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FLUOXETINE AND TWO OTHER SEROTONIN UPTAKE INHIBITORS WITHOUT AFFINITY FOR NEURONAL RECEPTORS

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Abstract—Fluoxetine and nine other antidepressant drugs which interact with brain receptors for neurotransmitters were studied *in vitro* using radioligand-binding techniques and transmitter-coupled adenylate cyclase assays. Tricyclic antidepressant drugs (desipramine, imipramine, clomipramine, amitriptyline and doxepin) had marked affinity for alpha-adrenergic, muscarinic cholinergic and histaminergic H_1 receptors, and lesser affinity for serotonin and dopamine receptors. Mianserin was relatively similar to some of the tricyclic compounds, whereas trazodone had less affinity for most receptors except serotonin and alpha-adrenergic receptors. Fluoxetine had little affinity for any of these receptors, and the same was true for zimelidine and fluvoxamine, two other selective inhibitors of serotonin uptake. None of the compounds showed much affinity for beta-adrenergic receptors, opiate receptors, gamma-aminobutyric acid receptors, or benzodiazepine receptors. The present findings with fluoxetine are consistent with the virtual absence of anticholinergic or other side effects often observed with tricyclic antidepressant drugs in animal models or during the treatment of depressed patients.

A specific neuronal uptake process for serotonin (5-HT) was demonstrated in brain tissues [1–3] and found to be localized mainly in the synaptosomal fraction of brain homogenates [1, 4, 5]. The uptake of 5-HT was inhibited by tricyclic antidepressant drugs, especially those that are tertiary amines [2, 6], and by *p*-chloroamphetamine [5]. We found fluoxetine (LY110140) to be the first selective inhibitor of 5-HT uptake by synaptosomal preparations from rat brain [7, 8]. Fluoxetine was effective and selective as an inhibitor of brain 5-HT uptake *in vivo* [8, 9] and enhanced serotonergic neurotransmission without affecting catecholamine uptake or catecholaminergic neurotransmission centrally or peripherally [8, 10–12].

Some antidepressant drugs have affinity for certain neurotransmitter receptors, in addition to inhibiting monoamine uptake, and these effects might be responsible for side effects often observed clinically. The present studies compared fluoxetine with other antidepressant drugs and found that fluoxetine and two other inhibitors of serotonin uptake, zimelidine and fluvoxamine, have comparatively little affinity for most of the neurotransmitter receptors.

MATERIALS AND METHODS

Male Sprague–Dawley rats weighing 110–150 g were decapitated. Brains were immediately removed and dissected into specific brain regions. Calf brains from the slaughterhouse were transferred in ice. Corpus striatum and cerebellum were dissected from brain within 1 hr of death.

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Table 1. Procedure for ^{3}H-labeled ligand binding

^{3}H-Labeled ligand	^{3}H-Labeled ligand concn (nM)	Brain tissue	Protein (mg/ml)	Incubation Time (min)	Incubation Temp (°)	Ref.
[^{3}H]WB4101	0.5	Rat cortex	0.6	15	20	13
[^{3}H]Clonidine	0.8	Rat cortex	0.5	30	20	13
[^{3}H]Dihydroalprenolol	2	Rat cortex	0.4	20	20	14
[^{3}H]Serotonin	2	Rat frontal cortex	0.4	10	37	15, 16
[^{3}H]Pyrilamine	2	Rat cortex	0.5	30	25	17
[^{3}H]Quinuclidinyl benzilate	0.2	Rat cortex	0.1	60	25	18
[^{3}H]Spiperone	0.5	Bovine striatum	0.2	10	37	19
[^{3}H]Dopamine	1	Bovine striatum	0.6	10	37	19
[^{3}H]Naloxone	2	Bovine striatum	0.5	15	25	20
[^{3}H]γ-Aminobutyric acid	2	Bovine cerebellum	0.4	20	4	21
[^{3}H]Flunitrazepam	2	Bovine cerebellum	0.2	40	4	22

Radioligand binding to various receptors was examined according to published methods (Table 1): [^{3}H]WB4101 (2,6-dimethoxyphenoxyethylaminomethyl-1,4-benzodioxane) and [^{3}H]clonidine (CLO) to alpha$_1$- and alpha$_2$-adrenergic receptors respectively [13]; [^{3}H]dihydroalprenolol (DHA) to beta-adrenergic receptors [14]; [^{3}H]5-hydroxytryptamine (5-HT) to 5-HT receptors [15, 16]; [^{3}H]pyrilamine (PYR) to histaminergic H_1 receptors [17]; and [^{3}H]3-quinuclidinyl benzilate (QNB) to muscarinic acetylcholine receptors [18]. Membranes isolated from crude synaptosomal fractions of rat cerebral cortex were used. The binding of [^{3}H]dopamine (DA) and [^{3}H]spiperone (SP) to dopamine receptors was determined with bovine striatal membranes [19] and [^{3}H]naloxone binding to opiate receptors in the same tissue [20], while the binding of [^{3}H]gamma-aminobutyric acid (GABA) and [^{3}H]flunitrazepam (FNT) to receptors of GABA and benzodiazepine, respectively [21, 22], was determined with bovine cerebellar membranes. Under the conditions as specified in Table 1, the pharmacological specificity of each radioligand binding was compared with that reported in the literature prior to the testing of antidepressants in the binding assay. Antidepressant concentrations which caused 50% inhibition of radioligand binding (IC_{50} values) were determined in at least two separate experiments; otherwise, $IC_{50} \pm$ S.E. values were obtained from three or more separate experiments.

After incubation under the specified conditions (Table 1), samples in triplicate were separated by filtration through GFB filters or by centrifugation at 10,000 *g* for 10 min and rinsed with cold buffer. Radioactivity of the sample was measured by liquid scintillation spectrometry. Specific binding of the above ^{3}H-labeled ligands was determined by computing the difference of radioactivity bound in the absence and presence of nonradioactive ligands: 100 and 10 μM *l*-norepinephrine for [^{3}H]WB4101 and [^{3}H]CLO binding respectively; 10 μM *l*-propranolol for [^{3}H]DHA binding; 10 μM 5-HT for [^{3}H]5-HT binding; 1 μM pyrilamine for [^{3}H]PYR binding; 1 μM atropine for [^{3}H]QNB binding; 10 μM dopamine for [^{3}H]DA binding; 1 μM *d*-butaclamol for [^{3}H]SP binding; 1 μM naltrexone for [^{3}H]naloxone binding; 1 mM GABA for [^{3}H]GABA binding and 10 μM diazepam for [^{3}H]FNT binding. Adenosine 3',5'-cyclic phosphate (cyclic AMP) formation activated by dopamine or norepinephrine in membranes of rat corpus striatum and cerebral cortex, respectively, was determined according to the described method [23].

Uptake of ^{3}H-labeled monoamines by synaptosomes [8] was determined as follows. Synaptosomes (equivalent to 1 mg protein) from a specific rat brain region were incubated at 37° for 3 min in 1 or 3 ml of Krebs bicarbonate medium containing also 10 mM glucose, 0.1 mM iproniazid, 1 mM ascorbic acid, 0.17 mM ethylenediamine tetraacetic acid (EDTA), 0.1 μM ^{3}H-labeled monoamine and various concentrations of antidepressant drugs in triplicate samples. The incubation mixtures were immediately diluted with 2 ml of ice-chilled Krebs bicarbonate buffer containing 1 mM nonradioactive monoamine but not dopamine, in the case of dopamine uptake. Synaptosomes were sedimented by centrifugation at 18,000 *g* for 10 min, rinsed with 5 ml of cold buffer, and transferred to counting vials containing 10 ml of scintillation fluid (PCS, Amersham). Radioactivity was measured by liquid scintillation spectrometers. Accumulation of ^{3}H-labeled monoamines at 4° represented the background and was subtracted from all samples. Antidepressant concentrations which caused 50% inhibition of ^{3}H-labeled monoamine uptake (IC_{50} values) were determined at least twice.

[^{3}H]-(−)-Norepinephrine [^{3}H]NE, 5 Ci/mmole; [^{3}H(G)]dopamine ([^{3}H]DA), 40 Ci/mmole; [^{3}H]5-hydroxytryptamine ([^{3}H]5-HT), 15 Ci/mmole; 2-[phenoxy-3-^{3}H(N)]WB4101, 24 Ci/mmole; [4-^{3}H]clonidine, 22.2 Ci/mmole; (−)-[propyl-2,3-^{3}H]dihydroalprenolol, 51.1 Ci/mmole; pyridinyl-5[^{3}H]pyrilamine, 27.3 Ci/mmole; [^{3}H]spiperone, 35.9 Ci/mmole; [(±)-benzilic-4',4'-^{3}H(N)]quinuclidinyl benzilate, 33.1 Ci/mmole; [methyl-^{3}H]flunitrazepam, 86.4 Ci/mmole; [2,3-^{3}H(N)]GABA, 39.2 Ci/mmole; and [^{3}H]naloxone, 38 Ci/mmole, were purchased from the New England Nuclear Corp. (Boston, MA).

Antidepressant drugs were gifts from the respective laboratories indicated within parentheses: amitriptyline HCl (Merck Sharp & Dohme, Rahway, NJ); imipramine HCl, clomipramine HCl and desipramine HCl (Ciba-Geigy, Summit, NJ); doxepin HCl (Pfizer, Groton, CT); zimelidine HCl (Astra, Lakemedel-Sodertalje, Sweden); fluvoxamine HCl (Philips-Duphar, Weesp, Netherlands); fluoxetine HCL (Lilly Research Laboratories, Indianapolis, IN); mianserin HCl (Organon, Newhouse, U.K.); and trazodone HCl (Mead Johnson, Evansville, IN).

RESULTS

As previously reported [7, 8], fluoxetine inhibited 5-HT uptake by 50% at a concentration (IC_{50} value) of 0.28 μM and NE uptake by 50% at a 29 times higher concentration in synaptosomes of rat cerebral cortex (Table 2). The two other selective inhibitors of serotonin uptake, zimelidine [24] and fluvoxamine [25], had selectivity ratios of 10 and 44 respectively. In agreement with earlier findings [6], the tertiary amine containing tricyclic antidepressant drugs clomipramine and imipramine preferentially inhibited 5-HT uptake over NE uptake by ratios of 12 and 18, respectively, whereas amitriptyline and doxepin were less effective as inhibitors of 5-HT uptake and also had a selectivity ratio approaching unity. Desipramine, demethylated imipramine, on the other hand, was over 100 times more effective as an inhibitor of NE uptake than of 5-HT uptake. These antidepressant drugs were even weaker inhibitors of DA uptake in synaptosomes of rat corpus striatum with IC_{50} values between 5.6 and 20 μM.

Effects on alpha-adrenergic receptors. In agreement with earlier reports [26–28], tricyclic antidepressants, including amitriptyline, doxepin, clomipramine, imipramine and desipramine were effective inhibitors of [^{3}H]WB4101 binding to alpha$_1$-adrenergic receptors in membranes of rat cerebral cortex. The IC_{50} values of the compounds varied between 0.06 and 0.6 μM (Table 3). The antidepressant drugs mianserin and trazodone also inhibited [^{3}H]WB4101

Table 2. Inhibition of monoamine uptake by antidepressant drugs in synaptosomes from rat brain regions*

Antidepressant drug	Inhibition of monoamine uptake IC_{50} (μM) 5-HT	NE	DA	IC_{50} (NE) / IC_{50} (5-HT)
Fluoxetine	0.28	7	6	29
Zimelidine	0.8	8	18	10
Fluvoxamine	0.16	7	NA†	44
Clomipramine	0.17	2	5.6	12
Imipramine	0.4	7	25	18
Amitriptyline	1	0.9	5.7	0.9
Doxepin	4	3	20	0.8
Desipramine	7	0.05	16	0.007

* Synaptosomal preparations from cerebral cortex were incubated at 37° for 3 min in Krebs bicarbonate medium also containing 10 mM glucose, 0.1 mM iproniazid, 1 mM ascorbic acid, 0.17 mM EDTA and 0.1 μM [^{3}H]5-HT or [^{3}H]NE. Striatal synaptosomal preparations were used for uptake of 0.1 μM [^{3}H]dopamine; otherwise, conditions were similar to above. Other conditions are described in the text.

† NA denotes compound not significantly active at a 10 μM concentration.

binding with IC_{50} values of 0.21 and 0.25 μM respectively. Fluoxetine and two other selective inhibitors of 5-HT uptake, zimelidine and fluvoxamine, were relatively weak inhibitors of [^{3}H]WB4101 binding, having IC_{50} values of 21, 2.3 and 4.5 μM respectively.

The binding of [^{3}H]clonidine (CLO) was inhibited most effectively by mianserin, followed by amitriptyline and doxepin with IC_{50} values of 0.09, 0.68 and 1.3 μM respectively (Table 3). Trazodone and the three other tricyclic drugs, desipramine, imipramine and clomipramine, inhibited [^{3}H]clonidine binding with IC_{50} values above 10^{-6} M. The three selective 5-HT uptake inhibitors also had IC_{50} values above 10^{-6} M, with fluoxetine being the least effective (IC_{50} value of 22 μM). The IC_{50} values of the antidepressive agents, except for fluoxetine and fluvoxamine, are in good agreement with previously reported values [26–28].

Effects on beta-adrenergic receptors. Consistent with earlier reports [14, 28], the tricyclic antidepressant drugs and mianserin were very weak inhibitors of [^{3}H]DHA binding. We also found that up to 10 μM concentrations of the ten antidepressant drugs failed to inhibit [^{3}H]DHA binding to the cortical membranes (Table 4). In addition, at 100 μM, almost all of the antidepressant drugs tested (Table 3) did not inhibit the norepinephrine activated adenylate cyclase to any significant extent.

Effects on histamine receptors. Among the radioligand bindings studied, [^{3}H]pyrilamine (PYR, Table 4) binding to the cortical membranes was most sensitive to tricyclic antidepressant drugs and mianserin, with IC_{50} values ranging from 0.004 to 0.8 μM. The three selective inhibitors of 5-HT uptake, fluoxetine, zimelidine and fluvoxamine, were relatively weak inhibitors with IC_{50} values of 1.9, 2.4 and 8.4 μM

Table 3. Effects of antidepressants on ^{3}H-labeled ligand binding to adrenergic receptors and norepinephrine activated adenylate cyclase (AC) in membranes of rat cerebral cortex

Antidepressants	Inhibition of ^{3}H-labeled ligand binding* WB4101	CLO	Norepinephrine activated AC†
	IC_{50} (μM)		Percent of control
Fluoxetine	21 ± 6.0	22 ± 7.4	89.7 ± 3.3
Zimelidine	2.3 ± 0.1	4.5 ± 0.3	100.9 ± 3.0
Fluvoxamine	4.5 ± 0.7	11.4 ± 0.9	96.7 ± 7.8
Desipramine	0.6 ± 0.1	9.2 ± 1.1	92.0 ± 5.8
Imipramine	0.25 ± 0.05	4.6 ± 0.2	73.9 ± 11.1
Clomipramine	0.08 ± 0.01	4.5 ± 0.2	77.2 ± 6.3
Amitriptyline	0.06 ± 0.01	0.68 ± 0.04	94.1 ± 10.4
Doxepin	0.06 ± 0.01	1.3 ± 0.1	65.6 ± 10.2‡
Mianserin	0.21 ± 0.01	0.09 ± 0.0	81.0 ± 1.9
Trazodone	0.25 ± 0.01	3.1 ± 0.3	72.2 ± 4.2

* Rat cerebral cortex was used for binding of [^{3}H]WB4101 and [^{3}H]clonidine (CLO).

† Cyclic AMP concentrations in the absence and presence of 100 μM norepinephrine and 10 μM GTP were 102.2 ± 8.2 and 195.4 ± 17.8 (control values) respectively.

‡ $P < 0.05$.

Table 4. Effects of antidepressants on ^{3}H-labeled ligand binding to putative neurotransmitter and pharmacological receptors in membranes of mammalian brains

Antidepressants	Inhibition of ^{3}H-labeled ligand binding* PYR	QNB	5-HT	NLX	DHA	GABA	FNT
	IC_{50} (μM)						
Fluoxetine	1.9 ± 0.4	6.6 ± 0.5	(27)†	(35)	NA‡		
Zimelidine	2.4 ± 0.3	NA	NA	(39)	NA		
Fluvoxamine	8.4 ± 0.4	NA	(31)	NA	NA		
Desipramine	0.8 ± 0.3	0.60 ± 0.03	(39)	(45)	NA		
Imipramine	0.03 ± 0.01	0.32 ± 0.01	(28)	9	NA		
Clomipramine	0.04 ± 0.01	0.23 ± 0.03	(33)	(45)	NA		
Amitriptyline	0.004 ± 0.001	0.043 ± 0.001	5.7	(42)	NA		
Doxepin	0.004 ± 0.001	0.19 ± 0.02	5.8	(42)	NA		
Mianserin	0.007 ± 0.4	1.5 ± 0.1	2	NA	NA		
Trazodone	1.1 ± 0.2	NA	4	NA	NA		

* Brain regions of animals used for ^{3}H-labeled ligand binding: rat cerebral cortex for binding of [^{3}H]pyrilamine (PYR), [^{3}H]quinuclidinyl benzilate (QNB) and [^{3}H]dihydroalprenolol (DHA); rat frontal cortex for [^{3}H]5-HT binding; bovine cerebellum for binding of [^{3}H]GABA and [^{3}H]flunitrazepam (FNT); and bovine corpus striatum for binding of [^{3}H]naloxone (NLX).

† Percent inhibition at a 10 μM concentration of drug tested.

‡ NA denotes compounds not significantly active at a 10 μM concentration.

respectively. Except for fluoxetine and fluvoxamine, IC_{50} values of other antidepressants agree well with those in previous reports [18, 28].

Effects on muscarinic receptors. Fluoxetine was relatively weak in inhibiting [^{3}H]QNB binding to muscarinic acetylcholine receptors in cortical membranes, with an IC_{50} value of 6.6 μM (Table 4). Up to a concentration of 10 μM, zimelidine, fluvoxamine and trazodone did not inhibit [^{3}H]QNB binding to any significant extent. The tricyclic antidepressant drugs were relatively effective inhibitors with IC_{50} values between 0.043 and 0.6 μM, while mianserin had an IC_{50} value of 1.5 μM (Table 4).

Effects on serotonin receptors. The antidepressant drugs are weak ligands for 5-HT receptors. They inhibited [^{3}H]5-HT binding with micromolar or higher IC_{50} values (Table 4), with mianserin being most effective, followed by trazodone, doxepin and amitriptyline. Fluoxetine at 10 μM inhibited [^{3}H]5-HT binding about 27%, while fluoxetine at 1 μM or lower concentrations had no effect.

Effects on opiate receptors. Similarly, the antidepressants were poor ligands for opiate receptors labeled by [^{3}H]naloxone (NLX, Table 4). The only drug which inhibited [^{3}H]naloxone binding by 50% at less than a 10 μM concentration was imipramine (IC_{50} at 9 μM), while fluoxetine at 10 μM caused a 35% inhibition.

Effects on GABA-benzodiazepine receptors. The antidepressant drugs, up to 10 μM failed to influence

Table 5. Effects of antidepressants on ^{3}H-labeled ligand binding to dopamine receptors in bovine striatal membranes and dopamine sensitive adenylate cyclase (AC) in rat striatal membranes

Antidepressants	Inhibition of ^{3}H-labeled ligand binding* DA	SP	Dopamine-AC† −GTP	+GTP
	IC_{50} (μM)		Percent of control	
Fluoxetine	23	2.1	93.2 ± 7.4	98.9 ± 2.8
Zimelidine	(47)‡	4.3	83.3 ± 4.3	104.7 ± 2.3
Fluvoxamine	(42)‡	2.73	105.0 ± 2.0	116.2 ± 4.3
Desipramine	8	0.9	102.5 ± 0.5	79.4 ± 12.6
Imipramine	3.9	0.7	92.6 ± 5.6	71.3 ± 5.88
Clomipramine	1.4	0.2	86.7 ± 2.3	72.9 ± 2.3
Amitriptyline	0.5	0.4	88.5 ± 3.6	62.9 ± 1.6
Doxepin	1.5	1.2	85.6 ± 2.7	60.7 ± 1.5¶
Mianserin	3.9	2	92.7 ± 5.4	61.0 ± 5.8
Trazodone	35	4.6	105.7 ± 3.6	64.9 ± 4.8¶

* Bovine corpus striatum was used for binding of [^{3}H]dopamine (DA) and [^{3}H]spiperone (SP).

† Cyclic AMP concentrations induced by 100 μM dopamine in the absence and presence of 10 μM GTP were 88.6 ± 6.0 and 148.2 ± 11.2 pmoles·min^{-1}·(mg protein)$^{-1}$, respectively, and the basal rate of cyclic AMP formation was 88.2 ± 10.0 pmoles·min^{-1}·(mg protein)$^{-1}$.

‡ Percent inhibition at a 100 μM concentration of tested drugs.

§ $P < 0.025$.

‖ $P < 0.01$.

¶ $P < 0.005$.

to any significant extent the binding of [^{3}H]GABA or [^{3}H]flunitrazepam (FNT) to their respective receptors in bovine cerebellar membranes (Table 4). These findings are in good agreement with those reported by Hall and Ogren [28].

Effects on dopamine receptors. The antidepressant drugs were more effective in inhibiting [^{3}H]SP than [^{3}H]DA binding to bovine striatal membranes (Table 5), except for amitriptyline and doxepin which had relatively equal and high affinities for the binding sites of both ^{3}H-labeled ligands. For fluoxetine, the differential ratio was 11, having IC_{50} values of 2.1 and 23 μM in the inhibition of [^{3}H]SP and [^{3}H]DA binding respectivly. In view of their higher affinities toward the dopamine antagonist [^{3}H]SP binding sites, we examined the effects of the drugs on dopamine-activated adenylate cyclase in rat striatal membranes. Doxepin, amitriptyline, imipramine, clomipramine, mianserin and trazodone at 100 μM significantly reduced the concentrations of cyclic AMP formed in the presence of dopamine and GTP by 30–40% while desipramine caused only a 20% inhibition of the activity. The three selective inhibitors of 5-HT uptake did not influence the formation of cyclic AMP in the absence or presence of GTP. However, fluoxetine at 500 μM caused a 40% reduction of cyclic AMP formation, whereas almost identical reduction of cyclic AMP formation occurred with the presence of 10 μM amitriptyline or doxepin.

DISCUSSION

Fluoxetine is a competitive inhibitor of 5-HT uptake, with an inhibitor constant (K_i value) of 0.06 μM in synaptosomes of rat brain; it is over two orders of magnitude weaker as indicated by the K_i values for the inhibition of catecholamine uptake [7, 8]. The present studies further illustrate the specificities of the three inhibitors of serotonin uptake, fluoxetine, zimelidine and fluvoxamine, since they have relatively weak affinity toward a number of receptors detected by radioligand assays. In fact, fluoxetine was first reported to be without affinity for postsynaptic 5-HT receptors [15]. Although we observed a 27% inhibition of 5-HT binding by fluoxetine at 10 μM, in a similar assay the concentration was at least two orders of magnitude greater than the IC_{50} required to inhibit [^{3}H]5-HT uptake. On the other hand, fluoxetine was effective at nanomolar concentrations in competing with [^{3}H]imipramine binding to the putative 5-HT uptake carrier in neuronal membranes [29]. Unlike the tricyclic antidepressants amitriptyline and clomipramine, fluoxetine *in vivo* failed to block the central stimulatory action of the 5-HT agonist lysergic acid diethylamide (LSD) in the spinal rat hind limb reflex preparation or the contractile action of 5-HT in rat stomach fundus strip *in vitro* [30]. These results indicate that fluoxetine *in vitro* and *in vivo* is devoid of affinity toward the central as well as the peripheral 5-HT postsynaptic receptors.

The effectiveness of fluoxetine in the enhancement of 5-HT neurotransmission is well documented. Fluoxetine alone suppressed the firing of the raphe 5-HT neurons [31] and lowered the rate of 5-HT turnover [9, 10]. However, the pharmacological responses of fluoxetine are usually potentiated or dependent on the coadministration of 5-hydroxytryptophan (5-HTP), a precursor in 5-HT synthesis. The combined administration of fluoxetine and 5-HTP lowers blood pressure in spontaneously hypertensive rats [32] and elevates plasma concentrations of prolactin [33] and corticosterone [34]. These results indicate that the blockade of 5-HT reuptake by fluoxetine has elevated the intrasynaptic concentration of 5-HT.

Antidepressants, including fluoxetine as demonstrated in the present studies, do not affect significantly the NE-activated adenylate cyclase or [^{3}H]DHA binding to the beta-adrenergic receptors in cortical membranes *in vitro*. Contrary to the findings with fluoxetine, however, the chronic administration of tricyclic antidepressant drugs and the antidepressants mianserin and zimelidine produces beta-adrenergic receptor subsensitivity [35–38]. Inhibition of NE uptake by the tricyclic antidepressant drugs and perhaps also by zimelidine presumably elevates intrasynaptic NE concentrations, which may be increased also by blocking the presynaptic alpha$_2$ receptors. Indeed, the time required for the development of receptor subsensitivity was reduced substantially upon coadministration of desipramine and an alpha$_2$-adrenergic antagonist, phenoxybenzamine [39] or yohimbine [40]. The inhibition of alpha$_2$ receptors may also explain the beta-adrenergic subsensitivity caused by chronic treatment with mianserin [27].

Fluoxetine exhibited little or no affinity toward alpha$_1$- or alpha$_2$-adrenergic receptors labeled by [^{3}H]WB4101 and [^{3}H]clonidine respectively. The absence of chronic effects of fluoxetine in the clonidine-induced aggressive behavior substantiates these findings [41] and further differentiates fluoxetine from other antidepressant drugs. The tricyclic antidepressants amitriptyline, doxepin, clomipramine, imipramine and desipramine (in order of decreasing effectiveness) and the antidepressants mianserin and trazodone were potent inhibitors of [^{3}H]WB4101 binding. Among these antidepressants, amitriptyline, imipramine and mianserin, after their chronic administration, have been found to enhance the aggressive behavior induced by clonidine in mice, suggesting hyperactivity of the noradrenergic system [41, 42]. Indeed, upon long-term treatment with amitriptyline, the number of [^{3}H]WB4101 binding sites in hippocampus is increased in mice [43] and the number of [^{3}H]clonidine binding sites in hippocampus is decreased in rats [44]. In addition, amitriptyline was next to mianserin in being the most effective ligand for the [^{3}H]clonidine binding sites. In contrast to the findings with fluoxetine, long-term treatment with another 5-HT uptake inhibitor zimelidine also potentiates aggressive behavior induced by clonidine [41]. Since zimelidine is a relatively weak ligand for the alpha-adrenergic receptors, the hyperactivity of the noradrenergic system may be a consequence of the adaptable change in NE–5-HT interaction caused by chronic administration with zimelidine.

Specific binding of the DA antagonist [^{3}H]spiperone was about ten times more sensitive to fluoxetine than [^{3}H]DA binding in bovine striatal

membranes, with IC_{50} values at greater than micromolar concentrations. However, fluoxetine even at 100 μM concentrations, failed to antagonize the DA-activated adenylate cyclase. Thus far, there is no evidence that fluoxetine administration alone alters dopaminergic functions, such as elevating plasma prolactin concentrations [33] which is seen with DA antagonists. In behavioral studies, chronic administration of fluoxetine up to 14 days also fails to attenuate the amphetamine-induced hyperactivity in rats, whereas similar chronic administration with desipramine produces greater amphetamine hyperactivity than in control rats [12]. The present studies confirm the earlier findings that the tricyclic antidepressants are potent inhibitors of the DA-sensitive adenylate cyclase [45]. Mianserin and trazodone have now been shown also to have this property. Fluoxetine was also a weak inhibitor of [^{3}H]spiperone binding to the putative 5-HT_2 receptors in rat frontal cortex *in vitro*, but chronic treatment with fluoxetine fails to produce any significant effect [46].

Although the dimethylated tricyclic antidepressants are more potent inhibitors of [^{3}H]QNB binding and 5-HT uptake than the monomethylated drugs [18], the two synaptic processes are unrelated. In the present studies, all three selective inhibitors of 5-HT uptake, fluoxetine, zimelidine and fluvoxamine, were either weak or inactive in inhibiting [^{3}H]QNB binding. Furthermore, among the antidepressants studied, amitriptyline was intermediate in effectiveness as an inhibitor of 5-HT uptake, but at the same time it was the most effective antagonist in [^{3}H]QNB binding. Consistent with the latter findings, chronic administration of amitriptyline in mice causes an increase in the muscarinic binding sites in hippocampus [43]. It is believed that the anticholinergic effects of the tricyclic antidepressants, most notably amitriptyline, cause the side effects of dry mouth and drowsiness in humans [47]. Preliminary clinical data showed that the antidepressive dose of fluoxetine did not produce similar drug-related side effects (P. Stark, personal communication).

By studying the inhibition of H_1- and H_2-histamine receptor-mediated adenylate cyclase activity in guinea pig brain, Psychoyos [48] confirmed the earlier findings [17, 49] that antidepressants are more potent antagonists of the H_1- than the H_2-histamine receptors, with amitriptyline and mianserin being most effective. In both the H_1 and H_2 systems, fluoxetine is relatively inactive, with IC_{50} values greater than 10 μM [47]. With [^{3}H]pyrilamine binding as an assay, we found that mianserin, amitriptyline and doxepin were 270–475 times more effective ligands than fluoxetine for the H_1 receptors. The antagonism of the H_1-receptors may have been related to the side effects of the tricyclic antidepressants such as sedation and appetite-stimulation [47]. The lack of any stimulation on weight gain and the lower incidence of sedation seen in the clinical studies with fluoxetine (P. Stark, personal communication) are consistent with its lack of interaction with the H_1 receptor.

In conclusion, in addition to being a selective inhibitor of 5-HT uptake, fluoxetine is practically devoid of affinity for neurotransmitter and pharmacological receptors, including alpha$_1$-, alpha$_2$- and beta-adrenergic receptors; 5-HT receptors; D-A receptors; histamine H_1 receptors; muscarinic acetylcholine receptors; opiate receptors; and GABA-benzodiazepine receptors. This neurochemical profile is reflected in a clinical profile of fluoxetine which has been relatively side-effect free (P. Stark, personal communication), unlike that of tricyclic antidepressant drugs. The unique pharmacological profile of fluoxetine may pave the way for a better insight into the treatment of depression since indeed fluoxetine has been found efficacious in the treatment of depressed patients.*

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