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N,N-DIMETHYL-α-[2-(*p*-TOLYLOXY)ETHYL]BENZYLAMINE HYDROCHLORIDE (LY125180)

EFFECTS ON SEROTONIN UPTAKE AND SEROTONIN SYNTHESIS IN RAT BRAIN *IN VITRO* AND *IN VIVO*

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Abstract—*N,N*-Dimethyl-α-[2-(*p*-tolyloxy)ethyl]benzylamine hydrochloride (LY125180) competitively inhibited the uptake of serotonin and norepinephrine by cortical synaptosomes and of dopamine by striatal synaptosomes, with K_i values of 0.06, 2.2 and 2.5 μM respectively. In platelets of human plasma, LY125180 blocked serotonin uptake by 50 per cent at 22 nM. The administration of LY125180 led to a reduction of serotonin uptake by rat hypothalamic synaptosomes with an ED_{50} value of 12 mg/kg, i.p., and a maximum effect within 1 hr. Prior treatment with β-diethylaminoethyl-2,2-diphenylvalerate, HCl (SKF-525A), an inhibitor of microsomal metabolism, enhanced the potency of LY125180 by 3-fold and prolonged its action for at least 4 hr. LY125180 *in vivo* blocked the neurotoxic effect of *p*-chloroamphetamine on serotonin uptake by cortical synaptosomes but did not prevent the neurotoxic effect of 6-hydroxydopamine on norepinephrine uptake by hypothalamic synaptosomes or the accumulation of radiolabeled norepinephrine in rat heart at 50 mg/kg, i.p. A reduction in brain level of 5-hydroxyindoleacetic acid but not of serotonin and tryptophan and a decrease in the conversion of [^{3}H]tryptophan to [^{3}H]serotonin and [^{3}H]-5-hydroxyindoleacetic acid after the administration of LY125180 suggest a decrease of serotonin turnover in rat brain. These data are consistent with the conclusion that LY125180 is effective and selective in the blockade of the serotonin pump *in vitro* as well as *in vivo*. Except for a much shorter duration of action *in vivo*, LY125180 exhibits properties similar to the earlier reported selective serotonin uptake inhibitor, fluoxetine.

Tricyclic antidepressant drugs such as imipramine and chlorimipramine, which are tertiary amine containing compounds, are selective inhibitors of serotonin uptake, while desipramine and chlordesipramine, which are secondary amine containing compounds, are selective inhibitors of norepinephrine uptake [1–4]. The tertiary amines, however, are readily *N*-demethylated upon administration *in vivo* and, therefore, become blockers of both serotonin and norepinephrine uptake *in vivo* [5]. Fluoxetine was the first bicyclic compound discovered to be a selective blocker of serotonin uptake *in vitro* as well as *in vivo* [3, 4], and its structurally related compound, nisoxetine, was a potent inhibitor of norepinephrine uptake [6, 7]. Both fluoxetine and nisoxetine are secondary amines. The primary amine analog of fluoxetine retained the selectivity and potency in blocking uptake of serotonin, while that of nisoxetine was less effective in blocking uptake of monoamines. Fluoxetine blocked serotonin uptake by synaptosomes [4] and platelets [8] for at least 24 and 12 hr, respectively, after an intraperitoneal injection in rats. The accumulation of desmethylfluoxetine [9] in laboratory animals may account for the prolonged effect of fluoxetine on amine uptake. In this communication, we report on a new bicyclic compound, *N-N*-dimethyl-α-[2-(*p*-tolyloxy)ethyl]benzylamine hydrochloride (LY125180), which exhibits the properties of a selective blocker of serotonin uptake *in vitro* and *in vivo* but has a shorter duration of action than fluoxetine.

MATERIALS AND METHODS

Male Sprague–Dawley rats weighing about 150 g were obtained from Harlan Industries, Cumberland, IN. They were decapitated and their brains were immediately removed and dissected [10]. Crude synaptosomes were prepared by differential centrifugation from a 10% homogenate in 0.32 M sucrose and 10 mM glucose [7]. The procedures for measuring uptake of radioactive monoamines at 0.1 μM (or concentrations otherwise indicated) by synaptosomes and uptake of [^{14}C]norepinephrine by heart were according to the published methods [4, 7]. Indole metabolites were extracted from brain [11]. 5-Hydroxyindoleacetic acid was isolated by the method of Contractor [12]. Brain levels of serotonin and 5-hydroxyindole acetic acid were determined spectrofluorometrically as condensates of *o*-phthalaldehyde [13]. The method of Denckla and Dewey [14] was used for the measurement of tryptophan. Radioactivity was determined by the liquid scintillation technique.

Platelet-rich plasma from human volunteers was prepared according to the method of Sneddon [15]. Platelet uptake of serotonin was determined by a modified method [8]. Human platelet-rich plasma in aliquots of 0.2 ml was mixed with 1.8 ml of Krebs bicarbonate buffer, pH 7.4, containing 0.1 μM

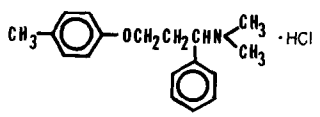

Fig. 1. Structure of LY125180, *N,N*-dimethyl-α-[2-(*p*-tolyloxyl)ethyl] benzylamine hydrochloride.

[^{3}H]serotonin (5-hydroxytryptamine, 5-HT), 10 mM glucose, 0.1 mM iproniazid, 0.2 mg/ml ascorbic acid and 0.05 mg/ml EDTA, and was incubated at 37° for 10 min. Uptake was terminated by chilling in ice and adding 0.1 ml of 20% formaldehyde. The platelets were harvested by centrifugation at 12,000 *g* for 25 min. After decanting the supernatant fraction, the platelets were digested in hydrogen peroxide, and radioactivity was determined by the liquid scintillation method. Accumulation at 4° was subtracted from each sample.

N-N,Dimethyl-α-[2-(*p*-tolyloxy)ethyl]benzylamine hydrochloride (LY125180, Fig. 1) and fluoxetine hydrochloride were synthesized at the Lilly Research Laboratories. Desipramine was provided by the Ciba–Geigy Corp., Summit, NJ. *p*-Chloroamphetamine and 6-hydroxydopamine were purchased from the Regis Chemical Co, Chicago, IL. 7-[^{3}H]-(−)-Norepinephrine ([^{3}H]NE), 15 Ci/mmole, [G-^{3}H]dopamine ([^{3}H]DA), 10 Ci/mmole, [G-^{3}H]-tryptophan, 5 Ci/mmole, 5-[1,2-^{3}H(N)]hydroxytryptamine ([^{3}H]5-HT), 30 Ci/mmole, and 7-[^{14}C]-(±)-norepinephrine ([^{14}C]NE), 40 mCi/mmole, were purchased from the New England Nuclear Corp., Boston, MA.

RESULTS

*Effects of LY*125180 in vitro. Synaptosomes, isolated from cerebral cortex of rat brain, accumulated 5-HT in a concentration-dependent and saturable manner with a dissociation constant, K_m value, of 0.09 ± 0.00 μM and a maximum velocity, V_{max} value, of 20.4 ± 0.20 pmoles/mg protein in 3 min of incubation. The effects of LY125180 at three concentrations (0.01, 0.05 and 0.1 μM) on the uptake of 5-HT at four concentrations were examined. The data of such an experiment were plotted according to the method of Dixon [16]. The convergence of lines at the $1/V_{max}$ value (horizontal line) in Fig. 2 suggested that LY125180 competitively inhibited the uptake of the substrate, 5-HT, with an inhibitor constant, K_i value, of 0.06 μM.

LY125180 was a relatively weak inhibitor of the uptake of NE by cortical synaptosomes and of the uptake of DA into striatal synaptosomes. Dixon analysis (Figs. 3 and 4) revealed that the compound competitively blocked the uptake of NE and DA with K_i values of 2.2 and 2.5 μM, respectively. The K_m constants for the two uptake processes were calculated according to the method of Wilkinson [17] with values of 0.18 ± 0.01 and 0.14 ± 0.05 μM, which are in agreement with the published values [7].

The effects of LY125180 and fluoxetine on uptake

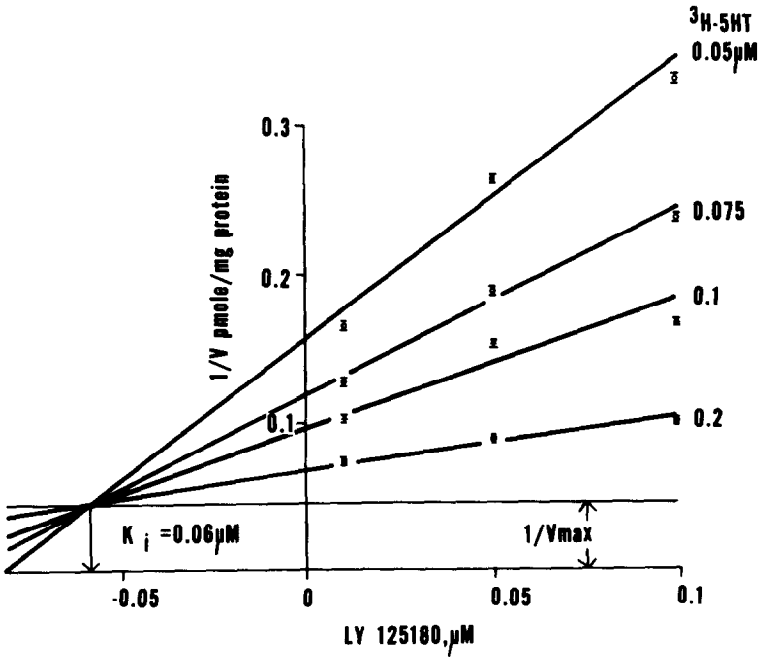

Fig. 2. Uptake of serotonin by cortical synaptosomes competitively inhibited by LY125180 as illustrated by a Dixon plot [14]. Cerebral cortex of rat brain was homogenized in 9 vol. of 0.32 M sucrose medium containing 10 mM glucose. The homogenate was centrifuged at 1085 *g* for 10 min. Crude synaptosomes were sedimented from the supernatant fraction after centrifugation at 17,000 *g* for 20 min. Aliquots of synaptosomes (1 mg protein) were incubated at 37° for 3 min in 1 ml of Krebs bicarbonate buffer, pH 7.4, containing 0.05–0.2 μM [^{3}H]-5-HT, 10 mM glucose, 0.1 mM iproniazid, 1 mM ascorbic acid, 0.17 mM EDTA, and various concentrations of LY125180. Other conditions were from the described methods [4, 6]. The V_{max} value was obtained from a double reciprocal plot of the control data in the same experiment.

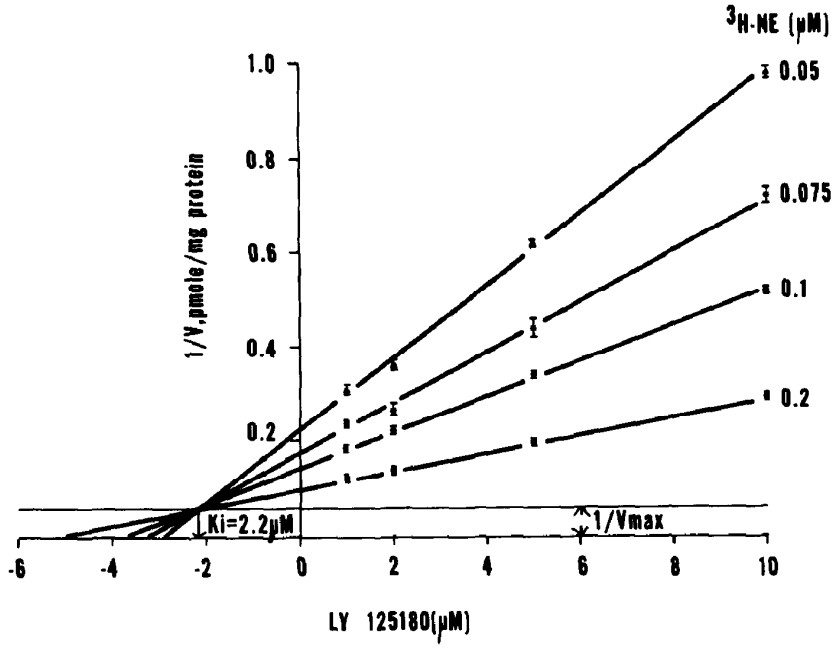

Fig. 3. Uptake of norepinephrine by cortical synaptosomes competitively inhibited by LY125180, as illustrated by a Dixon plot [14]. Experimental conditions were the same as those described in the legend of Fig. 2 except that [^{3}H]NE replaced [^{3}H]-5-HT in the reaction medium.

of 5-HT by platelets of human plasma were compared (Table 1). The two compounds were about equally effective in blocking the uptake of 5-HT, with estimated concentrations to cause 50 per cent inhibition (IC_{50} values) of 22 nM and 14 nM, respectively.

*Effects of LY*125180 in vivo. The uptake of 5-HT by hypothalamic synaptosomes was reduced significantly after an intraperitoneal injection of LY125180 in increasing doses (1–100 mg/kg, Table 2). The calculated ED_{50} value was 12 mg/kg. The compound at 100 mg/kg caused only a 24 per cent reduction in NE uptake, and its effects at lower doses were even less. After microsomal metabolism was prevented by β-diethylaminoethyl-2,2-diphenylvalerate, HCl (SKF-525A) (50 mg/kg, i.p.), LY125180 reduced 5-HT uptake with a lower ED_{50} value of 4.4 mg/kg, i.p.

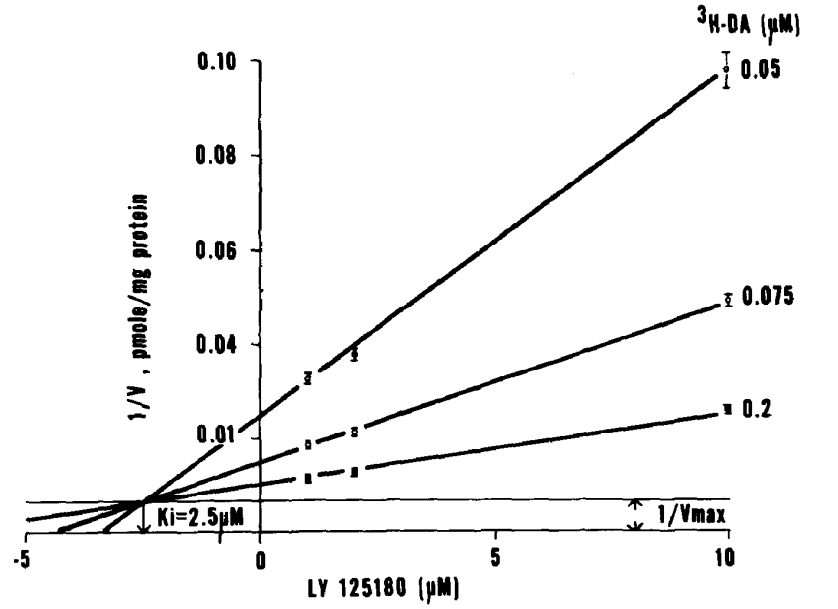

Fig. 4. Uptake of dopamine by striatal synaptosomes competitively inhibited by LY125180, as illustrated by a Dixon plot [14]. Crude striatal synaptosomes were isolated in the same manner as described for the crude cortical synaptosomes in the legend of Fig. 2. The conditions for [^{3}H]DA uptake were the same as those shown in Fig. 2 except that [^{3}H]DA was used instead of [^{3}H]-5-HT.

Table 1. Effects of LY125180 and fluoxetine on uptake of serotonin (5-HT) by platelets of human plasma*

Compounds	Concn (μM)	5-HT uptake (pmole/10^6 platelets)	Inhibition (%)
None	0	0.41 ± 0.01	0
LY125180	0.01	0.23 ± 0.03	43
	0.1	0.15 ± 0.01	64
	1	0.02	95
Fluoxetine	0.01	0.23 ± 0.01	44
	0.1	0.05 ± 0.00	89
	1	0.01	98

* Human platelet-rich plasma in aliquots of 0.2 ml (1.5×10^8 platelets) was incubated at 37° for 10 min in 1.8 ml of reaction medium containing 0.1 μM [^{3}H]-5-HT and the indicated concentrations of LY125180 or fluoxetine. Other conditions have been described in the text.

(Table 2). SKF-525A did not enhance the ability of LY125180 to inhibit the uptake of NE in the same preparations of synaptosomes.

The administration of SKF-525A also prolonged the duration of action of LY125180 in reducing uptake of 5-HT in synaptosomes of hypothalamus homogenates from 1 hr to at least 4 hr (Table 3). The uptake of NE in the same preparations was maintained near the control level during the entire time course study.

LY125180 selectively protected the neuronal uptake of monoamines by rat brain from the neurotoxic effect of *p*-chloroamphetamine (p-CA) but not from that of 6-hydroxydopamine (6-OHDA) (Table 4). Uptake of 5-HT by homogenates of cerebral cortex was reduced from 6.11 ± 0.22 to 2.41 ± 0.18 pmoles/mg protein by a single dose of p-CA (10 mg/kg, i.p.) given 4 days prior to killing. The neurotoxicity of p-CA was partially reversed by prior treatment with 25 mg/kg of LY125180, which by itself at 50 mg/kg had no effect on day 4.

6-Hydroxydopamine (50 μg), administered by an intraventricular injection 4 days earlier, reduced the uptake of NE by hypothalamic homogenates from

Table 2. Effects of LY125180 administration on uptake of serotonin and norepinephrine by synaptosomes of hypothalamus*

Treatment	Uptake of Serotonin (pmoles/mg protein)	Uptake of Norepinephrine (pmoles/mg protein)
Experiment 1		
Control	10.14 ± 1.15	7.68 ± 0.35
LY125180 (mg/kg)		
1	7.43 ± 0.10	7.02 ± 0.37
5	7.36 ± 0.88	6.39 ± 0.13
10	5.98 ± 0.51	6.32 ± 0.21
25	2.88 ± 0.37†	6.13 ± 0.15†
50	2.00 ± 0.21†	6.55 ± 0.41
100	0.99 ± 0.26†	5.87 ± 0.26†
ED_{50} (mg/kg)	12	
Experiment 2		
Control	7.51 ± 0.25	7.53 ± 0.47
SKF-525A (50 mg/kg, i.p.)	8.47 ± 0.25	7.83 ± 0.47
SKF-525A + LY125180 (mg/kg)		
1	6.88 ± 0.21†	6.81 ± 0.31
5	3.90 ± 0.18†	6.95 ± 0.30
10	2.69 ± 0.32†	6.61 ± 0.06
25	1.80 ± 0.08†	6.87 ± 0.24
ED_{50} (mg/kg)	4.4	

* Rats in groups of five were treated with saline or LY125180 at the indicated dosages for 1 hr before decapitation. Hypothalamus from individual rats was homogenized in 9 vol. of 0.32 M sucrose. The homogenate was incubated with 0.1 μM [^{3}H]-5-HT or 0.1 μM [^{3}H]NE at 37° for 3 min. Other conditions were identical to those described in the legend of Fig. 2 (experiment 1). Separate groups of rats were pretreated with SKF-525A at 50 mg/kg, i.p., 1 hr prior to treatment with LY125180 (experiment 2).

† $P < 0.005$, significantly different from control group.

Table 3. Duration in reduction of serotonin and norepinephrine uptake by homogenate of hypothalamus after an intraperitoneal injection of LY125180*

Time (hr) after LY125180 (25 mg/kg)	Uptake of Serotonin (pmoles/mg protein)	Norepinephrine (pmoles/mg protein)
(1) Control		
0	8.40 ± 0.36	8.01 ± 0.41
0.5	4.90 ± 0.58†	7.21 ± 0.28
1	6.69 ± 0.18†	7.58 ± 0.32
4	9.32 ± 0.28	7.91 ± 0.33
(2) SKF-525A (50 mg/kg, i.p.)		
4	3.76 ± 0.30†	7.37 ± 0.43
16	8.10 ± 0.69	7.09 ± 0.18

* Rats in groups of five were treated with LY125180 (25 mg/kg, i.p.) and were decapitated at the time specified. Two separate groups of five rats were pretreated with SKF-525A (50 mg/kg, i.p.) 1 hr before treatment with LY125180. All other conditions have been shown in Table 2.

† $P < 0.005$, significantly different from control zero time group.

Table 5. Effect of LY125180 on accumulation of [^{14}C]norepinephrine in rat hearts*

Treatment	[^{14}C]Norepinephrine accumulation (d.p.m./g)
Control	7942 ± 320
LY125180 (mg/kg, i.p.)	
5	7165 ± 555
10	7044 ± 474
25	7594 ± 350
50	7131 ± 411
DMI (10 mg/kg, i.p.)	1200 (756, 1644)

* Rats in groups of five were treated with various doses of LY125180 for 45 min before an intravenous injection of [^{14}C]NE (1 μCi/kg) and were decapitated 15 min later. For comparison, two rats were treated with desipramine (DMI) at 10 mg/kg, i.p. Hearts were removed and immediately frozen on dry ice. After weighing, hearts were digested in hydrogen peroxide before the determination of radioactivity by the liquid scintillation technique.

7.52 ± 0.14 to 3.06 ± 0.24 pmoles/mg protein. No significant protection against the neurotoxicity of 6-OHDA resulted from the prior treatment with LY125180 at doses up to 50 mg/kg, i.p. The administration of the uptake inhibitor alone at 50 mg/kg, i.p., had no effect on NE uptake.

LY125180 from 5 to 50 mg/kg, i.p., did not affect the accumulation of intravenously administered [^{14}C]norepinephrine in rat heart (Table 5). However, the secondary amine containing tricyclic antidepressant, desipramine (DMI), at 10 mg/kg, i.p., reduced the radioactivity from 7942 to 1200 d.p.m./g tissue weight.

Effects of 5-HT *turnover in rat brain.* The administration of LY125180 at 50 mg/kg, i.p., 1 hr prior to an intravenous injection of [^{3}H]tryptophan ([^{3}H]Try) and 2 hr before decapitation did not change brain levels of 5-HT and Try but reduced the 5-hydroxyindoleacetic acid (5-HIAA) level in brain by 33 per cent (Table 6). The reduction of the 5-HIAA level was an indication of a reduction in 5-HT turnover [18]. This was further supported by the

Table 4. Ability of LY125180 to block the neurotoxicity of *p*-chloroamphetamine (p-CA) and 6-hydroxydopamine (6-OHDA)*

Treatment	Serotonin uptake (pmoles/mg protein)	Blockade (%)
1 Control	6.11 ± 0.22	
p-CA (10 mg/kg, i.p.)	2.41 ± 0.18†	
p-CA + LY125180 (mg/kg, i.p.)		
1	2.97 ± 0.30†	15
5	2.26 ± 0.18†	0
10	2.19 ± 0.20	0
25	3.91 ± 0.35†	41†
LY125180 (50 mg/kg, i.p.)	7.01 ± 0.29	
Treatment	**Norepinephrine uptake (pmoles/mg protein)**	**Blockade (%)**
2 Control	7.52 ± 0.14	
6-OHDA (50 μg, i.v.)	3.06 ± 0.24†	
6-OHDA + LY125180 (mg/kg, i.p.)		
10	2.51 ± 0.23†	0
25	2.77 ± 0.21†	0
50	3.20 ± 0.50†	3
LY125180 (50 mg/kg, i.p.)	7.73 ± 0.22	

* Rats in groups of five were treated with either saline or various doses of LY125180 0.5 hr before an intraperitoneal injection of p-CA (10 mg/kg) or an intraventricular injection of 6-OHDA (50 μg) and were decapitated at 96 hr. Homogenates of cerebral cortex were used for the assay of 5-HT uptake and those of hypothalamus for NE uptake. Other conditions have been described in Table 2.

† $P < 0.005$, significantly different from control group.

Table 6. Effects of LY125180 on brain levels of Try, 5-HT and 5-HIAA and their labeling from [^{3}H]Try*

Measurement	Control	LY125180	Reduction (%)
5-HT, nmoles/g	3.97 ± 0.33	3.58 ± 0.34	10
[^{3}H]-5-HT, d.p.m./g	2491 ± 100	1958 ± 82	21†
d.p.m./nmole	644 ± 40	565 ± 56	12
5-HIAA, nmoles/g	4.27 ± 0.15	2.84 ± 0.10	33†
[^{3}H]-5-HIAA, d.p.m./g	1636 ± 85	743 ± 26	55†
d.p.m./nmole	383 ± 16	262 ± 8	32†
Try, nmoles/g	25.3 ± 1.2	27.0 ± 0.9	−7
[^{3}H]Try, d.p.m./g	23,084 ± 1905	23,129 ± 1070	0
d.p.m./nmole	909 ± 47	858 ± 38	6
Conversion factor: ([^{3}H]-5-HT + [^{3}H]-5-HIAA)/[^{3}HTry]	0.18 ± 0.01	0.12 ± 0.01	33†

* Rats were treated with saline (seven rats) or LY125180 at 50 mg/kg, i.p. (six rats) for 1 hr and injected with [^{3}H]Try (413 μCi/kg) into the tail vein. One hour after injection of isotope, rats were decapitated and brains were immediately removed. Brains after the removal of cerebellum and pons-medulla were frozen on dry ice. Methods for the determination of indole metabolites have been indicated in the text.

† $P < 0.005$, significantly different from control group.

finding that the labeling of [^{3}H]-5-HT and [^{3}H]-5-HIAA by [^{3}H]Try was reduced by 21 and 55 per cent, respectively, after treatment with the uptake inhibitor, although the accumulation of [^{3}H]Try was unchanged. Only the specific activity (d.p.m./nmole) of [^{3}H]-5-HIAA was reduced significantly by treatment with LY125180 that led to a significant decrease of the conversion factor, ratio of the sum of [^{3}H]-5-HT and [^{3}H]-5-HIAA to [^{3}H]Try, from 0.18 ± 0.01 to 0.12 ± 0.01.

DISCUSSION

LY125180 effectively inhibited 5-HT uptake by synaptosomes of rat brain with a K_i value of 60 nM. However, it was only $\frac{1}{40}$ as effective in inhibiting NE uptake by cortical synaptosomes and DA uptake by striatal synaptosomes. The inhibitor competed with the substrates for uptake. The uptake of 5-HT by human platelets was also inhibited with an IC_{50} value of 22 nM. The profile of inhibition by LY125180 resembles that of fluoxetine (Table 7), which was at least ten times more effective in blocking 5-HT uptake than in blocking catecholamine uptake *in vitro* [4, 8].

The administration of LY125180 *in vivo* caused a reduction in 5-HT uptake but not NE uptake by synaptosomes of hypothalamus. This selective inhibition of 5-HT uptake appeared within 1 hr. Because it is a tertiary amine, LY125180 is expected to be *N*-demethylated by the liver endoplasmic reticulum. Indeed, pretreatment with SKF-525A, an inhibitor of microsomal metabolism, prolonged the duration of LY125180 action from 1 to at least 4 hr and increased the potency in lowering 5-HT uptake by synaptosomes from an ED_{50} of 12 to 4.4 mg/kg, i.p., whereas NE uptake remained unchanged. Therefore, inhibition of microsomal metabolism of LY125180 had enhanced its potency to equal that of fluoxetine but had lengthened the duration of action from $^1/_{24}$ to only $^1/_6$ that of fluoxetine (Table 7). The shorter acting drug may be more readily disposed of by the peripheral tissues.

Before the discovery of fluoxetine, the only selective inhibitors of 5-HT uptake were the tertiary amine-containing tricyclic antidepressants, imipra-

Table 7. Comparison of LY125180 and fluoxetine in blockade of monoamine uptake

Uptake of monoamines		LY125180	Fluoxetine
		IC_{50} (M)	
Cortical nerve endings	5-HT	8×10^{-8}	2×10^{-7}
	NE	5×10^{-6}	1×10^{-5}
Striatal nerve endings	DA	7×10^{-6}	4×10^{-6}
Platelets (human)	5-HT	2×10^{-8}	1×10^{-8}
		ED_{50} (mg/kg, i.p.)	
Heart	NE	50 (11%)	50 (0%)
Hypothalamic nerve endings	5-HT	12	
	NE	100 (25%)	
Hypothalamic nerve endings pretreated with SKF-525A	5-HT	4.4	
	NE	25 (12%)	
Blockade of *p*-chloroamphetamine in brain	5-HT	25	3.5
Blockade of 6-hydroxydopamine in brain	NE	50 (3%)	50 (0%)
Time course *in vivo*			
Hypothalamic nerve endings	5-HT	Uptake returned to control values in 4 hr	Inhibition lasts for at least 24 hr

mine and chlorimipramine [2, 3]. However, these two tricyclic compounds are readily *N*-demethylated *in vivo*. The *N*-demethylated products were more effective inhibitors of NE uptake by synaptosomes and by heart [4]. Thus, imipramine and chlorimipramine, *in vivo*, effectively antagonized the neurotoxicity of 6-OHDA with respect to NE uptake [5]. On the other hand, LY125180 and fluoxetine [4] at higher doses (50–100 mg/kg) failed to block NE uptake by rat heart or to protect the loss of NE uptake from intraventricularly administered 6-OHDA.

Like fluoxetine [18, 19], LY125180 also lowered the turnover rate of serotonin in rat brain. The compound decreased brain levels of 5-HIAA without changing the brain levels of 5-HT and Try and lowered the conversion of [^{3}H]Try into [^{3}H]-5-HT and [^{3}H]-5-HIAA. Thus, the decrease in brain level of 5-HIAA is not due to an inhibition of monoamine oxidase (since 5-HT levels were unaltered) but probably to a reduction in synthesis of its precursor, 5-HT.

The compound, LY125180, represents a new structure that has selective affinity for the uptake site of 5-HT, although it is also a bicyclic phenoxyphenylpropylamine like fluoxetine [3] and nisoxetine [5]. The phenyl group is moved from the phenoxy-end to the amino carbon of the propyl side chain. When the phenyl group is attached to the middle carbon of the propyl side chain, the affinity for uptake of monoamines is much reduced, with IC_{50} values greater than 10 μM (unpublished data). LY125180 has a methyl group in place of a trifluoromethyl group at the *para*-position of the phenoxy ring of fluoxetine. In both series of compounds represented by fluoxetine [3] and LY125180, substitution at the *para*-position of the phenoxy ring is preferred over the *meta*- or *ortho*-positions for the compounds to be selective toward inhibition of 5-HT uptake. Thus, substitution on the phenoxy ring plays an important factor in directing the compound to the specific amine pumps.

Since this laboratory originally reported the unique properties of fluoxetine on monoamine uptake in 1974 [3], at least eight other compounds have been described as selective 5-HT uptake inhibitors including FG 4963 [20], ORG 6582 [21], pirandamine [22], zimelidine [23], citalopram [24], fluvoxamine [25], paroxetine [26] and LY125180 (this paper). Among them, only zimelidine [27] and fluvoxamine [28] have been reported thus far to have clinical efficacy in the treatment of depression as originally intended for a selective 5-HT uptake inhibitor like fluoxetine [3]. Fluvoxamine seemed to produce a therapeutic response sooner than the tricyclic antidepressants [29]. The new inhibitors are perhaps more direct in their action on the uptake of 5-HT at the nerve terminals while the tricyclic antidepressants are better blockers of NE uptake than of 5-HT uptake *in vivo* [5].

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