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FLUVOXAMINE, A SPECIFIC 5-HYDROXYTRYPTAMINE UPTAKE INHIBITOR

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1 On the basis of both *in vitro* and *in vivo* experiments fluvoxamine has been characterized as a potential anti-depressant drug with almost exclusively 5-hydroxytryptamine (5-HT) uptake inhibiting properties.

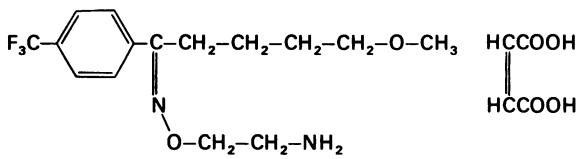
2 Fluvoxamine is effective in inhibiting 5-HT uptake by blood platelets and brain synaptosomes. Due to inhibition of the membrane pump the compound prevents 5-HT depletion by the tyramine-derivatives H 75/12 and H 77/77. As a result of the interference with the neuronal re-uptake mechanism for 5-HT, fluvoxamine produces a decreased 5-HT turnover in the brain. Effects of 5-hydroxytryptophan (5-HTP) are potentiated in mice and in combination with pargyline, fluvoxamine induces 5-HT-like behavioural effects.

3 In contrast to tricyclic antidepressants, noradrenaline uptake processes are either unaffected or only slightly inhibited by fluvoxamine. The noradrenaline depleting effects of tyramine derivatives are not influenced by fluvoxamine. Reserpine effects, such as ptosis are affected only at very high doses of the test compound. The antagonism by fluvoxamine of the reserpine-induced lowering of the pentamethylenetetrazole convulsive threshold can be regarded as due to an effect upon 5-HT uptake. In contrast to the effects of desmethylimipramine and imipramine, no stimulatory effects are found in rats when rapidly acting reserpine-like compounds are given following a dose of fluvoxamine.

Introduction

Growing support has been accumulating during recent years for the view that a disturbance in 5-hydroxytryptamine (5-HT) metabolism may be an important contributory factor in the development of depressive illness. It is to be expected that in the next few years significant improvement of the diagnostic classification of depressive disorders by biochemical means will clarify which subtypes of depression will benefit most by a normalization of 5-HT metabolism. On this basis, it can be postulated that, in addition to the available tricyclic antidepressant compounds which primarily affect the noradrenaline re-uptake, there is a need for a drug with a specific inhibitory effect on neuronal 5-HT re-uptake.

Compounds in the series of 2-aminoethyloximethers of aralkylketones possess inhibiting properties both with regard to neuronal noradrenaline (NA) and 5-HT re-uptake. The relative activity with respect to NA and 5-HT re-uptake processes is quite structure-specific. Fluvoxamine was selected from the series as being a compound with a rather high 5-HT re-uptake inhibiting activity but which has little effect on NA re-uptake.



Fluvoxamine

(E)-5-methoxy-4′-(trifluoromethyl)valerophenone

O-(2-aminoethyl)oxime maleate (1 : 1).

In this paper pharmacological and biochemical studies are presented which focus primarily on the characterization of the compound, with respect to inhibition of the neuronal re-uptake processes of 5-HT and NA.

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Methods

Potentiation of 5-hydroxytryptamine: inhibition of uptake processes

Inhibition of 5-hydroxytryptamine uptake by blood platelets. A blood platelet suspension in Sneddon's incubation medium (Sneddon, 1969) was prepared from guinea-pig blood. To ultramicrotubes containing 100 µl of a platelet-suspension, 10 µl of various concentrations of solutions of the test compound were added. The tubes were allowed to stand for 5 min at 37°C. After the addition of 20 µl of [^{14}C]-5-HT solution giving a final concentration of 5×10^{-6} M, the mixtures were incubated for 60 min at 37°C with occasional mixing. 5-HT uptake was terminated by cooling the tubes and immediate separation of the platelets by rapid centrifugation (Beckman-Sanz, micro-angle, 10,000 *g* for 4 minutes). The supernatant was decanted and the pellet washed twice with ice-cold medium and centrifuged. The resulting pellet was resuspended in 200 µl of a 4% solution of Triton-X-100 for complete lysis. A 150 µl sample was taken for liquid scintillation counting. Using as references the zero-time and the incubated control values, the pI_{50}-values, indicating the negative logarithm of the molar concentration causing 50% inhibition of the uptake, were calculated graphically for the test compounds.

Inhibition of 5-hydroxytryptamine uptake by rat brain synaptosomes. Male adult Wistar rats (240–300 g) were killed by cervical dislocation, their brains rapidly removed and a crude synaptosomal fraction (P_2) prepared by homogenization and differential centrifugation by the method of Whittaker (1969); 0.2 ml portions of the resuspended P_2-pellet, equivalent to approximately 25 mg original brain wet weight were pre-incubated for 4 min at 37°C. Tritium labelled 5-HT was then added and the incubation was continued for another 5 min (final volume 1.0 ml, inhibitor concentration 10^{-6} M, 5-HT concentrations 10^{-6}, 2×10^{-7} and 6.7×10^{-8} M). The incubation was stopped by cooling followed by filtration (Sartorius filter, pore size 0.6 µm). The residue was washed and the filter containing synaptosomes dissolved with 2 ml of 2-ethoxyethanol. The radioactivity was measured by liquid scintillation counting. Uptake data, corrected for 'uptake' at 0°C, were evaluated by construction of a Lineweaver-Burk plot. As a measure for the relative activity of the uptake inhibition, the K_i-value was calculated according to the formula $K_i = (i/K_{mi}/K_m - 1)$ where i is the molar concentration of the inhibitor and K_m and K_{mi} are the intercepts on the abscissa scale of the control and the inhibitor graphs respectively.

For displacement studies the crude synaptosomal fraction (P_2) was preloaded with [^{3}H]-5-HT by incubation at a concentration of 5×10^{-8} M for 20 min at 37°C. After centrifugation at 14,000 *g* for 10 min at 4°C the pellet was resuspended in fresh incubation medium. Displacement of [^{3}H]-5-HT was brought about by addition of unlabelled amine to aliquot samples and further incubation during 15 min at 37°C (final volume 1.0 ml, synaptosomal protein content approximately 1 mg, 5-HT concentrations 10^{-7}, 10^{-6} and 10^{-5} M). The effect of the uptake inhibitor on the displacement was studied by incorporation into the incubation medium of the test compound at a final concentration of 10^{-6} or 10^{-5} M. The content of [^{3}H]-5-HT remaining in the synaptosomes was determined as described above and was expressed as a percentage of the content in synaptosomes which were incubated without addition of unlabelled amine.

An estimate of the *in vivo* inhibition of the uptake process was obtained by studying the uptake properties *in vitro* of synaptosomes isolated from rats that had been treated with the inhibitor compounds. The test compound was administered intraperitoneally to male Wistar rats (250–280 g) at a dose of 25 mg/kg. Untreated animals were used as controls. The treated animals were killed after 30, 60 and 90 min respectively. The cerebral hemispheres were removed and homogenized in 6 ml of 0.32 M sucrose per g tissue as described above. Heavy debris was removed by centrifugation for 10 min at 1,000 *g*. The supernatant was used as the crude synaptosomal fraction (SN_1). Aliquots of the supernatant (2.0–2.6 mg protein) were preincubated for 4 minutes. 5-HT uptake was estimated by incubation with [^{3}H]-5-HT at concentrations of 5×10^{-8} and 10^{-7} M as described. The results were expressed as pmol 5-HT taken up per mg of synaptosomal protein during 5 minutes.

Inhibition of 5-hydroxytryptamine release by the tyramine derivatives H 75/12 and H 77/77. H 77/77 was administered intramuscularly to male Wistar rats (220–270 g) in two doses of 12.5 mg/kg; there was a 2 h interval between doses. The test compound was injected intramuscularly at a dose of 25 mg/kg 30 min before each H 77/77 injection. H 75/12 was injected with the same dosing schedule but using two doses of 25 mg/kg. Four hours after the first injection of the releasing substance the animals were killed, the brain removed and homogenized in acid *n*-butanol. The extract was analysed spectrofluorimetrically for 5-HT according to a modification of the method of Shore & Olin (1958).

Effect upon 5-hydroxytryptamine turnover in rat brain. The compound was administered intramuscularly to rats in a dose of 25 mg/kg together with an intraperitoneal injection of 200 mg/kg probenecid; 2 h later a second dose of probenecid was injected. Four hours after the first administration, the animals were killed. The brain was removed, cooled, and the cerebellum separated. The tissue was homogenized in acid *n*-butanol; 5-HT and 5-hydroxyindoleacetic acid

(5-HIAA) were separated by the normal acid extraction procedure. The cerebellum homogenate was used as a tissue blank. The concentration of 5-HT and 5-HIAA was measured fluorimetrically using an Aminco Bowman spectrofluorimeter. Correction factors for the calculation of the tissue concentration were obtained by performing the total procedure simultaneously with standard solutions.

The turnover rate was derived from the formula:

$$\frac{\text{(5-HIAA) treated} - \text{(5-HIAA) control}}{4 \times 191} \times 1000 \text{ nmol g}^{-1} \text{ h}^{-1}$$

In similar experiments the activity of fluvoxamine was demonstrated after oral application. The test compound was administered orally 15 min before the administration of probenecid at a dose of 200 mg/kg intraperitoneally. The animals were killed 2 h later, the total brain was removed and homogenized in acid *n*-butanol. After the usual separation procedures, 5-HIAA was determined fluorimetrically as its ortho-phthalicaldehyde fluorophor (Neff, Tozer & Brodie, 1967; Curzon & Green, 1970; Maickel, Cox, Saillant & Miller, 1968).

Potentiation of 5-hydroxytryptophan effects in mice. The test compound was administered orally in a range of doses to isolated male Swiss albino mice (5 mice per test dose), 1 h or 6 h respectively before the intraperitoneal injection of 150 mg/kg of DL-5-hydroxytryptophan (5-HTP), this being a threshold dose of 5-HTP. Thirty min after the 5-HTP injection, the mice were individually observed and the following parameters scored: stereotyped head-searching movements (on a 0 to 4 scale), hind limb abduction (0 to 2), tremor (0 to 4), escape tendency (0 or 1), fore limb clonus (0 to 2), lordosis (0 to 2). From the results, the ED_{50} values were determined by plotting log dose against total group score on a linear scale. The ED_{50} value is the dose required to potentiate the 5-HTP effect to 50% of the maximal score.

Potentiation of pargyline effects in mice. The induction of 5-HT-like effects was studied by administering fluvoxamine, in a series of doses, 4 h after administration of pargyline at a dose of 100 mg/kg intraperitoneally. The various parameters described in the previous section were scored 1 h after administration of the test compound, and the ED_{50} value was again determined graphically.

Antagonism of the reserpine-induced lowering of the pentamethylenetetrazole convulsive threshold. The test compound or 1% tragacanth was administered orally to groups of 10 male mice, 1 h before subcutaneous injection of reserpine (5 mg/kg); 2 h after the reserpine injection, a 0.45% solution of pentamethylenetetrazole was injected intravenously at the rate of 0.05 ml every 10 s (Orloff, Williams & Pfeiffer, 1949). The volume of solution producing the tonic extensor component of the convulsion was noted.

Potentiation of noradrenergic effects—inhibition of uptake processes

Inhibition of noradrenaline and dopamine uptake by rat brain synaptosomes. The effect of fluvoxamine on NA and dopamine uptake by rat brain synaptosomes was studied in the same manner as described for 5-HT.

Inhibition of NA and dopamine was also studied by displacement experiments as described for 5-HT.

The *in vivo* inhibition of NA and dopamine uptake processes was also studied with synaptosomes prepared from animals treated with the antidepressant compound. These experiments were performed in the same way as those described for 5-HT except, that [^{3}H]-NA and [^{3}H]-dopamine were used in place of [^{3}H]-5-HT.

Inhibition of noradrenaline release by H 75/12 and H 77/77. The method and dosage regimen used were as described for the experiments with these tyramine derivatives on 5-HT release. The acid *n*-butanol extract of brain was analysed for NA by a modification of the method of Welch & Welch (1969).

Potentiation of noradrenaline effects on rat vas deferens. The test was performed according to the method of Ursillo & Jacobson (1965).

Antagonism of tetrabenazine-induced ptosis in mice. The test compound was administered intraperitoneally or orally to groups of 5 male mice in a range of doses. After a time interval of 45 min tetrabenazine was injected subcutaneously at a dose of 80 mg/kg. Ptosis was scored 45 min after tetrabenazine administration by use of a ptosis chart (Rubin, Maline, Waugh & Burke, 1957). The dose (ED_{50}) of the test compound required to reduce the ptosis score to half that of the control tetrabenazine group was determined graphically by plotting responses on a probit scale against test doses on a logarithmic scale.

Antagonism of tetrabenazine-induced ptosis and induction of compulsive hyperactivity in rats. The method was as described in the preceding paragraph except that male albino rats were employed, a dose of 40 mg/kg of tetrabenazine was used and ptosis was scored by 2 observers at 90 min after tetrabenazine administration. During this test, the animals were also scored for the appearance of a hyperactive state. On the basis of the number of rats per dose group showing such behaviour, the hyperactivity ED_{50} was calculated (Horn, 1956).

Drugs

The following drugs were used: pargyline (Abbott Labs); tetrabenazine (Hoffman La Roche); chlorimipramine, desmethylimipramine (DMI), imipramine and reserpine (Ciba Geigy); probenecid (Merck, Sharp & Dohme); 5-HTP and 5-HT (Koch Light Labs); H 75/12 (Labkemi); H 77/77 (Axel Kistner).

5-Hydroxytryptamine creatinine sulphate [side chain 2-^{14}C], 56 mCi/mmol was obtained from Radiochemical Centre, Amersham.

The following radiochemicals were obtained from NEN chemicals: (−)-noradrenaline[7-^{3}H], 6.41 Ci/mmol; 3,4-dihydroxyphenylethylamine ethyl-[2-^{3}H], 5.9 Ci/mmol; 5-hydroxytryptamine bioxalate[1,2-^{3}H], 1.0 Ci/mmol.

Results

Potentiation of 5-hydroxytryptamine effects: inhibition of uptake processes

Inhibition of 5-hydroxytryptamine uptake by blood platelets. The activity of fluvoxamine in inhibiting 5-HT uptake by blood platelets was comparable to the activity of imipramine (Table 1). Both compounds were markedly more active than tricyclic secondary amines such as DMI.

Inhibition of 5-hydroxytryptamine uptake by rat brain synaptosomes. The Lineweaver-Burk plots for fluvoxamine, imipramine and DMI demonstrated a common intercept on the ordinate indicating no change in V_{max}. From this it may be concluded that the three compounds caused a competitive inhibition of the uptake process. Fluvoxamine showed the highest activity (Table 2).

Table 1 Inhibition of 5-hydroxytryptamine uptake in guinea-pig blood platelets

Compound	pI_{50}
Fluvoxamine	6.4 ± 0.20 (4)
Chlorimipramine	8.2 ± 0.55 (3)
Imipramine	6.8 ± 0.12 (14)
Desmethylimipramine	4.9 ± 0.20 (2)

Values are the means ± s.e. mean; the number of determinations is given in parentheses. The values of pI_{50}, indicating the negative logarithm of the molar concentration causing 50% inhibition of the uptake, were obtained from the graph relating log dose and percentage inhibition.

Increasing concentrations of unlabelled 5-HT produced an increasing release of labelled 5-HT from preloaded rat brain synaptosomes. In the concentrations used, fluvoxamine and imipramine themselves caused a small but significant release. Under these experimental conditions fluvoxamine inhibited the release of radioactive material by 5-HT up to a 5-HT concentration of 10^{-6} M. Fluvoxamine was in this respect at least 10 times more active than imipramine (Table 3).

Fluvoxamine or imipramine administration to rats 30, 60 or 90 min before they were killed, produced a significant inhibition of the uptake process of the synaptosomes when studied *in vitro*. The inhibitory effect was already apparent after 30 min and did not diminish during the longer time-intervals. In accordance with the results obtained in the *in vitro* experiments, fluvoxamine showed a markedly stronger inhibition than imipramine (Table 4).

Inhibition of 5-hydroxytryptamine release by the tyramine derivatives H 75/12 and H 77/77. Both H 75/12 and H 77/77 produced a marked depletion of brain 5-HT. Fluvoxamine, given twice at 25 mg/kg was very active in inhibiting the depleting action of both compounds. A clear protective effect against depletion by H 75/12 was found for imipramine at the same dose though the inhibitory effect was much less than for fluvoxamine. DMI caused a significant but rather small inhibition of the depletion by H 77/77 (Table 5).

Effect upon 5-hydroxytryptamine turnover rate. Probenecid produced a marked increase in the brain 5-HIAA content, compared to the untreated control animals, by inhibition of the transport of 5-HIAA out of the brain. The turnover rate of 5-HT calculated from these results, amounted to 1.31 nmol g^{-1} h^{-1}, a value closely corresponding to values reported by other authors (Neff, Lin, Ngai & Costa, 1969).

Table 2 Inhibition of 5-hydroxytryptamine (5-HT) uptake in rat brain synaptosomes *in vitro*

Compound	$K_i \times 10^6$ *M*
Fluvoxamine	0.084
Imipramine	0.23
Desmethylimipramine	1.2

Crude synaptosome fraction was incubated for 5 min with [^{3}H]-5-HT at final concentrations of 10^{-6}, 2×10^{-7} and 6.7×10^{-8} M; inhibitor concentration 10^{-6}M. Uptake data plotted as Lineweaver-Burk graph; K_i value is calculated according to $Ki = (i/K_{mi}/K_m - 1)$ where i is the molar concentration of the inhibitor and K_m and K_{mi} are the intercepts on the abscissae of the control and inhibitor graphs respectively.

Table 3 Inhibition of displacement of [^{3}H]-5-hydroxytryptamine from rat brain synaptosomes by unlabelled amine.

5-HT concentration in incubation fluid	*[^{3}H]-5-HT content remaining in synaptosomes (% control)*				
(M)	*Control*	*Fluvoxamine*		*Imipramine*	
		10^{-6} M	10^{-5} M	10^{-6} M	10^{-5} M
0	100.0	91.0	82.7	90.8	86.7
10^{-7}	77.0	84.8	83.9	82.4	87.4
10^{-6}	24.7	80.1	84.1	42.8	74.5
10^{-5}	18.4	63.4	78.7	22.4	34.7

Values are the means of two incubation experiments performed in parallel. Crude synaptosomal fraction was preloaded with [^{3}H]-5-HT by *in vitro* incubation. Effect of inhibitor on displacement by unlabelled amine was measured during 15 min incubation.

Table 4 Inhibition of 5-hydroxytryptamine (5-HT) uptake in rat brain synaptosomes after *in vivo* dosing

Compound	*Uptake of [^{3}H]-5-HT (pmol mg^{-1} synapt. protein 5 min^{-1})*		
	30 min	*60 min*	*90 min*
Control		6.10 ± 0.14 (12)	
Fluvoxamine	1.25* ± 0.08 (4)	1.07* ± 0.07 (4)	0.98* ± 0.10 (4)
Imipramine	3.13* ± 0.19 (4)	3.26* ± 0.60 (4)	3.02* ± 0.21 (4)

Values are the means ± s.e. mean; the number of determinations is given in parentheses. Test compounds were administered intraperitoneally to male rats in a dose of 25 mg/kg. Brains were removed at indicated post-dose times. The crude synaptosomal fraction was incubated for 5 min with [^{3}H]-5-HT in a final concentration of 5×10^{-8} M.
* *P* value < 0.001 when compared to control group (Student's *t* test).

Fluvoxamine decreased the turnover rate to 0.84 nmol g^{-1} h^{-1}. Imipramine also produced a decrease in turnover rate, although to a lesser extent than fluvoxamine. DMI was inactive in this respect (Table 6).

After oral administration, fluvoxamine also greatly reduced 5-HT turnover as was apparent from the very strong inhibition of the increased 5-HIAA accumulation by probenecid, being 62% at a dose of 50 mg/kg and 95% at a dose of 70 mg/kg ($n = 5$).

Potentiation of 5-hydroxytryptophan behavioural effects. Fluvoxamine was effective in potentiating the central effects of 5-HT as observed when administered together with 5-HTP. The compound was clearly more active than the most active of the clinically used tricyclic antidepressants, namely chlorimipramine. Imipramine was clearly less active than chlorimipramine while DMI showed no activity.

From the ratio of the ED_{50} values measured 6 and 1 h respectively after administration of the test

Table 5 Inhibition of 5-hydroxytryptamine (5-HT) release from rat brain by H 75/12 and H 77/77

Test compound (2 × 25 mg/kg i.m.)	*Releasing agent*	*Brain 5-HT content (µg/g tissue)*	P *value*
Control	—	0.67 ± 0.03 (8)	
Fluvoxamine	—	0.66 ± 0.03 (7)	
Imipramine	—	0.55 ± 0.01 (6)	<0.01
Desmethylimipramine	—	0.54 ± 0.01 (6)	<0.01
—	H 75/12	0.23 ± 0.02 (8)	
Fluvoxamine	H 75/12	0.53 ± 0.03 (8)	<0.001
Imipramine	H 75/12	0.37 ± 0.02 (8)	<0.001
—	H 77/77	0.27 ± 0.02 (8)	
Fluvoxamine	H 77/77	0.51 ± 0.01 (8)	<0.001
Desmethylimipramine	H 77/77	0.32 ± 0.02 (8)	<0.05

Values are the means ± s.e. mean; the number of determinations is given in parentheses. H 77/77 was injected at two doses of 12.5 mg/kg with a 2 h interval between doses. Test compounds were injected intramuscularly 30 min before each H 77/77 injection. H 75/12 was injected using the same dosing schedule but using two doses of 25 mg/kg.
P values refer to comparison with appropriate control group (Student's *t* test).

Table 6 Effect upon 5-hydroxytryptamine (5-HT) turnover in rat brain

Probenecid pretreatment (2 × 200 mg/kg i.p.)	*Test compound (25 mg/kg i.m.)*	*Brain 5-HIAA conc (μg/g tissue)*	*Turnover rate (nmol g^{-1} h^{-1})*
—	Control	0.31 ± 0.018 (14)	
Probenecid	—	1.32 ± 0.058 (16)	1.31
Probenecid	Fluvoxamine	0.95* ± 0.042 (8)	0.84
Probenecid	Imipramine	1.04* ± 0.069 (8)	0.94
Probenecid	Desmethylimipramine	1.14 ± 0.043 (8)	1.08

Values for 5-hydroxyindoleacetic acid (5-HIAA) content are the means ± s.e. mean; the number of determinations is given in parentheses. Turnover rate calculated as

$$\frac{\text{(5-HIAA) treated} - \text{(5-HIAA) control}}{4 \times 191} \times 1000 \text{ nmol g}^{-1} \text{ h}^{-1}.$$

Test compound administered intramuscularly at same time as first dose of probenecid; second dose of probenecid after 2 h; animals killed 4 h after administration of test compound.
* P value < 0.01 when compared to probenecid control group (Student's t test).

compound, it is apparent that fluvoxamine had a long duration of action (Table 7).

Potentiation of pargyline effects. Fluvoxamine induced marked 5-HT-like effects when combined with pargyline. The tricyclic antidepressants showed no activity in this test (Table 8).

Antagonism of reserpine-induced lowering of the pentamethylenetetrazole convulsive threshold. Fluvoxamine at oral doses of 10, 20 and 40 mg/kg caused a statistically significant antagonism of the reserpine-induced lowering of the pentamethylenetetrazole convulsive threshold. Chlorimipramine was active in this respect at a dose of 20 mg/kg; imipramine and DMI, however, were inactive at this dose (Table 9).

Potentiation of noradrenergic effects: inhibition of uptake processes

Inhibition of noradrenaline and dopamine uptake by rat brain synaptosomes. Fluvoxamine, as well as the tricyclic antidepressants, inhibited the uptake processes of NA and dopamine in synaptosomes from total brains. The Lineweaver-Burk plots indicated a competitive mechanism for the inhibition. Fluvoxamine was almost as active as DMI in this experiment in inhibiting NA uptake, though the effect of both compounds was rather weak.

Increasing concentrations of unlabelled amine

Table 7 Potentiation of 5-hydroxytryptophan (5-HTP) effects in mice

Test compound	*Oral ED_{50} (mg/kg)*	
	90 min	*390 min*
Fluvoxamine	36 ± 4.3 (7)	141 ± 21 (3)
Chlorimipramine	84 ± 6.6 (5)	~240 (2)
Imipramine	135 ± 18 (4)	
Desmethylimipramine	> 320 (3)	

Values are the means ± s.e. mean; the number of determinations is given in parentheses. Test compound was administered to groups of 5 mice in a range of doses 60 min and 360 min respectively before the administration of an intraperitoneal dose of 150 mg/kg of 5-HTP. Behavioural parameters were scored after a further 30 min: stereotyped headsearching movements on a 0 to 4 scale, hindlimb abduction (0 to 2), tremor (0 to 4), escape tendency (0 or 1), forelimb clonus (0 to 2), lordosis (0 to 2). ED_{50} value, the dose of the test compound required to potentiate the 5-HTP effects to 50% of the maximum score, was obtained from the graph relating log dose and total group score.

Table 8 Potentiation of pargyline effects in mice

Test compound	*Oral ED_{50} (mg/kg)*
Fluvoxamine	20 ± 1.2 (4)
Chlorimipramine	> 320 (2)
Imipramine	> 320 (2)
Desmethylimipramine	> 320 (2)

The values are the mean ± s.e. mean; the number of determinations is given in parentheses. Pargyline was administered intraperitoneally 4 h before orally dosing the test compound to groups of 5 mice. For details of behavioural parameters see footnote to Table 7. ED_{50} value, the dose of the test compound required to induce a behavioural score equal to 50% of the maximum score, was obtained from the graph relating log dose and total group score.

Table 9 Antagonism of the reserpine-induced lowering of the pentamethylenetetrazole convulsive threshold

Test compound	*Oral dose (mg/kg)*	*Reserpine (5 mg/kg s.c.)*	*Convulsive dose pentamethylenetetrazole (ml 0.45% solution)*
Experiment 1			
Vehicle	—	—	0.66 ± 0.03
Vehicle	—	Reserpine	0.18 ± 0.03
Fluvoxamine	5	Reserpine	0.20 ± 0.03
Fluvoxamine	10	Reserpine	0.28 ± 0.05*
Fluvoxamine	20	Reserpine	0.39 ± 0.06*
Fluvoxamine	40	Reserpine	0.36 ± 0.06*
Experiment 2			
Vehicle	—	—	0.53 ± 0.06
Vehicle	—	Reserpine	0.17 ± 0.01
Chlorimipramine	5	Reserpine	0.22 ± 0.03
Chlorimipramine	10	Reserpine	0.18 ± 0.01
Chlorimipramine	20	Reserpine	0.28 ± 0.05*
Chlorimipramine	40	Reserpine	0.30 ± 0.06*
Experiment 3			
Vehicle	—	—	0.46 ± 0.06
Vehicle	—	Reserpine	0.18 ± 0.01
Imipramine	20	Reserpine	0.20 ± 0.04
Desmethylimipramine	20	Reserpine	0.19 ± 0.03

The values are the mean doses (ml ± s.e. mean) of a 0.45% pentamethylenetetrazole solution required to produce tonic extensor seizures. Test compounds were administered orally to groups of 10 mice 1 h before a subcutaneous dose of 5 mg/kg of reserpine. Seizure susceptibility was tested 2 h after reserpine administration by intravenous injection of the pentamethylenetetrazole solution at the rate of 0.05 ml every 10 seconds.
* P value $\leqslant 0.05$ when compared to reserpine-treated control group (Wilcoxon).

produced an increasing release of tritium-labelled NA and dopamine respectively from preloaded rat brain synaptosomes. In the concentrations used, fluvoxamine and DMI caused a small but significant release. Fluvoxamine at a concentration of 10^{-5} M inhibited NA displacement with an amine concentration of 10^{-7} M; at higher amine concentrations no inhibition was apparent. In contrast DMI at a concentration of 10^{-5} M caused a marked inhibition at all three NA concentrations. Fluvoxamine is at least 10 times less active than DMI in these experiments. Neither fluvoxamine nor DMI showed an effect against displacement of dopamine (Table 10).

Both fluvoxamine and imipramine, administered to

Table 10 Inhibition of displacement of [^{3}H]-noradrenaline and [^{3}H]-dopamine from rat synaptosomes by unlabelled amine

Amine concentration in incubation fluid	*[^{3}H]-noradrenaline content remaining in synaptosomes (% control)*				*[^{3}H]-dopamine content remaining in synaptosomes (% control)*			
		Fluvoxamine	*DMI*			*Fluvoxamine*	*DMI*	
(M)	Control	10^{-5} M	10^{-6} M	10^{-5} M	Control	10^{-5} M	10^{-6} M	10^{-5} M
0	100.0	93.6	93.2	92.8	100.0	94.9	97.0	94.4
10^{-7}	79.3	86.7	91.6	91.2	62.6	53.6	57.7	58.9
10^{-6}	61.4	63.1	68.1	73.5	21.6	20.1	23.9	23.8
10^{-5}	45.4	43.8	50.4	55.8	11.5	11.0	13.8	15.1

Values are the means of two incubation experiments performed in parallel. Crude synaptosome fraction was preloaded with [^{3}H]-noradrenaline or [^{3}H]-dopamine respectively by *in vitro* incubation. Effect of inhibitor on displacement by unlabelled amine was measured during 15 min incubation.

Table 11 Effect on uptake of noradrenaline and dopamine by rat brain synaptosomes after *in vivo* dosing

Compound	*Uptake of [^{3}H]-noradrenaline (pmol mg^{-1} synapt. protein 5 min^{-1})*			*Uptake of [^{3}H]-dopamine (pmol mg^{-1} synapt. protein 5 min^{-1})*		
	30 min	*60 min*	*90 min*	*30 min*	*60 min*	*90 min*
Control		3.70 ±0.16			16.3±0.47	
Fluvoxamine	3.55 ±0.06	3.56 ±0.12	3.38*±0.26	16.3±0.84	17.1±0.38	13.9*±0.61
Imipramine	3.36*±0.13	3.26*±0.18	2.91*±0.37	19.2±0.72	17.6±0.60	15.0 ±1.08

Values are the means ± s.e. mean; the number of determinations was 12 for the control group and 4 in all other cases. Test compounds were administered intraperitoneally to male rats at a dose of 25 mg/kg. Brains were removed at indicated post-dose times. The crude synaptosome fraction was incubated for 5 min with [^{3}H]-noradrenaline and [^{3}H]-dopamine in a final concentration of 5×10^{-8} M.
* P value < 0.05 when compared to control group (Student's t test).

rats at a dose of 25 mg/kg intraperitoneally, caused a significant inhibition of the uptake of noradrenaline by isolated synaptosomes; however in contrast to imipramine, which was active at all three time intervals studied, fluvoxamine exhibited only a small and non-significant effect at 90 min post-dose (Table 11).

A small significant inhibition of 15% of dopamine uptake was found with synaptosomes of animals treated with fluvoxamine 90 min before they were killed.

Inhibition of noradrenaline release by H 75/12 and H 77/77. The results of the depleting action of the tyramine derivatives on brain noradrenaline and of the interfering effects of the test compounds are summarized in Table 12. As can be seen from these results H 75/12 and H 77/77 both caused a substantial decrease in brain NA content. Fluvoxamine, in two doses of 25 mg/kg, did not protect against the depleting action of these agents. In contrast, DMI was very effective in preventing the depeletion of NA by H 77/77. None of the test compounds administered alone affected the NA content of the brain.

Potentiation of noradrenaline effects on rat vas deferens in vitro. Fluvoxamine was only weakly active in potentiating NA when compared with DMI and imipramine. Chlorimipramine produced only α-lytic effects (Table 13).

Antagonism of tetrabenazine-induced ptosis in mice. Fluvoxamine was rather inactive in this test in comparison with the tricyclic antidepressants. The finding that imipramine was more active orally than intraperitoneally might be due to the fact that the metabolism of imipramine to the more active DMI occurs to a larger extent after oral than after intraperitoneal administration (Table 14).

Table 12 Effect on noradrenaline release from rat brain by H 75/12 and H 77/77

Test compound (2 × 25 mg/kg i.m.)	*Releasing agent*	*Brain noradrenaline content (μg/g tissue)*
Control	—	0.34±0.009 (8)
Fluvoxamine	—	0.34±0.027 (7)
Imipramine	—	0.34±0.011 (6)
Desmethylimipramine	—	0.33±0.022 (6)
—	H 75/12	0.18±0.008 (8)
Fluvoxamine	H 75/12	0.16±0.006 (8)
Imipramine	H 75/12	0.20±0.008 (8)
—	H 77/77	0.13±0.007 (8)
Fluvoxamine	H 77/77	0.14±0.006 (8)
Desmethylimipramine	H 77/77	0.28*±0.008 (8)

Values are the means ± s.e. mean; the number of determinations is given in parentheses. H 77/77 was injected at two doses of 12.5 mg/kg with a 2 h interval. The test compounds were injected intramuscularly 30 min before each H 77/77 injection. H 75/12 was injected using the same dosing schedule but with two doses of 25 mg/kg.
* P value < 0.001 in comparison with appropriate control group (Student's t test).

Table 13 Potentiation of effect of noradrenaline (NA) on rat vas deferens *in vitro*

Test compound	*Minimal conc. causing potentiation (ng/ml)*	*Conc. causing optimal potentiation (ng/ml)*	*Degree of optimal potentiation*
Fluvoxamine	200 (2)	2000 (2)	3.4 (4) 2.8–4.0
Imipramine	7 (2)	70 (2)	3.8 (2) 3.4–4.4
Desmethylimipramine	2 (2)	70 (2)	19 (2) 12–31
Chlorimipramine	only α-lytic effects		

Minimum concentration value indicates lowest concentration of test compound at which NA-induced contraction of vas deferens was increased. Optimal concentration value indicates concentration above which no further potentiation of the NA-induced contraction could be obtained. In parentheses the number of experiments; for fluvoxamine in 2 experiments spontaneous contractions were observed at 2000 ng/ml. The minimal and optimal concentration were estimated by testing the test compounds in a concentration range of 2, 7, 20 etc. ng/ml in combination with a suprathreshold dose of NA. Degree of optimal potentiation: apparent relative potency of NA when measured in presence of optimal concentration of test compound in comparison to NA without test compound in a series of four-point assays; 95% confidence limits indicated.

Antagonism of tetrabenazine-induced ptosis and induction of compulsive hyperactivity in rats. Fluvoxamine had only a very weak activity against tetrabenazine-induced ptosis in the rat, in contrast to the tricyclic antidepressants (Table 15). Fluvoxamine was not active in inducing hyperactivity when administered at doses up to 215 mg/kg in combination with tetrabenazine. DMI and imipramine showed a clear activity, DMI being the most active compound in this respect. Chlorimipramine failed to induce compulsive hyperactivity at the highest dose tested, namely 46 mg/kg (Table 15).

Table 14 Antagonism of tetrabenazine-induced ptosis in mice

Test compound	ED_{50} *(mg/kg) Intraperitoneally administered*	ED_{50} *(mg/kg) Orally administered*
Fluvoxamine	35 ± 3.0 (2)	107 ± 25 (9)
Chlorimipramine	8.0 ± 1.0 (2)	12 ± 2.7 (4)
Imipramine	8.7 ± 1.0 (3)	5.2 ± 0.3 (5)
Desmethyl-imipramine	0.47 ± 0.17 (2)	0.80 ± 0.09 (7)

Values are the means ± s.e. mean; the number of determinations is given in parentheses. Test compound was administered to groups of 5 mice in a range of doses intraperitoneally or orally, 45 min before a subcutaneous dose of 80 mg/kg of tetrabenazine. Ptosis was scored 45 min after tetrabenazine administration using a ptosis chart (Rubin, Malone, Waugh & Burke, 1957). ED_{50} value, the dose of the test compound required to reduce the ptosis score to half that of the tetrabenazine control group, was obtained from the graph relating percentage response on a probit scale and log dose.

Discussion

There is increasing support for the view that different subgroups of patients with depressive disorders may

Table 15 Antagonism of tetrabenazine-induced ptosis and induction of hyperactivity in rats

Test compound	*Oral* ED_{50} *(mg/kg) Ptosis antagonism*	*Oral* ED_{50} *(mg/kg) Hyperactivity*
Fluvoxamine	⩾215 (2)	>215 (3)
Chlorimipramine	19 ± 2.4 (4)	>46 (4)
Imipramine	4.0 ± 0.16 (16)	15 ± 3.0 (2)
Desmethyl-imipramine	5.1 ± 1.0 (7)	8.3 (1)

Values are the means ± s.e. mean; the number of determinations is given in parentheses. Test compound was administered to groups of 5 rats in a range of doses 45 min before a subcutaneous dose of 40 mg/kg of tetrabenazine. Ptosis was scored 90 min after tetrabenazine administration using a ptosis chart. ED_{50} value, the dose of the test compound required to reduce the ptosis score to half that of the control tetrabenazine group, was obtained from the graph relating percentage response on a probit scale and log dose. The number of rats per dose group showing hyperactivity after tetrabenazine administration was scored. ED_{50} value, the dose of test compound required to induce, in combination with tetrabenazine, hyperactivity in 50% of the animals, was calculated according to Horn (1956).

exhibit different specific abnormalities in the metabolism of biogenic amines. This may be the reason why various clinically defined subtypes of depression respond differently to antidepressant treatment (Hollister, 1972).

From recent investigations it is clear that the catecholamine hypothesis does not adequately explain the depressed state. For instance the catecholamine precursor L-DOPA has been a failure in therapy, except for a small subgroup of retarded depressives. Lapin & Oxenkrug (1969) suggested an important involvement of 5-HT in the pathogenesis of affective disorders. According to this view, activation of the central adrenergic functions is responsible for the psychoenergetic and motor-stimulating effects of antidepressants, whereas activation of serotoninergic functions is responsible for their mood-elevating properties. Confirmation of a possible role of a disturbance of 5-HT metabolism in depression comes from studies in which a decreased 5-HT content in the brain and a decreased 5-HT turnover has been demonstrated in patients (Shaw, Camps & Eccleston, 1967; van Praag, Korf & Puite, 1970; van Praag, Korf & Schut, 1973). It must be stated however that not all studies are in agreement concerning the occurrence of a reduced level of 5-HT metabolites in the cerebrospinal fluid of depressed patients (Papeschi & McClure, 1971).

Since the significance of 5-HT in the pathogenesis of affective disorders has not been clarified, there seems to be an urgent need for a drug with a specific effect on the 5-HT re-uptake processes, both as a specific therapeutic agent in suitable patients as well as a tool in classifying depressed patients. Fluvoxamine promises to be a member of such a new class of drugs.

The marked activity of fluvoxamine as a 5-HT uptake inhibitor was demonstrated in a number of test systems. Blood platelets may be used as a model for storage-, release- and uptake-processes of 5-HT in the nerve ending. Todrick & Tait (1969) compared the inhibitory properties of a large series of tricyclic antidepressants on 5-HT uptake. They found that the tertiary amines were more potent inhibitors than their demethylated derivatives; this is in accordance with the *in vivo* results of Carlsson, Fuxe & Ungerstedt (1968). In our experiments, the inhibitory activity of fluvoxamine on 5-HT uptake by blood platelets was found to be comparable to the activity of imipramine (Table 1). Chlorimipramine appeared to be clearly more active, a result also obtained by Todrick *et al.* (1969). However, such a superior activity for chlorimipramine, was not found in a recent investigation by Tuomisto (1974) who studied the effects at very low substrate concentrations and with very short incubation times. Although the experiments of Tuomisto may be criticized on the basis of a badly controlled incubation temperature, this result of a comparable activity of imipramine and chlorimipramine would agree better with the results of our *in vivo* experiments, in which for chlorimipramine no outstanding 5-HT potentiating properties were found.

The 5-HT uptake inhibiting properties of fluvoxamine, imipramine and DMI were compared in studies with rat brain synaptosomes (Tables 2–4). All three compounds showed a competitive inhibition of the uptake process. Under both *in vitro* conditions and in studies in which synaptosomes from animals pretreated with the test compounds were used, it was apparent that fluvoxamine produced a markedly stronger inhibition of 5-HT uptake than imipramine. Imipramine in its turn was more active than DMI.

An indirect indication of the effectiveness of fluvoxamine in inhibiting the uptake mechanism of serotoninergic neurones was obtained in the *in vivo* experiments with H 75/12 and H 77/77. These tyramine derivatives utilize the neuronal membrane pump to enter the neurone, where they cause a depletion of 5-HT. Here a marked inhibitory activity of fluvoxamine was apparent (Table 5). Imipramine also showed a protective effect at the same dose level although to a lesser extent than did fluvoxamine; DMI was only weakly active. The change in turnover rate of the neurotransmitter *in vivo* after administration of the test compound is generally used as an indication of the change in the neuronal activity of that aminergic system. A direct or an indirect increase of the activity of postsynaptic fibres produces a negative feedback in the presynaptic system, which expresses itself by a lowering of the turnover rate of the transmitter. Both after intramuscular as well as after oral administration, fluvoxamine produced a marked decrease in 5-HT turnover rate as measured by the probenecid test (Table 6). Against the background of the effects of fluvoxamine shown in the various experiments, it may be concluded that this decrease of the turnover rate of 5-HT probably results from the inhibition of the re-uptake of 5-HT from the synaptic cleft by the presynaptic neurone. Imipramine showed the same effect on 5-HT turnover rate, but possibly to a lesser extent.

Further evidence for the prominent activity of fluvoxamine as a 5-HT uptake inhibitor was obtained in mice by the potentiation of the induction of behavioural effects by 5-HTP (Table 7). These 5-HTP-induced effects are probably due to the formation of an increased amount of 5-HT in the brain, since we found that these signs are suppressed by centrally acting anti-5-HT compounds and also by high doses of centrally acting decarboxylase inhibitors. In comparison with the tricyclic antidepressants, fluvoxamine possesses outstanding oral activity. This is demonstrated to an even greater extent by the induction of 5-HT-like effects in mice, pretreated with a MAO-inhibitor. All the tested tricyclic antidepressants were almost inactive in this test (Table 8).

Further suggestive evidence, for a potentiation of effects of 5-HT by fluvoxamine, was obtained from the

experiments on the antagonism of the reserpine-induced lowering of the pentamethylenetetrazole convulsive threshold (Table 9). Although most reserpine effects which are used for demonstrating antidepressant activity are dependent upon NA-mechanisms, the convulsion-facilitating effect may be attributable to the depletion of 5-HT (Boggan, 1973). In this test fluvoxamine also showed the highest activity; of the tricyclic antidepressants tested, only chlorimipramine was active. These results therefore support the hypothesis of Boggan (1973).

Whereas tricyclic antidepressants are characterized by a pronounced inhibition of the NA-uptake process, fluvoxamine in general demonstrated only a very weak NA-uptake inhibiting activity. A comparable, rather weak, uptake inhibiting activity was noted for fluvoxamine and DMI in experiments in which the uptake of labelled NA was measured. However, the use of total brain synaptosomes in this way does not differentiate very well between activities of NA uptake inhibitors (Schacht & Heptner, 1974). Fluvoxamine produced only a marginal NA-uptake inhibiting effect when the compound was intraperitoneally administered to the animal in a dose of 25 mg/kg (Table 11). Also in experiments in which the displacement of labelled NA from the synaptosomes by unlabelled amine in the incubation medium was studied, fluvoxamine was again much less active than the tricyclic antidepressant drugs (Table 10). The same ineffectiveness is apparent from the fact that in the rat, no protection was produced by fluvoxamine against the NA-depleting action of H 75/12 and H 77/77 (Table 12).

The NA effect on the rat vas deferens was potentiated by fluvoxamine only at much higher concentrations than those at which tricyclic antidepressants were active (Table 13).

Fluvoxamine is only weakly active in antagonizing reserpine-like effects; this is again in contrast to the tricyclic antidepressants (Tables 14–17). For example, fluvoxamine possesses an ED_{50} value of 107 mg/kg in antagonizing tetrabenazine-induced ptosis in mice and thus is 9 to 130 times less active than the tested tricyclic drugs. In antagonizing tetrabenazine-induced ptosis in rats, fluvoxamine was even less active. Experiments, not presented, have also shown that reserpine-induced hypothermia in mice was reversed by fluvoxamine at much higher dose levels than those at which tricyclic antidepressants showed a significant effect. In contrast to most tricyclic antidepressants fluvoxamine did not produce compulsive hyperactivity in the rat when administered in combination with tetrabenazine (Table 15).

Since reserpine-induced effects can be antagonized by monoamine oxidase (MAO) inhibitors, experiments were performed to show that fluvoxamine did not inhibit this enzyme. Fluvoxamine, at concentrations up to 10^{-3} M, did not cause significant inhibition of MAO using kynuramine and 5-HT as substrates and ox brain and liver as enzyme sources. In contrast to MAO inhibitors, fluvoxamine, at an oral dose of 460 mg/kg in mice, failed to potentiate the toxicity due to tryptamine (250 mg/kg s.c.) administered either 1 or 24 h later. This lack of *in vivo* MAO inhibitory activity was also confirmed by the finding that the compound did not cause a significant change in the NA, dopamine or 5-HT contents of rat brain or heart after daily dosing during either 7 or 35 days at a dose of 100 mg/kg per day.

Based on the biochemical and pharmacological studies reported here, fluvoxamine can be characterized as a potential anti-depressant drug with almost exclusively 5-HT-uptake inhibiting properties and the compound should therefore offer better possibilities for the treatment of depression in cases where disturbances of 5-HT metabolism are prominent.

The role of 5-HT in a number of physiological processes and possible deficiencies of serotoninergic functions in various pathological conditions seem to indicate that fluvoxamine should be tested in a number of additional disease states.

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