Effect of Fluvoxamine on 5-Hydroxytryptamine Uptake, Paroxetine Binding Sites and Ketanserin Binding Sites in the Japanese Monkey Brain and Platelets, In Vivo and In Vitro

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ABSTRACT—We investigated the in vitro effects of fluvoxamine on \(^3\)H-paroxetine binding and \(^3\)H-monoamine uptake in monkey cerebral cortex in comparison with those of other antidepressants. Fluvoxamine selectively inhibited \(^3\)H-5-hydroxytryptamine (5-HT) uptake and \(^3\)H-paroxetine binding. However, it did not alter \(^3\)H-norepinephrine or \(^3\)H-dopamine uptake. In addition, we examined the effects of chronic treatment with fluvoxamine (5 mg/kg per day, p.o.) on 5-HT uptake sites that bind \(^3\)H-paroxetine and 5-HT\(_2\) receptors that bind \(^3\)H-ketanserin, in monkey brains and platelets. Chronic treatment with fluvoxamine affected neither the paroxetine binding sites nor the ketanserin binding sites of the brains and platelets. These results suggest that long-term treatment with fluvoxamine does not affect either the 5-HT uptake sites or 5-HT\(_2\)-receptors of 5-HT neurons in monkey brain in spite of its strong inhibitory effect on 5-HT uptake in vitro.

Keywords: Fluvoxamine, 5-Hydroxytryptamine uptake, Paroxetine binding, Monkey brain, Monkey platelet

5-Hydroxytryptamine (5-HT) is thought to participate in the genesis of affective disorders, a theory based on observed actions of the serotonergic system (1). Biochemical support for this hypothesis includes findings that lower levels of 5-hydroxyindoleacetic acid (5-HIAA) are found in the cerebral spinal fluid of depressed patients (2) and the brain of suicide victims (3), as compared with healthy controls. It has also focused attention on the relationship between 5-HT and antidepressants, since the antidepressive action of these drugs is often accompanied by a parallel inhibition of monoamine oxidase or 5-HT uptake. Therefore, in recent years, several potent and selective 5-HT uptake inhibitors have become available for the treatment of affective disorders. However, some antidepressants have an affinity for certain neurotransmitter receptors, in addition to inhibiting monoamine uptake, and these effects might be responsible for the often observed clinical side effects (4).

Fluvoxamine, a potent 5-HT uptake inhibitor, increases serotonergic neurotransmission by selectively inhibiting the uptake of 5-HT (5, 6). Fluvoxamine does not alter norepinephrine (NE) uptake, nor does it bind to most types of neurotransmitter receptors. Moreover, the long term effects of fluvoxamine on 5-HT receptors and enzyme expression are still unclear, although fluvoxamine reduced the number of cortical \(\beta\)-adrenoreceptors and decreased the expression of tyrosine hydroxylase in rats (7). Unfortunately, little attention seems to have been paid to the possibility of species differences in these effects of fluvoxamine. Therefore, investigation of the effects of this drug on the central nervous system in monkeys should provide information more directly applicable to humans.

This report compares the influences of fluvoxamine and other antidepressants on paroxetine binding and monoamine uptake in monkey brain. In addition, we studied the effects of chronic administration of fluvoxamine on the kinetic parameters of paroxetine binding; \(^3\)H-paroxetine binding to 5-HT-uptake sites (8); and ketanserin binding, whose binding sites represent 5-HT\(_2\) receptor sites (9), in platelets and membranes of the cerebral cortex in monkeys.

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MATERIALS AND METHODS

Chronic treatment with fluvoxamine

Eight adult, male, Japanese monkeys (Macaca fuscata, 3- to 6-years-old; donated from the Animal Center, Oita Medical University) were used in the study. Each was housed in an individual cage for 30 days before treatment to allow them to adjust to the experimental conditions. Monkeys were divided into two groups. The first group (control, three monkeys) was given only saline. The second group (five monkeys) was given 5 mg/kg per day of fluvoxamine (p.o.) for 8 weeks. Each of the monkeys in both groups was anesthetized with 50 mg/kg (s.c.) of ketamine, and 5 ml of blood was taken from the femoral artery within 10 min after the ketamine injection. Starting the next day, each monkey in the experimental group was given 5 mg/kg per day of fluvoxamine (p.o.) for 8 weeks, and blood samples were taken during the second, fourth, sixth and eighth weeks. Following the 8 weeks of fluvoxamine administration, the monkeys were killed by blood depletion under ketamine anesthesia, and the brains were quickly removed. With the exception of the brains used for the monoamine uptake assay, all other platelet and brain samples were stored at —80°C until use. For the monoamine uptake assay, the brains were immediately homogenized and then the crude synaptosomes were obtained by differential centrifugation. This study was performed according to the Oita Medical University Guidelines for the Care and Use of Laboratory Animals.

In vitro binding of paroxetine in the monkey brain

The characterization of paroxetine binding to monkey cortical membranes was performed according to the method of Habert et al. (10). Control monkey frontal cortices were homogenized in 25 vol. of ice-cold 50 mM Tris-HCl buffer containing 100 mM NaCl and 5 mM KCl (pH 7.4). The P2 fractions obtained by centrifugation of the homogenate at 12,000 × g for 20 min were used as crude membrane preparations (final concentration of approximately 0.1 mg protein/tube) for the assays. Aliquots of the crude membrane suspension were incubated with 50 pM [3H]-paroxetine at 22°C in a final volume of 250 µl for 180 min. Fluoxetine (final concentration of 10 µM) was used to determine nonspecific binding. To investigate the effects of various antidepressants on paroxetine binding, we preincubated the crude membranes with reagents at concentrations of 10^4—10^9 M before adding [3H]-paroxetine. The incubation was terminated via rapid filtration of the membrane suspension through Whatman GF/B glass fiber filters under reduced pressure. Each filter was rapidly washed three times with 5 ml of ice-cold saline. The filters were then dried, and their radioactivities were measured in Triton X-100-toluene scintillation fluid by using a liquid scintillation spectrometer.

Monoamine uptake assay, in vitro

The study of 5-HT, NE and dopamine (DA) uptake into synaptosomes was conducted essentially according to the method of Snyder and Coyle (11), with some minor modifications. The cerebral cortices from normal monkeys were homogenized in 0.32 M sucrose, and the crude synaptosomes were obtained by differential centrifugation as described earlier (12). A 100-µl aliquot of crude synaptosomes (final concentration of approximately 0.2 mg protein/tube) was preincubated at 37°C for 5 min with Krebs-Henseleit buffer (121 mM NaCl, 25 mM NaHCO3, 11.1 mM glucose, 4.7 mM KCl, 1.4 mM CaCl2, 1.2 mM KH2PO4, 1.1 mM ascorbic acid, 130 µM EDTA 2Na and 78 µM pargyline; pH 7.4; bubbled with a 95% O2, 5% CO2 gas mixture). Then the [3H]-labeled monoamines were added (final concentration: 188 nM [3H]-5-HT, 32 nM [3H]-NE, 25 nM [3H]-DA) and incubated at 37°C for either 2 min for NE uptake or 10 min for 5-HT and DA uptake. Nonspecific uptake was calculated from data obtained during incubation at 0°C. In order to investigate the effects of various antidepressants on monoamine uptake, membrane suspensions were preincubated with the reagents at concentrations of 10^-4—10^-9 M before adding the monoamines. The incubation was terminated via rapid filtration of the membrane suspension through Whatman GF/B glass fiber filters under reduced pressure. Each filter was rapidly washed three times with 5 ml of ice-cold saline. The filters were dried, and their radioactivities were measured as described above.

Determination of the kinetic parameters of paroxetine binding and ketanserin binding

Monkey brain: Monkey brains subjected to 8 weeks of fluvoxamine or saline administration were used in this study. Paroxetine binding to monkey cortical membrane preparations was performed with [3H]-paroxetine (final concentration of approximately 0.1 mg protein/tube), and nonspecific binding was determined by using fluoxetine (final concentration of 10 µM). This procedure was performed according to the method of Habert et al. (10). Ketanserin binding to monkey cortical membrane preparations was performed with [3H]-ketanserin (final concentration of approximately 0.2 mg protein/tube) according to the method of McKeith et al. (13) with minor modifications. Unlabeled spiperone at a final concentration of 1.0 µM was used to determine nonspecific binding. Aliquots of the membrane preparations were incubated with [3H]-paroxetine (final volume of 250 µl) at 22°C for 180 min or with [3H]-ketanserin (final volume of 500 µl) at 37°C for 15 min. The incubation was terminated by
rapid filtration of the membrane suspension under reduced pressure through Whatman GF/B glass fiber filters. Each filter was then rapidly washed three times with 5 ml of ice-cold 50 mM Tris-HCl buffer, pH 7.4. The filters were then dried, and the radioactivity was determined as described above. Specific binding represents the total binding minus the binding in the presence of 10 μM fluoxetine or 1.0 μM spiperone, at concentrations of 13–570 pM 3H-paroxetine or 0.25–4.0 nM 3H-ketanserin. Values of B_{max} and K_{d} were estimated by Scatchard analysis of the specific binding. The binding assays were performed in triplicate.

**Monkey platelets:** To obtain monkey platelets, monkey blood was collected into plastic centrifuge tubes containing heparin (final concentration of 10 U/ml) and then centrifuged at 200 x g for 10 min to yield platelet-rich plasma. Platelets were isolated from the platelet-rich plasma by centrifugation at 3000 x g for 10 min. The platelets were washed twice with 50 mM Tris-HCl buffer, pH 7.4, and they were kept at -80°C until analysis. Binding assays of 3H-paroxetine and 3H-ketanserin in monkey platelets were performed by the method of Mellerup et al. (14) and Biegon et al. (15), respectively, with minor modifications. The incubation mixture was 300 μl in total volume and contained 100 μl of membrane suspension (approximately 50 μg membrane protein) and 50 μl of 3H-paroxetine (final concentrations of 0.1–3.0 nM) and either a) 150 μl of 50 mM Tris-HCl buffer, pH 7.4 (total binding) or b) 150 μl of 50 mM Tris-HCl buffer, pH 7.4 containing 10 μM fluoxetine (to define non-specific binding). The 3H-ketanserin binding procedure consisted of incubating 800 μl of membrane suspension and 100 μl 3H-ketanserin (0.25–4.0 nM) with either a) 100 μl 50 mM Tris-HCl buffer, pH 7.4 (total binding) or b) 100 μl 50 mM Tris-HCl buffer, pH 7.4 containing 1.0 μM spiperone (to define non-specific binding). After incubation at 20°C for either 180 min (for paroxetine binding) or 25°C for 60 min (for ketanserin binding), 5 ml of ice-cold 50 mM Tris-HCl buffer, pH 7.4 was added, and each of the samples was rapidly filtered through a Whatman GF/B glass fiber filter. The filters were washed three times with 5 ml of ice-cold 50 mM Tris-HCl buffer, pH 7.4 and then dried. The radioactivity was determined as described above. Values of B_{max} and K_{d} were estimated by Scatchard analysis of the specific binding. The binding assays were performed in triplicate.

**Chemicals**

Paroxetine, [phenyl-6-3H]- (555 GBq/mmol−1.11 TBq /mmol); hydroxytryptamine creatinine sulfate, 5-[1,2-3H(N)]- (555 GBq/mmol−1.11 TBq /mmol); norepinephrine, levo-[ring-2,5,6-3H]- (1.48–2.22 TBq /mmol); dihydroxyphenylethylamine, 3,4-[7-3H]- (740 GBq/mmol −1.48 TBq /mmol); and ketanserin hydrochloride, [ethylene-3H]- (2.22–3.33 TBq /mmol) were purchased from New England Nuclear (Boston, MA, USA). Fluvoxamine (Solvey-Meiji Yakuhin Co., Ltd., Tokyo) and fluoxetine (Lilly Research Laboratories, Indianapolis, IN, USA), spiperone (Eisai Co., Ltd., Tokyo), zimeldine (Fujisawa, Osaka), maprotiline (Ciba-Geigy, Takarazuka), and nomifensine (Hoechst, Frankfurt, Germany) were donated by the respective companies. All other chemicals were obtained from Wako Pure Chemical Industries, Ltd. (Osaka).

**Statistics**

Results are each expressed as the mean ± S.E. for three or five monkeys. The significance of the difference between means was determined by Student's t-test for unpaired data.

**RESULTS**

**Inhibition of monoamine uptake in the monkey brain by various antidepressants**

We tested the in vitro effects of fluvoxamine, maprotiline, desipramine, imipramine and nomifensine on monoamine uptake in monkey brain synaptosomes. Table 1 shows that fluvoxamine inhibited 5-HT uptake by 50%
at a concentration (IC50 value) of $8.2 \times 10^{-9}$ M and inhibited NE uptake by 50% at concentrations greater than $10^{-5}$ M. In synaptosomes of the monkey cerebral cortex, therefore, fluvoxamine inhibited 5-HT uptake 1,000 times more effectively than it inhibited NE uptake. On the other hand, desipramine, nomifensine, maprotiline and imipramine inhibited NE uptake 10–1,000 times more effectively than they inhibited 5-HT uptake. With the exception of nomifensine, these antidepressants inhibited DA uptake more weakly than they did 5-HT and NE uptakes in the synaptosomes of monkey cerebral cortex.

**Inhibition of paroxetine binding in the monkey brain by various antidepressants**

We investigated the effects on in vitro paroxetine bind-
ing to monkey cerebral membranes by a) various concentrations (100 μM to 1.0 mM) of fluvoxamine; b) two other selective inhibitors of 5-HT uptake, fluoxetine and zimeldine; and c) a tricyclic antidepressant, imipramine. Figure 1 shows that the paroxetine binding to monkey cerebral membranes was inhibited by antidepressants, in the order: fluvoxamine > fluoxetine > imipramine > zimeldine.

Effects of chronic treatment with fluvoxamine on paroxetine binding and ketanserin binding in monkey platelets

We investigated the effects of chronic treatment with fluvoxamine on paroxetine binding and ketanserin binding in monkey platelets. We detected no significant changes in the Bmax or Kd values of paroxetine binding during the 8 weeks of fluvoxamine treatment when compared with the values obtained during saline treatment (Fig. 2). Also, we observed no significant changes in either the Bmax or Kd values of ketanserin binding (Fig. 3).

Effects of chronic treatment with fluvoxamine on paroxetine binding and ketanserin binding to monkey cerebral membranes

We compared the Bmax and Kd values of paroxetine binding and ketanserin binding to cerebral membranes in monkeys undergoing fluvoxamine treatment (8 weeks) against those of controls. Chronic treatment with fluvoxamine did not produce any significant changes in the kinetic constants of paroxetine or ketanserin binding sites as compared with the controls, when the chronically treated monkeys were sacrificed 24 hr after the last fluvoxamine administration (Table 2).

The binding parameters for paroxetine binding obtained from 5 different monkeys indicated that the Bmax values and Kd values of the platelets exhibited a highly significant correlation with the respective parameter in the brain (r = 0.85, P < 0.007 for Bmax; r = 0.81, P < 0.009 for Kd). Similarly, for ketanserin binding, the Bmax values and Kd values of the platelets showed a good linear correlation with the respective value in the brain (r = 0.89, P < 0.05 for Bmax; r = 0.87, P < 0.05 for Kd).

Table 2. Bmax and Kd values of paroxetine and ketanserin binding in monkey brains

|                  | Control         | FV treatment   |
|------------------|-----------------|----------------|
| 3H-Ketanserin binding | Bmax 96.2±8.1  | 114.0±15.9     |
|                  | Kd 19.2±0.42    | 1.75±0.48      |
| 3H-Paroxetine binding | Bmax 526.2±56.2| 605.3±38.3     |
|                  | Kd 1.80±0.43    | 2.16±0.33      |

The Bmax and Kd values of paroxetine and ketanserin binding were determined graphically by Scatchard analysis and are expressed as the mean ± S.E. of the values obtained for five fluvoxamine-treated monkeys or three control monkeys. FV treatment: Fluvoxamine (5 mg/kg per day, p.o.) was administered to monkeys for 8 weeks. Bmax: fmol/mg protein, Kd: nM.
DISCUSSION

We found fluvoxamine to be the most potent selective inhibitor of 5-HT uptake among the antidepressants used for the experiments on synaptosomal preparations from monkey cerebral cortex. Moreover, in agreement with earlier reports (17, 18), zimeldine and desipramine were relatively weaker inhibitors and maprotiline and nomifensine were the weakest inhibitors of 5-HT uptake in the monkey cerebral cortex. Fluvoxamine inhibited 5-HT uptake with an IC50 of $8.2 \times 10^{-9}$ M, over 1,000 times more effectively than it inhibited NE uptake or DA uptake. This selectivity towards monoamine uptake by fluvoxamine is in good agreement with results from previous studies using rat cerebral cortices (18–20). However, as an inhibitor of 5-HT uptake, the affinity of fluvoxamine in monkey cerebral cortex was about 100 times that in rat cerebral cortex; this difference may be related to a difference in the transport system. To our knowledge, our present study is the first one to investigate the effects of fluvoxamine using monkeys. Our data indicate that there are species differences in the drug effects that must be taken into account, although which preparation is preferable for studying remains to be determined.

The existence of high-affinity binding sites for imipramine has been demonstrated in membrane preparations from rat (21) and human brains (22). These recognition sites, labeled with $^3$H-imipramine, have been shown to be distinct, yet allosterically related to the 5-HT transport system (23, 24). Moreover, several authors have suggested that these sites are clinically important, since platelets of depressed patients show a decrease in the number of imipramine binding sites (25, 26) as well as a decrease in 5-HT uptake (27). However, it has been also reported that paroxetine binding sites appear to be associated with neuronal 5-HT uptake sites when $^3$H-paroxetine is used as a ligand (28). In a recent paper, $^3$H-paroxetine was used as a new and highly selective ligand to study the neuronal 5-HT transport complex (29). In the present study, fluvoxamine markedly inhibited $^3$H-paroxetine binding as compared to other 5-HT uptake inhibitors. The results of the present study also indicate that fluvoxamine acts at the recognition site labeled by $^3$H-paroxetine and inhibits the 5-HT uptake process.

High-affinity sites for imipramine (30) or paroxetine (31) are located presynaptically on serotonergic neurons and are related to the 5-HT uptake mechanism inside the nerve terminals (32). Published data have shown that chronic administration of antidepressants can produce either a down regulation (30, 33, 34) or no change (35–37) in imipramine binding sites. Paroxetine possesses very high affinity and selectivity for sites on or near the 5-HT uptake region of the 5-HT terminal. Occupation of paroxetine binding sites by fluvoxamine might modify the sensitivity of 5-HT uptake. It has been reported that fluvoxamine competitively displaces imipramine in platelet membranes, in vitro (38). Therefore, it is possible that long-term occupation of these sites by fluvoxamine might result in fewer paroxetine binding sites. In the present study, however, we did not observe any changes in the density and affinity of paroxetine binding sites in monkey brains and platelets following long-term administration of fluvoxamine. Brunello et al. (39) reported that fluvoxamine induced an increase in the $B_{max}$ for high-affinity 5-HT uptake in rat cortex slices and that this increase could be due to a rebound phenomenon following withdrawal from drugs that acutely inhibit 5-HT uptake. On the other hand, there have been reports that daily-treatment with fluvoxamine for 7 days reduced 5-HT uptake by crude synaptosomes by 56% in rat brains (40). It is unclear whether these variances reflect species differences or regional differences in the adaptability of 5-HT uptake sites (41), or whether they can be attributed to the conditions used in these in vitro experiments.

Further work is needed to clarify whether long-term treatment with fluvoxamine alters the sensitivity of 5-HT receptors. Because the 5-HT1 receptor sites implicated in ketanserin-inhibited 5-HT-induced aggregation of platelets have been reported to have binding characteristics similar to the central 5-HT2 receptors, they would be a useful tool for investigations (9). We therefore studied the effects of chronic treatment with fluvoxamine on ketanserin binding sites. In the present study, however, chronic treatment with fluvoxamine did not produce any significant changes in the kinetic constants of ketanserin-inhibited 5-HT binding sites in either monkey brains or platelets. In addition, Benfield and Ward reported that long-term administration of fluvoxamine at the dose of 10 mg/kg also did not affect the affinity or density of 5-HT receptors in rat frontal cortex (6). These results indicate that even if normal monkeys are subjected to long-term treatment of fluvoxamine at 5 mg/kg/day, this drug may not change the physiological levels of the central and peripheral 5-HT uptake sites and 5-HT2 receptors. However, there is report that another specific inhibitor of 5-HT uptake reduced the number of 5-HT receptors in the frontal cortex of rats after several weeks of administration (42). Therefore, additional studies are required to establish the exact nature of the long term effects of fluvoxamine on 5-HT receptors.

REFERENCES

1 Deakin JFW: Depression and 5-HT. Int Clin Psychopharmacol 6, Supp 3, 23–28 (1991)
2 Asberg M, Thoren P, Traskman L, Bertilsson L and Ringberger V: Serotonin depression, a biochemical subgroup within the affective disorder? Science 191, 478–480 (1976)
3 Korpi ER, Kleinman JE and Goodman SI: Serotonin and 5-hydroxyindoleacetic acid in brains of suicide victims: comparison in chronic schizophrenic patients with suicide as cause of death. Arch Gen Psychiatry 43, 594–600 (1986)
4 Guimaraes FS: New drugs, fluoxetine and fluvoxamine. Br J Hosp Med 45, 146–149 (1991)
5 Classen V: Review of the animal pharmacology and pharmacokinetics of fluvoxamine. Br J Pharmacol 15, 349s–355s (1983)
6 Benfield P and Ward A: Fluvoxamine: a review of its pharmacodynamic and pharmacokinetic properties, and therapeutic efficacy in depressive illness. Drugs 32, 313–334 (1986)
7 Wilde M, Plosker GL and Benfield P: Fluvoxamine: An updated review of its pharmacology, and therapeutic use in depressive illness. Drugs 46, 895–924 (1993)
8 Backstrom I, Bergstrom M and Marcussen J: High affinity 3H-paroxetine binding to serotonin uptake sites in human brain tissue. Brain Res 486, 261–268 (1989)
9 Leysen JE: Serotonin receptor binding sites. In Neuropharmacology of Serotonin, Edited by Gree AR, pp 106–108, Oxford University Press, London (1985)
10 Habert E, Graham D, Tahraoui L, Claustre Y and Langer SZ: Characterization of 3H-paroxetine binding to rat cortical membranes. Eur J Pharmacol 118, 107–114 (1985)
11 Snyder SH and Coyle JT: Regional differences in 3H-norepinephrine and 3H-dopamine uptake into rat brain homogenates. J Pharmacol Exp Ther 165, 75–86 (1969)
12 Egashira T, Yamamoto T and Yamanaka Y: Characteristics of mitochondrial and synaptosomal monoamine oxidase in monkey brain. Jpn J Pharmacol 34, 211–219 (1984)
13 McKeith IG, Marshall EF, Ferrier IN, Armstrong MM, Kennedy WN, Perry RH, Perry EK and Eccleston D: 5-HT receptor binding in post-mortem brain from patients with affective disorder. J Affect Disord 13, 67–74 (1987)
14 Mellerup ET, Plenge P and Engelstroft M: High affinity binding of 3H-paroxetine and 3H-imipramine to human platelet membranes. Eur J Pharmacol 96, 303–309 (1983)
15 Biegon A, Essar N, Israeli M, Elizur A, Bruch S and Bar-Nathan AA: Serotonin 5-HT2 receptor binding on platelets as a state dependent maker in major affective disorder. Psychopharmacology (Berlin) 102, 73–75 (1990)
16 Lowry OH, Rosebrough NJ, Farr AL and Randall RJ: Protein measurement with the Folin phenol reagent. J Biol Chem 193, 265–275 (1951)
17 Harms HH: The antidepressant agents desipramine, fluoxetine, fluvoxamine and norzimeldine inhibit uptake of 3H-noradrenaline and 3H-5-hydroxytryptamine in slices of human and rat cortical brain tissue. Brain Res 275, 99–104 (1983)
18 Wong DT, Bymaster FP, Reid LR and Thellend PG: Fluoxetine and two other serotonin uptake inhibitors without affinity for neuronal receptors. Biochem Pharmacol 32, 1287–1293 (1983)
19 Classen V, Davies JE, Hertting G and Placheta P: Fluvoxamine, a specific 5-hydroxytryptamine uptake inhibitor. Br J Pharmacol 60, 505–516 (1977)
20 Hyttel J and Lare J: Serotonin-selective antidepressants. Acta Pharmacol Toxicol 56, 146–153 (1985)
21 Raisman R, Briley MS and Langer SZ: Specific tricyclic antidepressant binding sites in rat brain. Nature 281, 148–150 (1979)
22 Rehavi M, Paul SM, Skolnick P and Goodwin FK: Demonstration of specific high affinity binding sites for 3H-imipramine in human brain. Life Sci 26, 2273–2279 (1980)
23 Briley MS, Langer SZ and Sette M: Allosteric interaction between the 3H-imipramine binding site and the serotonin uptake mechanism. Br J Pharmacol 74, 817–818 (1981)
24 Meyerson LR, Jeni JR and Wennogle LP: Allosteric interaction between the site labeled by 3H-imipramine and the serotonin transporter in human platelets. J Neurochem 48, 560–565 (1987)
25 Briley MS, Langer SZ, Raisman R and Sechter D: T ritiated imipramine binding sites are decreased in platelets untreated depressed patients. Science 209, 303–305 (1980)
26 Paul SM, Rehavi M, Skolnik P, Ballegger JC and Goodwin FK: Depressed patients have decreased binding of tritiated imipramine to platelet serotonin transporter. Arch Gen Psychiatry 38, 1315–1317 (1981)
27 Tuomisto J and Tukianen E: Decreased uptake of 5-hydroxytryptamine in blood platelets from depressed patients. Nature 262, 596–598 (1976)
28 Backstrom I, Bergstrom M and Marcussen J: High affinity 3H-paroxetine binding to serotonin uptake sites in human brain tissue. Brain Res 486, 261–268 (1989)
29 Hrdina PD, Foy B, Hepner A and Summers RJ: Antidepressant binding sites in brain: Autoradiographic comparison of 3H-paroxetine and 3H-imipramine localization and relationship to serotonin transporter. J Pharmacol Exp Ther 252, 410–418 (1990)
30 Raisman R, Briley MS and Langer SZ: Specific tricyclic antidepressant binding sites in rat brain characterized by affinity 3H-imipramine binding. Eur J Pharmacol 61, 373–380 (1980)
31 Langer SZ, Moret C, Raisman R, Dobociovich ML and Briley MS: High affinity 3H-imipramine binding in rat hypothalamus: association with uptake of serotonin but not of norepinephrine. Science 210, 1113–1135 (1980)
32 Moccetti I, Brunello N and Racagni G: Ontogenetic study of 3H-imipramine binding sites and serotonin uptake system: indication of possible interdependence. Eur J Pharmacol 83, 151–152 (1982)
33 Barbaccia ML, Gandolfi O, Chuang DM and Costa E: Modulation of neuronal serotonin uptake by a putative endogenous ligand of imipramine recognition sites. Proc Natl Acad Sci USA 80, 5134–5138 (1983)
34 Racagni G, Moccetti I, Calderini G, Battistella A and Brunello N: Temporal sequence of changes in central noradrenergic system of rat after prolonged antidepressant treatment: receptor desensitization and neurotransmitter interactions. Neuropharmacology 22, 415–424 (1983)
35 Olenge P and Mellerup ET: 3H-I mipramine high affinity binding sites in rat brain. Effects of imipramine and lithium. Psychopharmacology (Berlin) 77, 94–97 (1982)
36 Lee CM, Javitch JA and Snyder SH: Recognition sites for norepinephrine uptake. Regulation by neurotransmitter. Science 220, 626–629 (1983)
37 Gentisch C, Lichsteiner M and Feer H: 3H-I mipramine and 3H-cyanomipramine binding in rat brain tissue. Effect of long-term antidepressant administration. J Neural Transm 59, 257–264
Humphreys CJ, Levin J and Rudnick G: Antidepressant binding to the porcine and human platelet serotonin transporters. Mol Pharmacol 33, 657–663 (1988)

Brunello N, Riva M, Volterra A and Racagni G: Effect of some tricyclic and nontricyclic antidepressants on $^3$H-imipramine binding and serotonin uptake in rat cerebral cortex after prolonged treatment. Fundam Clin Pharmacol 1, 327–333 (1987)

Laperre YD, Rastogi RB and Singhal RL: Fluvoxamine influences serotonergic system in the brain: neurochemical evidence. Neuropsychobiology 10, 213–216 (1983)

Invernizzi R, Belli S and Samanin R: Citalopram’s ability to increase the extracellular concentrations of serotonin in the dorsal raphe prevents the drug’s effect in the frontal cortex. Brain Res 584, 322–324 (1992)

Ogren SO, Fuxe K, Agnati LF, Gustafsson JA, Jonsson G and Holm AC: Reevaluation of the indoleamine hypothesis of depression: evidence for a reduction of functional activity of central 5-HT systems by antidepressant drugs. J Neural Transm 46, 85–103 (1979)