Norfluoxetine Enantiomers as Inhibitors of Serotonin Uptake in Rat Brain

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Like fluoxetine, the N-demethylated metabolite norfluoxetine exists in R- and S-enantiomeric forms. S-Norfluoxetine inhibited serotonin (5-HT) uptake and [³H]paroxetine binding to 5-HT uptake sites with a pK_i of 7.86 and 8.88 or 14 and 1.3 nM, respectively, whereas R-norfluoxetine was 22 and 20 times, respectively, less potent. R- and S-Norfluoxetine were less potent than the corresponding enantiomers of fluoxetine as inhibitors of norepinephrine uptake and [³H]tomoxetine binding to norepinephrine uptake sites. Ex vivo studies showed that S-norfluoxetine inhibited 5-HT uptake with an ED_{50} of 3 mg/kg intraperitoneally, 4.7 mg/kg subcutaneously, and 9 mg/kg orally (7.3, 11.4 and 21.9 μmol/kg, respectively), while the ED_{50} for R-norfluoxetine exceeded 20 mg/kg intraperitoneally (48.6 μmol/kg). Inhibition of 5-HT uptake in cerebral cortex ex vivo and decrease in 5-HIAA levels in hypothalamus persisted for 24 hours after administration of S-norfluoxetine as demonstrated with the administration of fluoxetine. Thus, S-norfluoxetine is the active N-demethylated metabolite responsible for the persistently potent and selective inhibition of 5-HT uptake in vivo.
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KEY WORDS: *Fluoxetine; Norfluoxetine; Enantiomers; Serotonin 5-HT; Uptake; Inhibitors*

Fluoxetine, a selective inhibitor of serotonin (5-hydroxytryptamine, 5-HT) uptake (Wong et al., 1974, 1975), has been successfully developed as an antidepressant drug (Feighner 1983; Beasley et al. 1990). Fluoxetine is developed and is marketed as the racemate, i.e., the *R* (-) and *S* (+) enantiomers of equal amounts. Both enantiomers inhibit 5-HT uptake and effectively produce functional responses associated with an increased 5-HT transmission. There are no major differences in potency between *R*- and *S*-fluoxetine, and their eudismic ratio is close to unity (Wong et al., 1985, 1990; Robertson et al. 1988). Enzymatic N-demethylation is an early step of fluoxetine metabolism, and the demethylated compound norfluoxetine (Fig. 1) is a major metabolite (Parli and Hicks 1974; Lemberger et al. 1978; Beasley et al. 1990) in laboratory animals and in man. Norfluoxetine is also a potent and selective inhibitor of 5-HT uptake (Wong et al. 1975; Horng and Wong 1976; Fuller et al. 1978). In the present communication, we report the pharmacologic profiles of *R*- and *S*-norfluoxetine, which have been recently synthesized in high enantiomeric purity. In contrast with the two enantiomers of fluoxetine, however, we have found that *S*-norfluoxetine is over 20-fold more potent than the *R* enantiomer as an inhibitor of 5-HT uptake both in vitro and in vivo.

MATERIALS AND METHODS

Male Sprague-Dawley rats weighing between 100 and 150 g (Harlan Industries, Cumberland, IN) were housed in a room with a 12-hour dark/light cycle at 23°C, and

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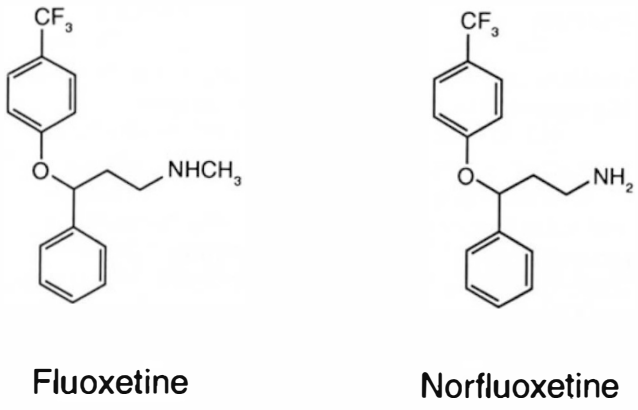

Figure 1. Chemical structures of fluoxetine and norfluoxetine.

had free access to Purina Chow pellets and water. Rats were killed by decapitation. Brain tissues of cerebral cortex, hypothalamus, or striatum were homogenized in 9 volumes of a medium containing 0.32 mol/L sucrose and 10 mmol/L glucose. Crude synaptosomal preparations were isolated after differential centrifugation at 1000 × *g* for 10 minutes and 17,000 × *g* for 28 minutes. The final pellets were suspended in the same medium and kept on ice until used within the same day.

Synaptosomal uptake of tritium-labeled 5-HT, norepinephrine (NE), or dopamine (DA) was determined as follows: synaptosomal preparations (equivalent to 0.5 to 1.0 mg of protein) were incubated at 37°C for 5 minutes in 1 ml of Krebs bicarbonate medium containing also 10 mmol/L glucose, 0.1 mmol/L iproniazid, 1 mmol/L ascorbic acid, 0.17 mmol/L EDTA, and [^{3}H]-monoamine at a specified concentration. The reaction mixture was immediately diluted with 2 ml of 0.9% saline and filtered using Whatman GF/B filters under vacuum with a cell harvester (Brandel, Gaithersburg, MD). Filters were rinsed twice with approximately 5 ml of ice-chilled 0.9% saline and were transferred to a counting vial containing 10 ml of scintillation fluid (PCS; Amersham, Arlington Heights, IL.). Radioactivity was measured by a liquid scintillation spectrophotometer. Accumulation of activity at 4°C represented the background and was subtracted from all samples. Uptake of 5-HT in human platelets was conducted according to previously described methods (Horng and Wong 1976; Lemberger et al. 1978).

Radioligand-binding assays were conducted according to previously described methods. Briefly, synaptosomal preparations of rat cerebral cortex, bovine choroid plexus, or striatum were suspended by homogenizing in 50 volumes of cold reaction medium (50 mmol/L Tris-HCl, pH 7.4, with or without 150 mmol/L NaCl, 50 mmol/L KCl) and centrifuging at 50,000 × *g* for 10 minutes. The process was repeated two times with a 10-minute incubation at 37°C between the second and third washes. The final pellet was stored at −70°C until use.

Binding of [^{3}H]paroxetine to 5-HT uptake sites was carried out in 2 ml of reaction medium containing the appropriate drug concentrations, 0.1 nmol/kg [^{3}H]-paroxetine and the cerebral cortical membranes (equivalent to 50 μg protein/tube) (Marcusson et al. 1988), and [^{3}H]tomoxetine at 0.2 nmol/kg was used to label NE uptake sites using a higher concentration of 300 mmol/kg NaCl as previously described (Wong et al. 1991). Samples were incubated at 37°C for 30 minutes, and those containing 1 μmol/kg fluoxetine or desipramine were used to determine nonspecific binding of [^{3}H]-paroxetine and [^{3}H]tomoxetine, respectively. After incubation, the tubes were filtered through Whatman GF/B filters, which were soaked in 0.05% polyethylenimine for 1 hour before use, using a cell harvester by adding about 4 ml of cold Tris buffer (pH 7.4), aspirating, and rinsing the tubes three additional times. Filters were then placed in scintillation vials containing 10 ml of scintillation fluid, and the radioactivity was measured by liquid scintillation spectrophotometry. Radioligand-binding assays for subtypes of receptors of 5-HT and other neurotransmitters were conducted according to previously described methods (Hoyer 1985; Wong et al. 1983, 1989, 1991b).

For in vivo studies, rats were fasted overnight prior to oral administration of drugs. Groups of five rats were given drugs by specified routes and doses, and were killed by decapitation at appropriate times. Brain areas were quickly dissected and placed either in cold 0.32 mol/L sucrose medium for uptake studies, or were frozen on dry ice for metabolite level determinations.

For measurement of 5-hydroxyindoleacetic acid (5-HIAA), brain tissues were homogenized in 5 to 10 volumes of 0.1 N trichloroacetic acid containing internal standard (5-hydroxyindolecarboxylic acid) and centrifuged at 10,000 × *g* for 10 minutes. The 5-HIAA in 20 μl of supernatant was determined by high-pressure liquid chromatography and electrochemical detection.

Enantiomers of fluoxetine and norfluoxetine were synthesized in the Lilly Research Laboratories (Indianapolis, IN). Serotonin[1,2-^{3}H(N)] (28.7 Ci/mmol); 1-NE[8-^{3}H] (57 Ci/mmol); DA[7-^{3}H(N)] (26.8 Ci/mmol); [phenyl-6-^{3}H]paroxetine (25 Ci/mmol); [N-methyl-^{3}H]tomoxetine (80.3 Ci/mmol); [propyl-2,3-ring 1,2,3-^{3}H]8-OHDPAT (169.9 Ci/mmol); [ethylene-^{3}H]ketanserin (64.9 Ci/mmol); [benzene-ring-^{3}H]spiperone (23.3 Ci/mmol); [pyridinyl-5-^{3}H]pyrilamine (27.3 Ci/mmol); [(±)benzilic-4′-^{3}H(N)quinuclidinyl benzilate, QNB (33.1 Ci/mmol); 2-[phenyoxy-3-^{3}H(N)]-WB4101 (24 Ci/mmol); [4-^{3}H]clonidine (22.2 Ci/mmol); and levo-[propyl-1,2,3-^{3}H]dihydroalprenolol (42.3 Ci/mmol) were purchased from New England Nuclear (Boston, MA). N-6[Methyl-^{3}H]mesulergine (84 Ci/mmol) and

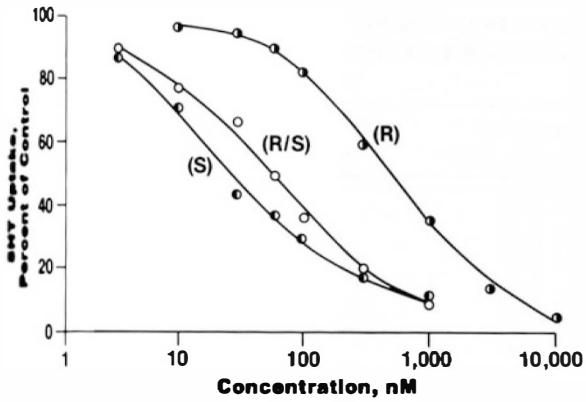

Figure 2. Inhibition of 5-HT uptake in synaptosomal preparation of rat cerebral cortex by norfluoxetine and its *R* and *S* enantiomers. Cortical synaptosomes equivalent to 1 mg protein (in triplicate samples) were incubated at 37°C for 3 minutes in Krebs bicarbonate medium containing 50 nmol/L [^{3}H]5-HT, 10 mmol/L glucose, 0.1 mmol/L iproniazid, 0.2 mg/ml ascorbic acid, 0.2 mmol/L EDTA, and concentrations of drugs as indicated. Other conditions were as described in Materials and Methods

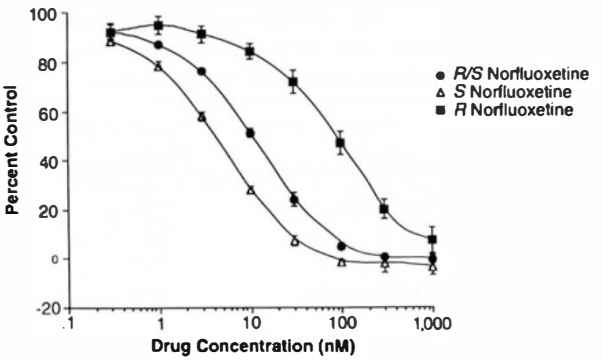

Figure 3. Inhibition of [^{3}H]paroxetine binding in cortical membranes by norfluoxetine and its *R* and *S* enantiomers. Cortical membranes equivalent to 50 µg of protein were incubated at 37°C for 30 minutes in 2 ml of reaction medium containing the appropriate drug concentrations, 0.1 nmol/L [^{3}H]paroxetine, 50 mmol/L Tris-HCl, pH 7.4, 150 mmol/L NaCl, and 5 mmol/L KCl. Fluoxetine at 1 µmol/kg was used to determine nonspecific binding. Other conditions were as described in Materials and Methods.

N-[methyl-^{3}H]-LY278584 (83.3 Ci/mmol) were prepared by Amersham (Arlington Heights, IL).

Statistical analysis was conducted by the use of Student's t-test to compare means of control and drug-treated samples. A probability of less than 0.05 was regarded as significant.

RESULTS

S-Norfluoxetine inhibits 5-HT uptake in synaptosomal preparations isolated from rat cerebral cortex with increasing concentrations from 3 nmol/kg, whereas R-norfluoxetine exerted its inhibitory effect at much higher concentrations beginning at about 100 nmol/kg, and as expected, the racemic mixture *R/S*-norfluoxetine had an intermediate potency (Fig. 2). From multiple determinations, the inhibitor constant (K_i values) were calculated from IC_{50} values (Cheng and Prusoff 1973) and are presented as pK_i ($-\log K_i$, M) values in Table 1. The mean pK_i for *S*-norfluoxetine of 7.86 ± 0.11 is significantly higher than the pK_i values for *R*-norfluoxetine and the racemate, indicating that the *S* enantiomer is significantly more potent. Contrary to these findings, the two enantiomers and the racemic mixture fluoxetine inhibited 5-HT uptake with almost equipotency (Table 1) as previously reported (Wong et al. 1985, 1990).

Consistent with its potency as an inhibitor of 5-HT uptake, *S*-norfluoxetine was more potent than the racemate or *R*-norfluoxetine as an inhibitor of [^{3}H]paroxetine binding to 5-HT uptake sites in cortical membranes (Fig. 3). The three compounds inhibited [^{3}H]paroxetine binding with mean pK_i values of 8.80 ± 0.04, 8.40 ± 0.04, and 7.49 ± 0.12, respectively (Table 2). In agreement with the inhibition of [^{3}H]fluoxetine binding

Table 1. Inhibition of 5-HT, NE, or DA Uptake in Synaptosomal Preparations by Enantiomers of Norfluoxetine and Fluoxetine

	Inhibition of Monoamine Uptake (pKi ($-\log K_i$, M))		
Enantiomer	**5-HT**	**NE**	**DA**
R/S-Norfluoxetine	7.35 ± 0.07	5.62 ± 0.14	5.66 ± 0.05
R-Norfluoxetine	6.51 ± 0.03[a]	5.43 ± 0.15	5.67 ± 0.03
S-Norfluoxetine	7.86 ± 0.11[b,*]	5.37 ± 0.14	5.56 ± 0.03
R/S-Fluoxetine	7.69 ± 0.04	5.91 ± 0.12	5.54 ± 0.02
R-Fluoxetine	7.46 ± 0.06	6.25 ± 0.22	5.55 ± 0.02
S-Fluoxetine	7.66 ± 0.05	5.69 ± 0.16	5.60 ± 0.01

Significant difference from racemate: [a] $p < .001$; [b] $p < .01$.
Significant difference between two enantiomers: * $p < .001$.

Table 2. Inhibition of [^{3}H]Paroxetine and [^{3}H]Tomoxetine Binding to Respective 5-HT and NE Uptake Carriers in Rat Cortical Membranes by Enantiomers of Fluoxetine and Norfluoxetine

Drug	Isomer	[^{3}H]Paroxetine (pK_i ($-\log K_i$, M))	[^{3}H]Tomoxetine (pK_i ($-\log K_i$, M))
Fluoxetine	*R/S*	8.51 ± 0.03	6.88 ± 0.15
	R	8.46 ± 0.02	7.08 ± 0.16
	S	8.53 ± 0.01	6.34 ± 0.15
Norfluoxetine	*R/S*	8.48 ± 0.04	5.84 ± 0.20
	R	7.58 ± 0.12	5.91 ± 0.13
	S	8.88 ± 0.04	5.82 ± 0.09

pK_i presented as means ± SE were calculated from IC_{50} values of at least three separate determinations.

(Wong et al. 1985), fluoxetine and its enantiomers are also potent inhibitors of [^{3}H]paroxetine binding to 5-HT uptake carriers (Table 2).

Uptake of 5-HT in platelets of human and rat plasma is known to be inhibited by fluoxetine and norfluoxetine (Horng and Wong 1976). *S*-Norfluoxetine inhibited 5-HT uptake in human platelets with increasing concentrations (Fig. 4), and its IC_{50} value was 7.5 nmol/kg; *S*-norfluoxetine was 2 and 14 times more potent than the racemate or the *R* enantiomer, respectively.

Like fluoxetine, norfluoxetine and its two enantiomers were weak inhibitors of NE uptake in synaptosomal preparations (Table 1). *S*-Norfluoxetine had the lowest pK_i of 5.23 ± 0.04, suggesting it is the least potent inhibitor of NE uptake among enantiomers of norfluoxetine and fluoxetine. Likewise, the two pairs of enantiomers are also weak inhibitors of DA uptake in striatal synaptosomes as previously reported for fluoxetine and norfluoxetine (Wong et al. 1990).

Consistent with being weak inhibitors of NE uptake, norfluoxetine and its enantiomers inhibited [^{3}H]tomoxetine binding to NE uptake carriers (Wong et al. 1991a) with pK_i values below 6, i.e., greater than micromolar concentrations (Table 2). Fluoxetine and its enantiomers were also weak inhibitors of [^{3}H]tomoxetine binding.

The abilities of *R*- and *S*-norfluoxetine to reduce 5-HT uptake ex vivo were compared (Table 3). *S*-Norfluoxetine, upon administration to rats at 3, 10, and 20 mg/kg (7.3, 24.3, and 48.6 μmol/kg, respectively) by either intraperitoneal or subcutaneous routes, significantly lowered 5-HT uptake in hypothalamus homogenates, with estimated ED_{50} values of 3 mg/kg IP and 4.7 mg/kg SC. *R*-Norfluoxetine lowered 5-HT uptake at all three doses administered, but the ED_{50} values exceeded 20 mg/kg by either route of administration.

S-Norfluoxetine administered orally at 3, 10, and 20 mg/kg to rats also effectively reduced 5-HT uptake in hypothalamus homogenates with an ED_{50} of approximately 9 mg/kg po an hour after its administration; the uptake of NE in the same tissue preparations remained unchanged (Table 4). In the same groups of rats, indole metabolites (5-HT and 5-HIAA) in cerebral cortex were measured, and 5-HT levels were essentially unchanged after treatment with the three doses of *S*-norfluoxetine; however, 5-HIAA levels were lower than control and this decrease in 5-HIAA levels became statistically significant at the 20-mg/kg dose (Table 4).

The duration of lowering of 5-HT uptake after a single oral administration of *S*-norfluoxetine at 20 mg/kg was found to be comparable to that caused by a similar treatment with fluoxetine and *S*-fluoxetine (Wong et al. 1975, 1985). Within an hour of treatment, 5-HT uptake in homogenates of cerebral cortex was reduced to below 30% of control activity, and the decrease persisted for 24 hours. Levels of 5-HIAA in hypothalamus were also significantly reduced at each time period of treat-

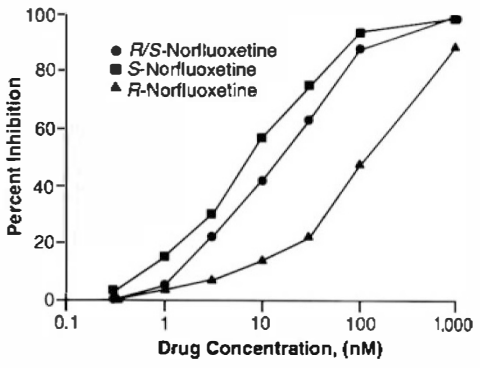

Figure 4. Inhibition of 5-HT uptake in human platelets by norfluoxetine and its *R* and *S* enantiomers. Aliquots of human platelet-rich plasma (1.24 × 107 cells) were incubated at 37°C for 3 minutes in 2 ml of Krebs bicarbonate buffer, pH 7.4, containing 10 mmol/L glucose, 0.1 mmol/L iproniazid, 1 mmol/L ascorbic acid, 0.17 mmol/L EDTA, 50 nmol/L [^{3}H]5-HT, and various concentrations of drug. Active 5-HT uptake was determined by subtracting the nonspecific uptake at 4°C. Other conditions were as described in Materials and Methods.

Table 3. Inhibition of 5-HT Uptake in Hypothalamus Homogenates Ex Vivo After an Intraperitoneal or Subcutaneous Administration of the Enantiomers of Norfluoxetine in Rats

Dose mg/kg (μmol/kg)	Route	5-HT Uptake, p mol/mg protein	
		R-Norfluoxetine	*S*-Norfluoxetine
0		4.70 ± 0.08	5.00 ± 0.29
3 (7.3)	IP	4.08 ± 0.13#	2.48 ± 0.11*
10 (24.3)	IP	3.50 ± 0.09*	1.47 ± 0.14*
20 (48.6)	IP	2.97 ± 0.07*	1.30 ± 0.11*
ED_{50}, mg/kg (μmol/kg)	IP	>20 (48.6)	3 (7.3)
0		3.29 ± 0.17	2.89 ± 0.19
3 (7.3)	SC	3.00 ± 0.21	1.81 ± 0.28#
10 (24.3)	SC	2.58 ± 0.20†	0.85 ± 0.10*
20 (48.6)	SC	2.20 ± 0.11*	0.37 ± 0.04*
ED_{50}, mg/kg (μmol/kg)	SC	> 20 (48.6)	4.7 (11.4)

Groups of five rats were treated with either saline or an enantiomer of norfluoxetine at dose and route of administration indicated for an hour before killing. Hypothalamus was dissected and homogenized in 0.32 mol/L sucrose. Aliquots of the homogenate in triplicate samples were incubated for uptake of 5-HT as described in Materials and Methods. Significant difference from control is indicated (* $p < .001$; # $p < .005$; † $p < .05$).

ment (Figure 5). As observed in previous experiments (Table 4), *S*-norfluoxetine treatment did not change 5-HT levels in hypothalamus during the entire time course of study (results not shown). A similar duration of decrease of 5-HT uptake occurred following administration of *S*-norfluoxetine at 10 mg/kg SC, but 5-HT uptake had returned to control levels by 48 hours after treatment (Table 5).

By means of radioligand-binding techniques, the two enantiomers of norfluoxetine were found to interact with receptors of transmitters only an micromolar or higher concentrations, including subtypes of 5-HT receptors (1A, 1B, 1D, 2, and 3); adrenergic alpha$_1$-, alpha$_2$-, and beta-receptors; dopaminergic D_1 and D_2 receptors; muscarinic acetylcholine receptors; and histamine H1 receptor (Table 6). Consistent with the findings on the enantiomers of fluoxetine (Wong et al. 1991b), *R*-norfluoxetine inhibited [^{3}H]mesulergine binding to 5-HT$_{1C}$ sites in membranes of bovine choroid plexus with a K_i of 0.18 ± μmol/kg, whereas *S*-norfluoxetine was much weaker, with a K_i of 3.5 μmol/kg (Table 6).

DISCUSSION

Contrary to the equipotency of *R*- and *S*-fluoxetine as inhibitors of 5-HT uptake (Wong et al. 1985, 1990), *S*-norfluoxetine is consistently more effective than *R*-norfluoxetine as an inhibitor of 5-HT uptake in synaptosomal preparations and human platelets in vitro. Indeed, *S*-norfluoxetine exhibited 20 times greater affinity than *R*-norfluoxetine for the 5-HT uptake sites

Table 4. Effect of *S*-Norfluoxetine Administered Orally on Uptake of 5-HT and NE in Hypothalamus Homogenates and Levels of Indole Metabolites in Cerebral Cortex

S-Norfluoxetine mg/kg (μmol/kg) po	Monoamine Uptake (p mol/mg protein)		Metabolite Level (nmol/g tissue[a])	
	5-HT	NE	5-HT	5-HIAA
Control	5.5 ± 0.4	4.5 ± 0.3	2.16 ± 0.05	2.30 ± 0.06
3 (7.3)	3.9 ± 0.3*	4.6 ± 0.3	2.14 ± 0.05	2.24 ± 0.04
10 (24.3)	2.5 ± 0.4*	4.6 ± 0.4	2.41 ± 0.23	2.12 ± 0.06
20 (48.6)	1.7 ± 0.2*	4.6 ± 0.3	2.19 ± 0.10	1.96 ± 0.05#

Groups of five rats were treated with saline or *S*-norfluoxetine at the indicated doses and killed an hour later. Hypothalamus and cerebral cortex were dissected. Hypothalamus was homogenized in 0.32 mol/L sucrose and used for measurement of 5-HT and NE uptake. Cerebral cortices were immediately frozen for subsequent measurement of indole metabolites. Other conditions were those described in Materials and Methods.

[a] Tissue weight was expressed in gram of wet weight.

Significant difference from control is indicated (* $p < .025$; # $p < .002$).

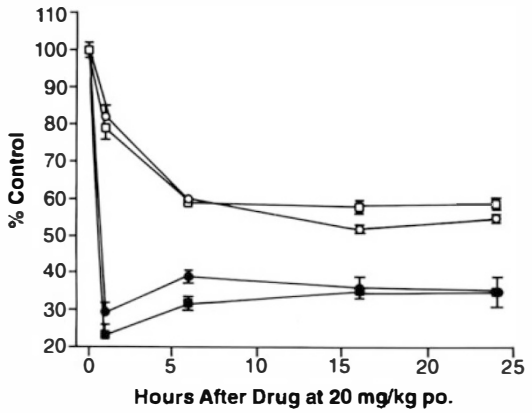

Figure 5. Duration of inhibition of 5-HT uptake in homogenates of cerebral cortex and lowering of 5-HIAA in hypothalamus of rats treated with fluoxetine or *S*-norfluoxetine in vivo. Groups of five rats were treated with fluoxetine (circles) or *S*-norfluoxetine (squares) at 20 mg/kg po and were killed by decapitation at the time intervals specified. A separate group of rats was treated with saline as control. The brain was removed, and the hypothalamus and cerebral cortex were dissected. Uptake of 5-HT in cortical homogenates (open symbols) and 5-HIAA levels in the hypothalamus (filled symbols) was measured as described in Materials and Methods. The drug-treated groups were significantly different from control groups ($p < .001$).

labeled by [^{3}H]paroxetine, and fluoxetine and its *R* and *S* enantiomers displaced [^{3}H]paroxetine binding with equal affinities, as shown previously, using [^{3}H]fluoxetine as radioligand binding to 5-HT uptake sites (Wong et al. 1985). In fact, the pK_i values of *S*-norfluoxetine are higher than the pK_i value of *S*-fluoxetine as an inhibitor of [^{3}H]paroxetine binding ($p < .01$) in cortical preparations, suggesting that N-demethylation of *S*-fluoxetine appears to increase intrinsic affinity for the 5-HT uptake carrier.

Table 5. Duration of Decrease on Uptake of 5-HT in Homogenates of Hypothalamus after Subcutaneous Administration of *S*-Norfluoxetine in Rats

Duration (Hour)	5-HT Uptake (pmol/mg protein)	Percent of Control (pmol/mg protein)
0	3.27 ± 0.15	100
½	1.29 ± 0.29*	39.5
1	1.03 ± 0.07*	31.5
6	0.95 ± 0.05*	29.1
24	2.73 ± 0.22	83.5
48	3.24 ± 0.15	99.1

Groups of four rats were treated with *S*-norfluoxetine at 10 mg/kg (24.3 μmol/kg) SC and were killed at the times indicated. A group of eight rats was treated with saline as a control group. Hypothalamus was dissected and homogenized in 0.32 mol/L sucrose. Aliquots of the homogenate in duplicate samples were incubated for 5-HT uptake as described under Materials and Methods. Significant difference from control values is indicated (* $p < .001$).

Table 6. Inhibition of Radioligand Binding to Receptors of Neurotransmitters by Enantiomers of Norfluoxetine

Receptor	*R*-Norfluoxetine (K_i μmol/kg)	*S*-Norfluoxetine (K_i μmol/kg)
5-HT_{1A}	>10	>10
5-HT_{1B}	12.6; 7.1	10.3 ± 2.4
5-HT_{1C}	0.18 ± 0.03	3.5 ± 0.5
5-HT_{1D}	57.5 ± 13.5	48.5 ± 9.1
5-HT_2	0.57 ± 0.03	3.8 ± 0.7
5-HT_3	3.1; 4.9	>10
Alpha-$_1$-adrenergic	>10	>10
Alpha-$_2$-adrenergic	>10	>10
Beta-adrenergic	>10	>10
DA-$_1$	>10	>10
DA-$_2$	>10	>10
Muscarinic-Ach	2.9	2.8
Histamine-H1	3	2.5

K_i in mean ± SE were calculated from IC_{50} values of at least three separate determinations. otherwise, values are shown as obtained from each determination.

Fluoxetine and norfluoxetine, as reported previously (Wong et al. 1975, 1990), and their respective enantiomers as demonstrated in the present study, are weak inhibitors of NE uptake. Using [^{3}H]tomoxetine as a radioligand of NE uptake carrier (Wong et al. 1991a), we further show that norfluoxetine and its enantiomers are indeed weaker ligands for the NE uptake carrier. Calculating the ratios of K_i inhibiting [^{3}H]tomoxetine binding to K_i inhibiting [^{3}H]paroxetine binding, the values for fluoxetine, *R*-fluoxetine, *S*-fluoxetine, norfluoxetine, *R*-norfluoxetine, and *S*-norfluoxetine (Table 2) are 33, 20, 219, 697, 58, and 1317, respectively. In agreement with the inhibition of 5-HT and NE uptake, inhibition of direct radioligand binding to the respective uptake sites shows a minimum of 20-fold greater affinity of *R*-fluoxetine for 5-HT than for NE uptake sites, whereas the selectivity of *S*-fluoxetine was 21 times higher, with a ratio of 219. Unexpectedly, N-demethylation of *S*-fluoxetine to *S*-norfluoxetine confers an even greater selectivity for the 5-HT uptake sites over NE uptake sites, with a K_i ratio of 1317. Even though *R*-norfluoxetine has one-eighth the affinity of *R*-fluoxetine for the [^{3}H]paroxetine-labeled 5-HT uptake site, it also has one-third the affinity of the parent drug for the [^{3}H]tomoxetine-labeled NE uptake.

The present study demonstrates that *S*-norfluoxetine is as potent as *S*-fluoxetine in inhibiting 5-HT uptake ex vivo (Wong et al. 1985). The closed ED_{50} values of *S*-norfluoxetine ranged from 3 to 9 mg/kg after intraperitoneal, subcutaneous, and oral routes of administration, suggesting excellent bioavailability and ready penetration into the brain. Consistent with the in vitro findings, *R*-norfluoxetine was relatively inactive as a 5-HT uptake inhibitor ex vivo with ED_{50} doses exceeding 20 mg/kg. In vivo, fluoxetine and norfluoxetine at

20 mg/kg IP, more than twice the ED_{50} inhibiting 5-HT uptake ex vivo, failed to inhibit the accumulation of radioactive NE in rat heart, whereas the tricyclic antidepressant drugs imipramine, desipramine, clomipramine, and chlorodesipramine at 5 mg/kg IP nearly maximally inhibited accumulation of NE radioactivity in heart (Wong et al. 1975).

The duration of 5-HT uptake inhibition was longer after an administration of *S*-fluoxetine than *R*-fluoxetine (Wong et al. 1985). Both enantiomers are readily metabolized to form norfluoxetine (Potts et al. 1989; Fuller et al. 1991; Potts and Parli 1992). The loss of inhibitory effects beginning 5 hours after *R*-fluoxetine administration can be explained by the generation of a relatively inactive metabolite, *R*-norfluoxetine, since both *R*- or *S*-norfluoxetine reached similar peak levels in brain between 4 and 8 hours after administration of the corresponding enantiomer of fluoxetine (Fuller et al. 1990). On the other hand, the N-demethylation of *S*-fluoxetine leads to an equally active and enduring metabolite, *S*-norfluoxetine, which was responsible for the long-lasting inhibition of 5-HT uptake after treatment with *S*-fluoxetine (Wong et al. 1985).

The K_i ratios of *S*-fluoxetine (219) and *S*-norfluoxetine (1317) are 11 and 22 times higher than the K_i ratios of *R*-fluoxetine (20 and *R*-norfluoxetine (58), representing a broad range of selectivity for the uptake carriers of 5-HT over those of NE. Despite having a low selectivity ratio of 20 and the submicromolar affinity of *R*-fluoxetine for NE uptake sites, administration of racemic fluoxetine to rats in vivo at doses up to 13 times the ED_{50} (3.8 mg/kg i.p.) for inhibition of 5-HT in vivo failed to inhibit NE uptake in brain and in heart (Wong et al. 1975; Wong and Bymaster 1976). Higher doses were rarely used and would be considered unnecessary. Since the enantiomers of the parent drug and the demethylated metabolite should bear comparable physical and chemical properties, their tissue distribution is most likely to be similar. Comparable brain levels of the enantiomers of fluoxetine and norfluoxetine were indeed achievable after a single dose of racemic fluoxetine in rat (Potts and Parli 1992). Thus, a 5-HT uptake inhibitor, having a selectivity ratio of 20 like *R*-fluoxetine, appears to provide adequate selectivity in vivo toward inhibiting 5-HT uptake without inhibiting NE uptake. In a recent clinical study, four individuals receiving racemic fluoxetine daily for 45 days showed nearly a fourfold higher rate of clearance for *R*-fluoxetine compared to *S*-fluoxetine (R. Bergstrom, personal communication). As a result, one can expect that *S*-fluoxetine accumulates to higher concentrations than *R*-fluoxetine and consequently ensures selectivity toward inhibiting 5-HT uptake in humans.

Besides fluoxetine and the tertiary amine containing tricyclic antidepressant drugs, including imipramine and clomipramine (Wong et al. 1975, Hyttel and Larsen 1985), N-demethylation is also involved in the metabolism of other selective inhibitors of 5-HT uptake. Sertraline, a secondary amine, inhibited 50% of 5-HT uptake with a concentration of 58 nmol/kg (IC_{50}) and NE uptake with an IC_{50} of 1200 nmol/kg, whereas the N-demethylated metabolite, CP 62,508, inhibited 5-HT and NE uptake with IC_{50}s of 450 and 4600 nmol/kg, respectively (Koe et al. 1983). Thus, the ratio of IC_{50} inhibiting NE uptake to the IC_{50} inhibiting 5-HT uptake was reduced from 20 for sertraline to 10 for the desmethyl metabolite of sertraline. In ex vivo studies, sertraline was shown to be a selective inhibitor of 5-HT uptake without significant inhibition of NE uptake (Koe et al. 1983).

Citalopram, a tertiary amine, and its N-demethylated metabolites desmethylcitalopram and didesmethylcitalopram inhibited 5-HT uptake with IC_{50}s of 1.8, 7.4 and 24 nmol/kg, respectively, and were 4889, 105, and 63 times, respectively, less potent as inhibitors of NE uptake (Hyttel and Larsen, 1985). At twice the ED_{50} dose inhibiting 5-HT uptake ex vivo, citalopram at 10 mg/kg IP did not reduce NE uptake in midbrain synaptosomes of drug-treated rats (Maitre et al. 1980).

Like other inhibitors of 5-HT uptake, including fluoxetine, oral administration of *S*-norfluoxetine at 20 mg/kg caused a decrease of 5-HIAA levels in hypothalamus and cerebral cortex, whereas 5-HT levels in these brain areas were essentially unchanged. It is believed that the decrease in 5-HIAA levels reflects a decrease of 5-HT turnover, a consequence of greater synaptic availability of 5-HT when presynaptic 5-HT uptake is inhibited.

The two enantiomers of norfluoxetine are similar to fluoxetine (Wong et al. 1983) and its two enantiomers (Wong et al. 1985), and have little affinity for receptors of neurotransmitters, including 5-HT, NE, DA, acetylcholine, and histamine. The relatively low affinity of the norfluoxetine enantiomers for receptors of acetylcholine (muscarinic class) and histamine is consistent with the low incidence of anticholinergic and antihistaminergic side-effect profiles of fluoxetine and norfluoxetine (Feighner 1983; Beasley et al. 1990). Among subtypes of 5-HT receptors, *R* enantiomers of norfluoxetine (present study) and fluoxetine (Wong et al. 1991) exhibit submicromolar affinity for 5-HT_{1C} receptors in membranes of bovine choroid plexus; however, it should be noted that the affinity of the two *R* enantiomers for the 5-HT uptake site is two orders of magnitude higher. Nevertheless, the pharmacologic effects of the two *R* enantiomers on 5-HT_{1C} receptors are an important area for further investigation.

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