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Relationship between brain serotonin transporter binding, plasma concentration and behavioural effect of selective serotonin reuptake inhibitors

[1]Kazufumi Hirano, [1]Ryohei Kimura, [2]Yumi Sugimoto, [2]Jun Yamada, [3]Shinya Uchida, [4]Yasuhiro Kato, [4]Hisakuni Hashimoto & *,[1]Shizuo Yamada

[1]Department of Biopharmaceutical Sciences and COE Program in the 21st Century, School of Pharmaceutical Sciences, University of Shizuoka, 52-1 Yada, Shizuoka 422-8526, Japan; [2]Department of Pharmacology, Kobe Pharmaceutical University, 4-19-1 Motoyamakita, Higashinada, Kobe 658-8558, Japan; [3]Department of Clinical Pharmacology, School of Medicine, Hamamatsu University, 1-20-1 Handayama, Hamamatsu 431-3192, Japan and [4]Department of Hospital Pharmacy, School of Medicine, Hamamatsu University, 1-20-1 Handayama, Hamamatsu 431-3192, Japan

1 The present study was undertaken to characterise the relationship between *in vivo* brain serotonin transporter (SERT) binding, plasma concentration and pharmacological effect of selective serotonin reuptake inhibitors (SSRIs) in mice. Oral administration of fluvoxamine, fluoxetine, paroxetine and sertraline at pharmacologically relevant doses exerted dose- and time-dependent binding activity of brain SERT as revealed by significant increases in K_D for specific [^{3}H]paroxetine binding, and the *in vivo* SERT-binding potency was in the order of paroxetine $\gg$ fluoxetine, sertraline $>$ fluvoxamine.

2 The time courses of brain SERT binding by SSRIs in mice were mostly in parallel to those of their plasma concentrations. Also, norfluoxetine (active metabolite) has been suggested to contribute largely to the long-lasting binding activity of brain SERT after the fluoxetine administration.

3 Oral administration of each SSRI suppressed significantly the marble-burying behaviour with no change in locomotor activity in mice, and the extent and time course of suppression agreed well with those of brain SERT binding. Thus, the pharmacological potencies of SSRIs in the attenuation of marble-burying behaviour correlated significantly with their brain SERT binding activities.

4 In conclusion, the present study has provided the first *in vivo* evidences to support that fluvoxamine, fluoxetine, paroxetine and sertraline orally administered bind to the pharmacologically relevant brain SERT in mice and that their SERT-binding characteristics is closely associated with the pharmacokinetics and inhibition of marble-burying behaviour.
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Abbreviations: OCD, obsessive-compulsive disorder; SERT, serotonin transporter; SSRI, selective serotonin reuptake inhibitor

Introduction

Selective serotonin reuptake inhibitors (SSRIs) such as fluvoxamine, fluoxetine, paroxetine and sertraline are currently among the most frequently prescribed therapeutic agents in all medicines and their therapeutic actions are diverse, ranging from efficacy in depression to obsessive-compulsive disorder (OCD), panic disorder and other conditions as well. Serotonin has been known to mediate such diverse behaviours as mood, anxiety, sleep, temperature, appetite, sexual behaviour and eating behaviour (Wong *et al*., 1995; Figgitt & McClellan, 2000), and serotonin transporter (SERT), a reuptake molecule that removes serotonin from the synapse, regulates the synaptic concentration of released serotonin. Depression and other psychiatric disorders are considered to be due to chronically low levels of serotonergic neurotransmission and this model is called to 'serotonin hypothesis' (Schafer, 1999). SSRIs potently interfere with the activity of SERT in the brain and subsequently increase the extracellular levels of serotonin, resulting in the enhancement of serotonergic neurotransmission. In addition, SSRIs have been characterised by low affinities for adrenergic, dopaminergic, muscarinic, histaminergic H_1, opiate, GABA and benzodiazepine receptors (Wong *et al*., 1995; Owens *et al*., 1997), and these properties make it possible to medicate psychiatric diseases with acceptable side effects and relatively safe cardiac effects, compared with imipramine and other tricyclic antidepressants.

With the advent of antidepressants, many investigators have attempted to develop appropriate animal models for the clarification of antidepressive mechanisms of these drugs. The forced swimming test and marble-burying test have been utilised as behavioural models to predict the therapeutic effects for depression and OCD, respectively (Porsolt *et al*., 1978; Njung'e & Handley, 1991). The determination of degree and duration of SERT occupancy by SSRIs under physiological conditions is important to characterise the pharmacological specificity in relation to the pharmacokinetics and pharmacodynamics, as reported previously for 1,4-dihydropyridine

*Author for correspondence;
E-mail: yamada@ys-7.u-shizuoka-ken.ac.jp
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calcium channel antagonists (Yamada *et al*., 2002), angiotensin II receptor antagonists (Nozawa *et al*., 1998) and α_1-adrenoceptor antagonists (Yamada *et al*., 2001). To date, the pharmacological effects of SSRIs in mice have been little investigated in relation to brain SERT binding and plasma concentrations. Therefore, the present study was carried out to clarify the relationship between brain SERT binding, plasma concentration and behavioural effects of SSRIs by determining simultaneously these parameters in mice after oral administration. Mice received orally fluvoxamine (6.91–69.1 μmol kg^{-1}), fluoxetine (2.89–28.9 μmol kg^{-1}), paroxetine (0.27–2.67 μmol kg^{-1}) and sertraline (2.92–29.2 μmol kg^{-1}), because each drug at these oral doses is anticipated to increase significantly the extracellular serotonin in rat brain (Rutter & Auerbach, 1993; Bosker *et al*., 1995; Malagié *et al*., 2000).

Methods

Materials

[^{3}H]Paroxetine (795.5 GBq mmol^{-1}) was purchased from Dupont-NEN Co. Ltd. (Boston, MA, U.S.A.). Fluvoxamine maleate was purchased from Tocris (U.K.). Fluoxetine hydrochloride, paroxetine hydrochloride and sertraline hydrochloride were kindly donated by Eli Lilly pharmaceuticals (Greenfield, IN, U.S.A.), GlaxoSmithKline pharmaceuticals (West Sussex, England) and Pfizer Inc. (Groton, CT, U.S.A.), respectively. All other drugs and materials were obtained from commercial sources.

Animals

Male ICR strain mice at 6–8 weeks of age (Japan SLC Inc., Shizuoka, Japan) were housed 10 per cage in the laboratory with free access to food and water, and were maintained on a 12 h dark/light cycle in a room with controlled temperature (24 ± 1°C) and humidity (55 ± 5%). This study was conducted according to guidelines approved by the Experimental Animal Ethical Committee of University of Shizuoka.

Measurements of specific binding of [^{3}H]paroxetine

Mice were fasted for 16 h before the administration of drugs, and received orally fluvoxamine (6.91–69.1 μmol kg^{-1}), fluoxetine (2.89–28.9 μmol kg^{-1}), paroxetine (0.27–2.67 μmol kg^{-1}) and sertraline (2.92–29.2 μmol kg^{-1}) dissolved in distilled water. At 0.25–48 h after the drug administration, mice were exsanguinated by taking the blood from descending aorta under light anesthesia with diethyl ether, and brain was perfused with 0.9% NaCl from the aorta. Then, the whole brain tissue was removed. The plasma from mouse blood was isolated by centrifugation, and stored at –80°C until the drug concentration was determined. The brain tissue was homogenised in 19 volumes of 50 mM Tris-HCl buffer (pH 7.4) containing 120 mM NaCl and 5 mM KCl with a Polytron homogeniser, and the homogenate was centrifuged at $40{,}000 \times g$ for 15 min. The pellet was resuspended in 24 volumes of the buffer. All steps for the tissue preparation were performed at 4°C. The binding assay for SERT in brain homogenates from mice was performed by using [^{3}H]paroxetine, as previously described (Habert *et al*., 1985). Briefly, the brain homogenates (approximately 400 μg protein) were incubated with six different concentrations (0.03, 0.1, 0.3, 0.5, 1.0, 2.0 nM) of [^{3}H]paroxetine for 2 h at 20°C in 50 mM Tris-HCl buffer (pH 7.4) containing 120 mM NaCl and 5 mM KCl. The reaction was terminated by rapid filtration (Cell Harvester, Brandel Co., Gaithersburg, MD, U.S.A.) through Whatman GF/B glass fibre filters, and filters were rinsed three times with 2 ml of ice-cold buffer. Tissue-bound radioactivity was extracted from filters overnight in scintillation fluid (21 of toluene, 1 l of Triton X-100, 15 g of 2,5-diphenyloxazole, 0.3 g of 1,4-bis[2-(5-phenyloxazolyl)]benzene) and it was determined in a liquid scintillation counter. Specific binding of [^{3}H]paroxetine was determined experimentally from the difference between counts in the absence and presence of 10 μM fluoxetine. All assays were conducted in duplicate. Every binding experiment was performed using fresh tissues. Protein concentration was measured according to the method (Lowry *et al*., 1951) using bovine serum albumin as standard.

Determination of SSRI and its active metabolite in plasma

The concentration of SSRIs and norfluoxetine in mouse plasma was determined by the column-switching HPLC with clomipramine as an internal standard, according to the previous method (Meineke *et al*., 1998) with some modification. Briefly, 400 μl of plasma was utilised, and 50 μl of internal standard (final concentration 500 ng ml^{-1}) and 50 μl of 2 M NaOH were added to the plasma. Samples were then vortex-mixed and consequently extracted into 2 ml of *n*-heptan/3-methyl-1-butanol (985/15, v v^{-1}) on a reciprocating shaker for 20 min. Then, the upper organic layer was transferred and dried under a gentle stream of nitrogen. The residue reconstituted in 110 μl of mobile phase, and it was analysed by HPLC. The HPLC system consisted of two pumps (model LC-9A, Shimadzu, Tokyo, Japan), a six-port switching valve (model FCV-2AH, Shimadzu, Tokyo, Japan) controlled by the use of a system controller (model SCL-6B, Shimadzu, Tokyo, Japan), an injector (SIL-6B, Shimadzu, Tokyo, Japan) and a variable wavelength UV detector (Hitachi, Tokyo, Japan). The UV detector was set at 254 nm (fluvoxamine), 225 nm (fluoxetine and norfluoxetine), 295 nm (paroxetine) and 214 nm (sertraline). Integration of peak area was performed by a computing integrator (model Chromatopac C-R4A, Shimadzu, Tokyo, Japan). Chromatography was performed on a reversed-phase. Column I was a Shim-pack SPC-RP3, 9 μm polyvinylalcohol resin, 30×4 mm inside diameter, pretreatment column (Shimadzu, Tokyo, Japan). Column II was a Shim-pack CLC-CN(M), 5 μm cyanopropyl group, 250×4.6 mm inside diameter, analytical column (Shimadzu, Tokyo, Japan). The columns were maintained at 50°C. The mobile phase consisted of 0.01 M phosphate buffer (pH 6.8), acetonitrile and methanol (100 : 123 : 40). The sensitivity limits of plasma assay were 5 ng ml^{-1} (fluvoxamine, fluoxetine and norfluoxetine) and 2.5 ng ml^{-1} (paroxetine and sertraline).

Behavioural tests

Mice received orally fluvoxamine (69.1 μmol kg^{-1}), fluoxetine (28.9 μmol kg^{-1}), paroxetine (2.67 μmol kg^{-1}) and sertraline (29.2 μmol kg^{-1}), and control animals received vehicle. At 0.25–48 h after the administration, mice were tested for the

marble-burying test. Briefly, an open cubic transparent plastic box ($22.5 \times 33.8 \times 14.0$ cm) was used, and 20 clean glass marbles (15 mm diameter) were evenly spaced (5 cm apart) on sawdust (5 cm deep) as previously described (Njung'e & Handley, 1991; Ichimaru *et al*., 1995). Mice were placed into the cubic box, and the number of marbles buried at least two-thirds were counted for 30 min. The total locomotor activity of animals for 30 min was also counted by an activity sensor (NS-AS01, Neuroscience Inc., Tokyo, Japan).

Analysis of binding data and estimation of SERT-binding activity

Analysis of binding data was performed as described previously (Yamada *et al*., 1980). The apparent dissociation constant (K_D) and maximal number of binding sites (B_{max}) for [^{3}H]paroxetine were estimated by Rosenthal analysis of the saturation data (Rosenthal, 1967). The SERT-binding activity (percent increase in K_D values by each SSRI) was calculated by the equation: K_D (drug)/K_D (control) $\times$ 100, where K_D (control) and K_D (drug) are K_D values for specific [^{3}H]paroxetine binding in the brains of vehicle- and drug-treated mice, respectively.

The ability of SSRIs and norfluoxetine to inhibit specific [^{3}H]paroxetine (0.3 nM) binding *in vitro* was estimated by IC_{50} values, which are the molar concentrations of unlabelled drugs necessary for displacing 50% of specific binding (estimated by log probit analysis). The inhibition constant, K_i, was calculated from the equation, $K_i = IC_{50}/(1 + L/K_D)$, where L equals concentration of the radioligand.

Pharmacokinetic analysis

The pharmacokinetic parameters of each drug were estimated with noncompartmental methods as previously described (Caccia *et al*., 1990; Yoon *et al*., 2000). The terminal elimination rate constant (β) was estimated from the least-square regression slope of terminal log-linear plasma concentration data points. The half-life ($t_{1/2}$) of the elimination phase was calculated as $\ln 2/\beta$. The total area under the plasma concentration *versus* time curve (AUC) was calculated by the trapezoidal rule, with extrapolation to the infinity by the terminal slope. The first moment of the plasma concentration *versus* time profile (AUMC) was determined similarly after multiplying each concentration by its time. Apparent total clearance (CL/F) and volume of distribution (Vd/F) were calculated by the equations:

$$CL/F = Dose/AUC,\ Vd/F = Dose \cdot AUMC/(AUC)^2$$

in which F indicates bioavailability. The $t_{1/2}$ and AUC of SERT-binding activity were also calculated as described above.

Statistics

The results were reported as mean $\pm$ s.e.m. Statistical analysis of the data was performed by one-way analysis of variance followed by Dunnett's test for multiple comparisons. A value of $P < 0.05$ was considered significant.

Results

Effects of oral administration of SSRIs on specific [^{3}H]paroxetine binding in mouse brain

In the *in vitro* binding experiment, fluvoxamine (1–100 nM), fluoxetine (1–100 nM), norfluoxetine (1–100 nM), paroxetine (0.1–10 nM) and sertraline (1–100 nM) inhibited specific [^{3}H]paroxetine binding in mouse brain homogenates in a concentration-dependent manner and their K_i values were 5.52 ± 0.82, 10.8 ± 1.9, 8.72 ± 1.30, 0.54 ± 0.04 and 3.39 ± 1.25 nM ($n = 3$–4), respectively.

The values of K_D and B_{max} for [^{3}H]paroxetine binding were determined in brains of mice at 0.25–48 h after oral administration of fluvoxamine, fluoxetine, paroxetine and sertraline (Table 1). Following oral administration of fluvoxamine at doses of 6.91, 23.0 and 69.1 μmol kg^{-1}, there were dose-dependent increases in K_D without a change in B_{max} for brain [^{3}H]paroxetine binding at 0.5 h after the administration, compared to the control values (Table 1). The enhancements at 23.0 (0.5 h) and 69.1 (0.25–4 h) μmol kg^{-1} of this drug were statistically significant and maximal (4.8- and 23.1-fold, respectively) at 0.5 or 1 h later.

Similarly, there were significant (3.2-, 7.0- and 5.7-fold, respectively) increases in K_D for [^{3}H]paroxetine in the mouse brain at 1, 4 and 12 h after oral administration of fluoxetine (8.68 μmol kg^{-1}). Higher dose (28.9 μmol kg^{-1}) of this drug brought about greater (5.5-, 10.4-, 19.6-, 22.7- and 12.7-fold, respectively) increases in K_D at 0.5, 1, 4, 12 and 24 h after the administration, and enhanced significantly (17–33%) B_{max} at 1–24 h. The maximal effect by fluoxetine tended to be seen at 4 or 12 h later.

Oral administration of paroxetine at relatively low dose (0.80 and 2.67 μmol kg^{-1}) caused dose-dependent increases in K_D for brain [^{3}H]paroxetine binding, and the extents of enhancement were statistically significant at 4 h (0.80 μmol kg^{-1}) and 1, 4 and 12 h (2.67 μmol kg^{-1}) after the oral administration. The maximal increases (5.0- and 15.1-fold, respectively) at each dose were seen at 4 h later. The significant decreases (29.7 and 28.3 %, respectively) of B_{max} were observed at 4 and 12 h after the oral administration of paroxetine (2.67 μmol kg^{-1}).

Sertraline at oral doses of 2.92, 8.75 and 29.2 μmol kg^{-1} brought about dose-dependent increases in K_D without a change in B_{max} for brain [^{3}H]paroxetine binding at 0.5–12 h later, and the enhancement (2.5-, 5.6- and 14.8-fold, respectively) at each dose was maximal at 4 h later.

Based on the linear regression analysis of oral doses of SSRIs *versus* their increased rates of K_D for brain [^{3}H]paroxetine binding (Figure 1), oral doses of fluvoxamine, fluoxetine, paroxetine and sertraline, which increased K_D for [^{3}H]paroxetine binding by 10-fold, were 31.3, 14.3, 1.74 and 18.7 μmol kg^{-1}, respectively.

Figure 2a illustrates the time courses of enhancement of K_D for brain [^{3}H]paroxetine binding in mice after oral administration of fluvoxamine (69.1 μmol kg^{-1}), fluoxetine (28.9 μmol kg^{-1}), paroxetine (2.67 μmol kg^{-1}) and sertraline (29.2 μmol kg^{-1}). The $t_{1/2}$ of elimination phase and AUC of the enhancement were largest for fluoxetine, followed by sertraline and paroxetine $\gg$ fluvoxamine (Table 2).

Table 1 Effects of oral administration of fluvoxamine, fluoxetine, paroxetine and sertraline on apparent dissociation constant (K_D) and maximal number of binding sites (B_{max}) of specific [^{3}H]paroxetine binding in mouse brain

Dose (μmol kg^{-1})	*Time* (h)	K_D (nM)	B_{max} (fmol mg protein^{-1})
Control		0.13±0.01	292±5
Fluvoxamine			
6.91	0.5	0.26±0.11	233±4
23.0	0.5	0.62±0.11**	272±19
	1	0.33±0.03	278±16
	4	0.24±0.05	315±13
69.1	0.25	1.94±0.29***	327±23
	1	3.00±0.36***	336±19
	4	0.96±0.15**	330±8
	8	0.36±0.02	293±8
	12	0.21±0.01	295±6
Control		0.13±0.01	292±5
Fluoxetine			
2.89	4	0.17±0.03	244±15
8.68	0.5	0.23±0.02	271±4
	1	0.41±0.04**	291±5
	4	0.91±0.15**	329±12
	12	0.74±0.09**	290±19
	24	0.23±0.04	290±10
28.9	0.5	0.71±0.01**	318±8
	1	1.35±0.15**	343±9*
	4	2.55±0.58***	343±5*
	12	2.95±0.31***	389±30***
	24	1.65±0.16**	361±16**
	48	0.22±0.05	301±8
Control		0.13±0.01	290±8
Paroxetine			
0.27	4	0.18±0.03	307±15
0.80	1	0.15±0.02	247±20
	4	0.65±0.13**	321±16
	12	0.16±0.01	299±8
2.67	0.5	0.53±0.13	247±14
	1	1.57±0.21***	261±16
	4	1.96±0.26***	204±20**
	12	1.02±0.06***	208±8***
	24	0.12±0.01	253±10
Control		0.14±0.01	316±10
Sertraline			
2.92	4	0.35±0.06**	328±5
8.75	1	0.53±0.10**	308±23
	4	0.79±0.07***	317±16
	12	0.37±0.05*	311±25
29.2	0.5	0.66±0.11**	327±11
	1	1.26±0.10***	282±17
	4	2.07±0.30***	272±43
	12	1.02±0.11***	286±12
	24	0.14±0.01	318±14

Mice received fluvoxamine (6.91–69.1 μmol kg^{-1}), fluoxetine (2.89–28.9 μmol kg^{-1}), paroxetine (0.27–2.67 μmol kg^{-1}) or sertraline (2.92–29.2 μmol kg^{-1}) orally, and were exsanguinated by taking blood from descending aorta at 0.25–48 h later. Specific [^{3}H]paroxetine (0.03–2.0 nM) binding in mouse brain was measured. Values are mean±s.e.m. of three to five (SSRI-treated group) and seven to eight (vehicle-treated group) mice. Asterisks show a significant difference from the control values, *$P<0.05$, **$P<0.01$, ***$P<0.001$.

Plasma concentration of SSRIs

Plasma concentrations of SSRIs and norfluoxetine were measured in mice after the oral administration of each SSRI

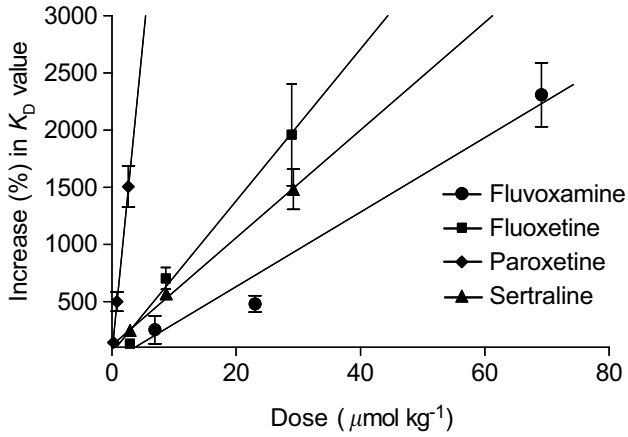

Figure 1 Relationship between oral doses of SSRIs and brain SERT-binding activity (percent increase in K_D values for specific [^{3}H]paroxetine binding). Mice received fluvoxamine (6.91–69.1 μmol kg^{-1}), fluoxetine (2.89–28.9 μmol kg^{-1}), paroxetine (0.27–2.67 μmol kg^{-1}) and sertraline (2.92–29.2 μmol kg^{-1}) orally, and the mean values at the times when plasma drug concentration and SERT-binding activity became maximal (0.5 or 1 h for fluvoxamine, 4 h for fluoxetine, paroxetine and sertraline) were utilised for linear-regression analysis. Each point represents mean±s.e.m. of four to five mice.

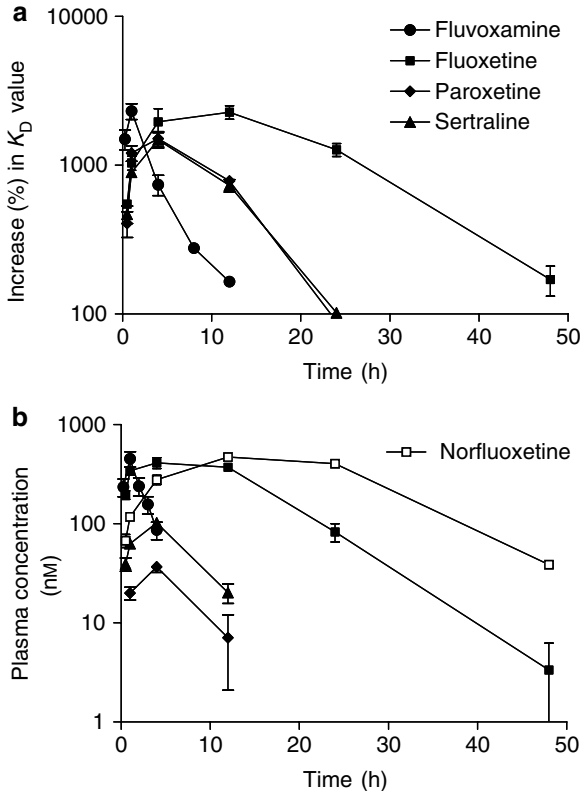

Figure 2 Time courses of brain SERT-binding activity (percent increase in K_D values for specific [^{3}H]paroxetine binding) (a) and plasma concentration (b) at 0.25 to 48 h after oral administration of fluvoxamine (69.1 μmol kg^{-1}), fluoxetine (28.9 μmol kg^{-1}), paroxetine (2.67 μmol kg^{-1}) and sertraline (29.2 μmol kg^{-1}). The plasma concentration of norfluoxetine was also plotted. Each point represents mean±s.e.m. of three to five mice.

Table 2 Pharmacokinetic parameters calculated from concentration of SSRI and its active metabolite in mouse plasma and SERT-binding activity (percent increase in K_D values for specific [^{3}H]paroxetine binding) after oral administration

Pharmacokinetic parameters	*Fluvoxamine* (*69.1* μmol kg^{-1})	*Fluoxetine* (*28.9* μmol kg^{-1})	*Norfluoxetine*	*Paroxetine* (*2.67* μmol kg^{-1})	*Sertraline* (*29.2* μmol kg^{-1})
Plasma concentration					
$t_{1/2}$ (h)	1.29	6.02	12.3	3.36	5.98
CL/F (ml min^{-1} kg^{-1})	1030	58.3	—	146	406
Vd/F (l kg^{-1})	138	79.0	—	50.6	319
AUC (nM h)	1220	8260	15,800	306	1200
SERT-binding activity					
$t_{1/2}$ (h)	1.83	9.43	—	4.79	5.09
AUC (% h)	9110	60,500	—	19,000	17,900

Pharmacokinetic parameters of SSRIs and norfluoxetine were calculated from the mean value of plasma concentration and SERT-binding activity at each time point shown in Figure 2.

(Figure 2b). The plasma concentration of fluvoxamine after the oral administration of 69.1 μmol kg^{-1} peaked at 1 h (453 nM) and decreased with the time. Fluoxetine undergoes extensive metabolic conversion, leading to the active metabolite norfluoxetine and other nonactive metabolites (Hiemke & Härtter, 2000). After the oral administration of fluoxetine (28.9 μmol kg^{-1}) in mice, plasma concentrations of fluoxetine and norfluoxetine peaked at 4 h (450 nM) and 12 h (439 nM), respectively, and norfluoxetine decreased more slowly than fluoxetine. In the case of oral administration of paroxetine (2.67 μmol kg^{-1}) and sertraline (29.2 μmol kg^{-1}) in mice, the plasma concentrations peaked at 4 h later (36.9 nM and 103 nM, respectively), and decreased with the time. The time courses of rise and decline of plasma concentrations of SSRIs were reasonably parallel to those of increases of K_D for brain [^{3}H]paroxetine binding (Figure 2a and b).

Pharmacokinetic parameters were estimated from the plasma concentrations of SSRIs and norfluoxetine in mice. As shown in Table 2, the $t_{1/2}$ value for plasma concentration was largest in norfluoxetine, followed by fluoxetine, sertraline > paroxetine > fluvoxamine. The AUC values were much greater in norfluoxetine and fluoxetine than other SSRIs. The CL/F values for fluvoxamine, paroxetine and sertraline were greater (17.7-, 2.5- and 7.0-fold, respectively) than that of fluoxetine, and Vd/F value for sertraline was the greatest.

Behavioural effects

The marble-burying behaviour was significantly (66.7, 69.4 and 36.7%, respectively) suppressed at 0.25, 1 and 4 h after the oral administration of fluvoxamine (69.1 μmol kg^{-1}) compared with the control values (Figure 3a), and it was recovered to the control value at 12 h. At 1, 4 and 24 h after oral administration of fluoxetine (28.9 μmol kg^{-1}), there was significant (31.5, 84.6 and 18.6%, respectively) attenuation of marble-burying behaviour (Figure 3b). In the case of paroxetine (2.67 μmol kg^{-1}), significant (40.3 and 46.6%, respectively) suppression of marble-burying behaviour was also observed at 1 and 4 h after the oral administration, and the behaviour was recovered to the control value at 12 and 24 h later (Figure 3c). Similarly, oral administration of sertraline (29.2 μmol kg^{-1}) attenuated significantly (18.8, 75.5 and 23.3%, respectively) the marble-burying behaviour at 1, 4 and 12 h later (Figure 3d). Thus, the maximal suppression by SSRIs was observed at 0.25 and 1 h (fluvoxamine), 4 h (fluoxetine), 1 and 4 h (paroxetine) and 4 h (sertraline), respectively.

On the other hand, the oral administration of SSRIs at the doses that suppressed significantly the marble-burying behaviour had no change in locomotor activity in mice (Figure 4).

Discussion

The major findings of our study are that fluvoxamine, fluoxetine, paroxetine and sertraline orally administered bind to brain SERT and their SERT-binding characteristics is closely associated with both plasma drug concentrations and inhibitory effects on marble-burying behaviour. Thus, the measurement of SERT occupancy after oral administration of SSRIs may offer better understanding of pharmacodynamics and pharmacokinetics of these drugs.

[^{3}H]Paroxetine has been shown to bind selectively to brain SERT with a high affinity (K_D: 0.13 nM) in agreement with previous report (Habert *et al*., 1985). Fluvoxamine, fluoxetine, paroxetine and sertraline competed with [^{3}H]paroxetine for the binding sites in the mouse brain in a concentration-dependent manner under the *in vitro* condition. Paroxetine was the most potent inhibitor of [^{3}H]paroxetine-binding sites, followed by sertraline ⩾ fluvoxamine > fluoxetine. These *in vitro* data have confirmed that SSRIs bind to the brain SERT (Owens *et al*., 1997).

Oral doses of fluvoxamine (69.1 μmol kg^{-1}), fluoxetine (28.9 μmol kg^{-1}), paroxetine (2.67 μmol kg^{-1}) and sertraline (29.2 μmol kg^{-1}) were considered to be pharmacologically equipotent in exerting similar magnitude of maximal increases in the extracellular serotonin levels measured by brain microdialysis (Rutter & Auerbach, 1993; Bosker *et al*., 1995; Malagié *et al*., 2000). In the present study, the oral administration of fluvoxamine (6.91–69.1 μmol kg^{-1}), fluoxetine (2.89–28.9 μmol kg^{-1}), paroxetine (0.27–2.67 μmol kg^{-1}) and sertraline (2.92–29.2 μmol kg^{-1}) caused dose- and time-dependent suppression of specific [^{3}H]paroxetine binding in mouse brain as revealed by significant increases in K_D. The inhibitory effects by these SSRIs of [^{3}H]paroxetine binding tended to peak at 1 h (fluvoxamine), 4–12 h (fluoxetine), 4 h (paroxetine) and 4 h (sertraline) after the oral administration, and significant increases in K_D by the highest doses lasted up to at least 4 h for fluvoxamine, 24 h for fluoxetine and 12 h for

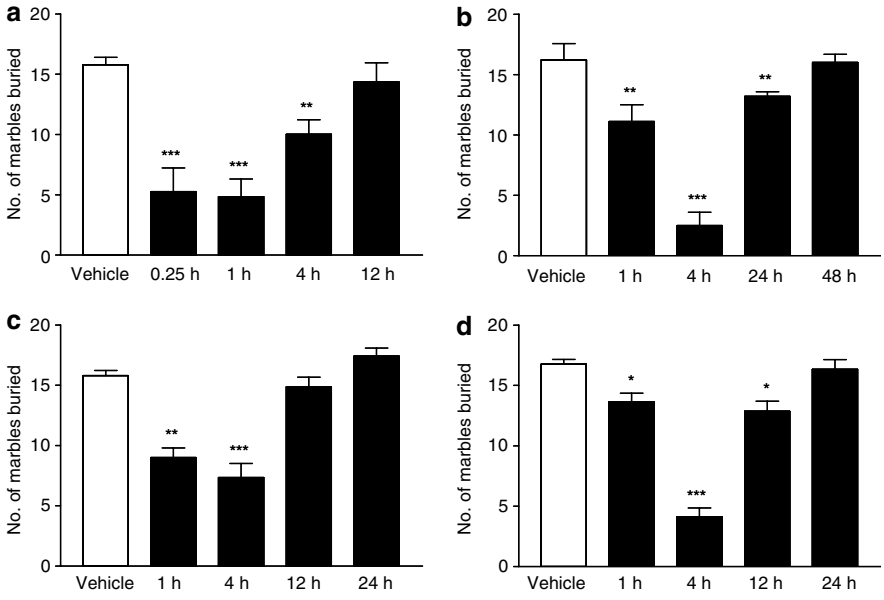

Figure 3 Effects of oral administration of fluvoxamine (a, 69.1 μmol kg^{-1}), fluoxetine (b, 28.9 μmol kg^{-1}), paroxetine (c, 2.67 μmol kg^{-1}) and sertraline (d, 29.2 μmol kg^{-1}) on the marble-burying behaviour in mice. At 0.25–48 h after the oral administration of these drugs, mice were placed into the cubic plastic box in which 20 glass marbles were evenly spaced on sawdust, and the number of marbles buried at least two-thirds was counted for 30 min. Each column represents mean ± s.e.m. of five to eight (vehicle-treated group) and six to nine (SSRI-treated group) mice. Asterisks show a significant difference from the vehicle control values, *$P<0.05$, **$P<0.01$, ***$P<0.001$.

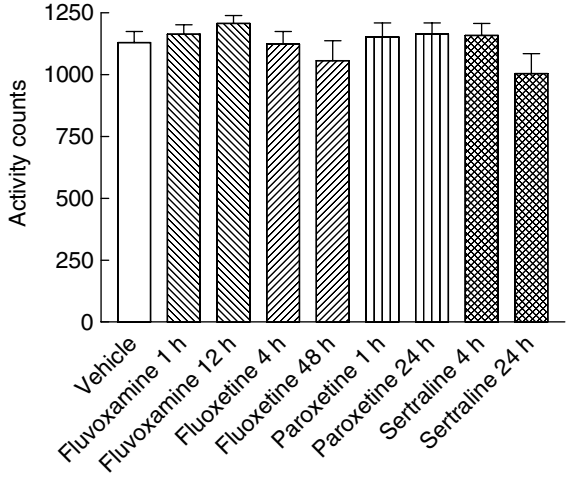

Figure 4 Effects of oral administration of fluvoxamine (69.1 μmol kg^{-1}), fluoxetine (28.9 μmol kg^{-1}), paroxetine (2.67 μmol kg^{-1}) and sertraline (29.2 μmol kg^{-1}) on the locomotor activity in mice. At 1–48 h after the oral administration of SSRIs, the total locomotor activity of mice for 30 min was measured by an activity sensor. Each column represents mean ± s.e.m. of eight (vehicle-treated group) and five to eight (SSRI-treated group) mice.

paroxetine and sertraline. Our preliminary data have also confirmed that each SSRI at oral doses used here enhanced significantly K_M for specific [^{3}H]5-HT uptake into mouse brain synaptosomes (Hirano *et al*., unpublished observation). Hence, these data suggest strongly that SSRIs orally administered bind selectively to the pharmacologically relevant brain SERT. Further, it has been shown that the time courses of brain SERT-binding activities by SSRIs are mostly in parallel to those of plasma concentrations of these agents including the active metabolite (Figure 2a and b).

Based on oral doses of fluvoxamine, fluoxetine, paroxetine and sertraline, which increased K_D for brain [^{3}H]paroxetine binding by 10-fold, the *in vivo* SERT-binding potency of SSRIs was shown to be in the order of paroxetine $\gg$ fluoxetine, sertraline $>$ fluvoxamine. This relative potency of fluoxetine did not necessarily coincide with the order of *in vitro* binding affinity (K_i) for SERTs, that is, paroxetine > sertraline > fluvoxamine > fluoxetine. The discrepancy between *in vivo* and *in vitro* in the binding potency of fluoxetine at the SERT may stem largely from pharmacokinetic factors such as oral bioavailability, plasma half-life, formation of active metabolites and permeability through the blood–brain barrier. In fact, the value of AUC for SERT-binding activity of fluoxetine was largest among four SSRIs (Table 2), due to the relatively longer-lasting occupation of brain SERT. Fluoxetine is metabolised by N-demethylation to a major metabolite, norfluoxetine (Hiemke & Härtter, 2000), which is pharmacologically comparable to fluoxetine (Wong *et al*., 1995) and is readily permeable through the blood–brain barrier (Fuller & Snoddy, 1993). In fact, the K_i of norfluoxetine for inhibiting *in vitro* brain [^{3}H]paroxetine binding was similar to that of fluoxetine, and AUC and $t_{1/2}$ of this metabolite in the mouse plasma were approximately two times larger than those of fluoxetine (Table 2). Thus, it is likely that norfluoxetine contributes largely to the long-lasting occupation of brain SERT after oral administration of fluoxetine. The present study provides the first *in vivo* evidence to support the idea that fluoxetine, paroxetine and sertraline orally administered may bind to brain SERT more potently than fluvoxamine, and such differences among SSRIs may reflect the dissimilarity in their clinical dosages for the treatment of psychiatric diseases (DeVane, 1999).

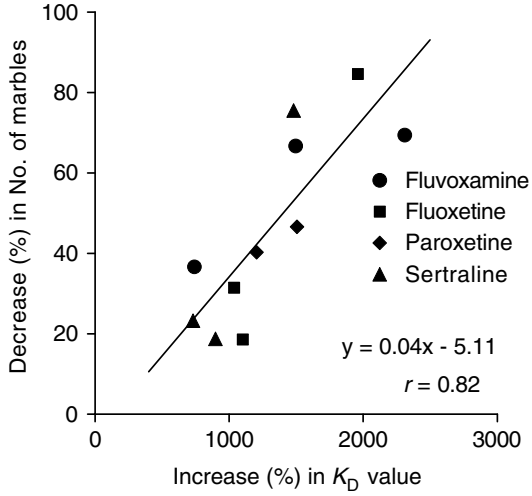

Figure 5 Relationship between binding activity of brain SERT (percent increase in K_D values for specific [^{3}H]paroxetine binding) and inhibitory effect of marble-burying behaviour (percent decrease in the number of marbles buried by mice) after oral administration of fluvoxamine (69.1 μmol kg^{-1}), fluoxetine (28.9 μmol kg^{-1}), paroxetine (2.67 μmol kg^{-1}) and sertraline (29.2 μmol kg^{-1}). The data for brain SERT-binding activity and suppression of marble-burying behaviour were derived from Figure 2 and Figure 3, respectively. The mean values at each time point were utilised for correlation analysis, where y, x and r represent inhibitory effect of marble-burying behaviour, brain SERT-binding activity and correlation coefficient, respectively.

It was reported that the suppressive effect of fluvoxamine on the marble-burying behaviour was more potent than that of clomipramine (a dual inhibitor of serotonin and norepinephrine reuptake), and that desipramine (selective inhibitor of norepinephrine reuptake) was ineffective in this model (Ichimaru *et al*., 1995). In the present study, marble-burying behaviour was significantly suppressed by fluvoxamine, fluoxetine, paroxetine and sertraline at oral dosages and times that occupied significantly brain SERT. On the other hand, each SSRI had no change in locomotor activity in mice, suggesting that the inhibitory effects on marble-burying behaviour are not derived from the suppression of locomotor activity. As shown in Figure 5, the inhibitory potencies of marble-burying behaviours after the oral administration of SSRIs correlated significantly ($P<0.01$) with their binding activities of brain SERT. Therefore, it is evident that the suppression of marble-burying behaviour after oral administration of SSRIs may be predominantly attributable to the enhancement of serotonergic neurotransmission due to the selective blockade of brain SERT. In fact, such notion is also supported by clinical findings that SSRIs have greater therapeutic efficacy on OCD than antidepressants without selective serotonin reuptake inhibitory property (Piccinelli *et al*., 1995).

After oral administration of paroxetine, there was not only increase in K_D for brain [^{3}H]paroxetine binding but also significant decrease in B_{max} at 4 and 12 h, suggesting competitive and noncompetitive blockade of SERT. Such antagonism is considered to be due to the slowly dissociating blockade by paroxetine of SERT in mouse brain, as previously demonstrated in the noncompetitive blockade of brain nicotinic receptors by neosurugatoxin (Yamada *et al*., 1985). There was slight but significant increase in B_{max} for [^{3}H]paroxetine binding in mouse brain after oral administration of fluoxetine. Although we have no clear explanation for this enhancement, it is possible that the long-lasting occupancy by fluoxetine brought about upregulation of SERT itself. In fact, we have previously reported that fluoxetine reduced B_{max} in brain [^{3}H]paroxetine-binding sites *in vitro* (Hirano *et al*., 2002), suggesting the sustained occupancy of SERT. Further detailed studies are required to clarify this issue.

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