was seen in cortical levels of indoles (Table 1). Almost complete recovery was observed at 2.5 mg/kg, the content of 5-HT and 5-HIAA averaging 90% of the vehicle-treated rats (0.27 ± 0.02 and 0.13 ± 0.01 μg/g for 5-HT and 5-HIAA). At 5 mg/kg DF reduced both indoles by about 60% and reductions were still significant eight weeks after treatment (vehicle 0.35 ± 0.05 and 0.19 ± 0.03 μg/g and pair-fed control 0.36 ± 0.04 and 0.18 ± 0.02 μg/g for 5-HT and 5-HIAA), although recovery was obvious compared to the depletions shortly after dosing. Catecholamines were never affected by these doses of DF.

FL doses up to 12.5 mg/kg had no significant effects on cortical contents of indoles (and DA), two weeks after the

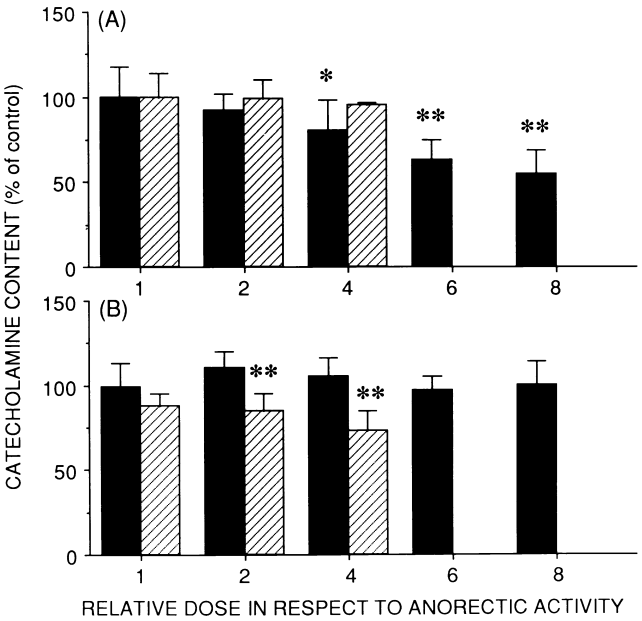

FIG. 4. Dose-dependent reductions of hippocampal noradrenaline (A) and striatal dopamine (B) 4 h after the last dose of three-week twice-daily regimens of oral dexfenfluramine (1.25, 2.5, 5, 7.5 and 10 mg/kg; ■) and fluoxetine (6.25, 12.5 and 25 mg/kg; ▨). $^{**}p < 0.01$; $^{*}p < 0.05$ vs. vehicle.

last dose. This is consistent with previous studies suggesting that the duration of the neurochemical effects of FL parallels the kinetics of elimination of the drug, particularly its active nor-metabolite, in rat brain (2,26). However, unlike DF, higher doses could not be used because of this drug's toxicity. In this study two rats given 25 mg/kg FL died on day 8 and two others had convulsions just after dosing on day 8–9 and were therefore immediately killed. Higher doses of DF and obviously FL were not considered in this study.

DISCUSSION

In rats DF is quite effective in reducing food consumption, possibly because of its ability to enhance serotoninergic transmission by potentiating the release of 5-HT and inhibiting its inactivation by reuptake. Its ED_{50} in an acute food deprivation paradigm is generally of the order of 0.6–1.25 mg/kg (10,22, 24). In similar paradigms FL is less active than DF, with anorectic ED_{50} in the range of 7–15 mg/kg (5,9,18). FL too blocks 5-HT's presynaptic neuronal reuptake of 5-HT (9) and through a different mechanism from DF enhances 5-HT release (10). However FL is currently thought to be a more specific 5-HT uptake blocker than DF (8). FL counteracts the 5-HT release induced by DF (21) and prevents its high-dose indole-depleting effect (17).

According to the known mechanisms of interaction within the serotoninergic system, repeated low doses of DF (1.25 mg/kg, twice daily) slightly and transiently lowered brain 5-HT. Comparable doses of FL (6.25–12.5 mg/kg, twice daily) caused greater initial indole lovering than DF, probably reflecting qualitative and quantitative differences in the mechanisms by which these drugs and their active nor-derivatives affect 5-HT release and block its uptake (8–10). FL was also more active than DF in slowing rats' weight gain, although it is difficult to distinguish FL's anorectic effect from its toxic action. However, the lower activity of DF than FL on rats' weight gain may be partly related to the fact that the anorectic activity of DF in rats is short-lasting and partial tolerance develops rapidly with repeated dosing (22–24).

Regional NA and DA contents were not affected by low doses of DF, confirming the relative specificity of the effect on 5-HT in rat brain (10). Higher doses dose-dependently reduced NA, with regional differences. Hippocampal NA content was negatively related to total drug concentration. In other brain areas (hypothalamus) NA has been implicated in the control of eating behaviour (15,24) but whether the reductions partly mediate the high-dose effects of DF remains to be established. As also observed after repeated intraperitoneal doses (2,12) FL did not affect regional NA but slightly reduced DA, again distinguishing this drug's neurochemical profile in rat from that of DF.

TABLE 1

RECOVERY OF CORTICAL SEROTONIN (5-HT) AND 5-HYDROXYINDOLEACETIC ACID (5-HIAA) WITH TIME IN RATS AFTER REPEATED ORAL DOSES OF DEXFENFLURAMINE AND FLUOXETINE

Time (weeks)	Relative Dose	Indole Content (% of control)			
		DF		FL	
		5-HT	5-HIAA	5-HT	5-HIAA
2	1[a]	102 ± 5	94 ± 6	97 ± 5	91 ± 9
	2	90 ± 2*	91 ± 3	102 ± 8	104 ± 13
	4	39 ± 2**	38 ± 8**	(b)	
8	4	51 ± 7**	45 ± 5**	(−)	

Rats were given dexfenfluramine (DF) or fluoxetine (FL) orally twice daily for 21 days and killed 2 or 8 weeks after the last dose ([a]1.25 and 6.25 mg/kg for DF and FL respectively).

Means ± SD of five rats; (b) = Convulsions/death; (−) = Not tested; $^{**}p < 0.01$; $^{*}p < 0.05$ vs control.

Reduction of brain 5-HT was more marked at higher drug doses, as expected (2,26,14,16,27). With both drugs there was a significant relationship between cortical 5-HT and log total drug concentrations and the indole was reduced approximately 50% at total drug levels about 50 times lower with DF than FL. However, the ratio between the drug levels causing marked indole lowering and those considered effective in reducing food intake was not so different between DF (about 3) and FL (between 3 and 15) because FL and NFL brain levels at the dose of 6.25 mg/kg were approximately 10 times the total drug brain concentrations found after 1.25 mg/kg DF and even higher (about 50 times) at 12.5 mg/kg because of the marked dose-dependent behaviour of FL in the rat.

DF also caused dose-dependent, prolonged lowering of 5-HT and 5-HIAA in a particularly sensitive region such as the cortex. However, persistent depletion of indoles (>8 weeks) only occurred from the dose of 5 mg/kg which resulted in six times higher brain exposure to total drug than after 1.25 mg/kg, most likely because of saturation of hepatic clearance with the higher doses (3). These long-term depletions are weaker than reported in squirrel monkeys (16) but stronger than in mice (7) given similar doses of DF parenterally. This may be related to the different initial depletions which in turn may depend on the different total drug concentrations achieved in the brain of these species (1). This study should provide further information for a better risk assessment of this drug's toxicological profile and contribute, with other pharmacokinetics and neurochemical studies, to a better extrapolation of animal findings to the therapeutic situation.

In summary, while the mechanism(s) underlying the long lasting effects of DF remain to be clarified, in rats there appears to be a relatively ample margin in favour of the pharmacological activity. Unfortunately, comparable doses of FL could not be tested because of the drug's general toxicity which led to the death of rats after only 2–4 multiples of the pharmacologically effective doses. This could be a consequence of the disproportionate rises in total drug brain concentrations, possibly due to both saturation and hepatic clearance and—unlike DF and DNF—enhanced brain uptake of the parent compound and its active metabolite at high repeated doses.

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