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# Comparative Affinity of Duloxetine and Venlafaxine for Serotonin and Norepinephrine Transporters *in vitro* and *in vivo*, Human Serotonin Receptor Subtypes, and Other Neuronal Receptors

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*The blockade of serotonin (5-HT) and norepinephrine (NE) transporters in vitro and in vivo by the dual 5-HT/NE reuptake inhibitors duloxetine and venlafaxine was compared. Duloxetine inhibited binding to the human NE and 5-HT transporters with $K_i$ values of 7.5 and 0.8 nM, respectively, and with a $K_i$ ratio of 9. Venlafaxine inhibited binding to the human NE and 5-HT transporters with $K_i$ values of 2480 and 82 nM, respectively, and with a $K_i$ ratio of 30. Duloxetine inhibited ex vivo binding to rat 5-HT transporters and NE transporters with $ED_{50}$ values of 0.03 and 0.7 mg/kg, respectively, whereas venlafaxine had $ED_{50}$ values of 2 and 54 mg/kg, respectively. The depletion of rat brain 5-HT by p-chloramphetamine and depletion of rat hypothalamic NE by 6-hydroxydopamine was blocked by duloxetine with $ED_{50}$ values of 2.3 and 12 mg/kg, respectively. Venlafaxine had $ED_{50}$ values of 5.9 and 94 mg/kg for blocking p-chloramphetamine– and 6-hydroxydopamine–induced monoamine depletion, respectively. Thus, duloxetine more potently blocks 5-HT and NE transporters in vitro and in vivo than venlafaxine.*
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KEY WORDS: *Duloxetine; Venlafaxine; Depression; Norepinephrine uptake; Serotonin uptake; 5-HT receptors*

Serotonin (5-HT) and norepinephrine (NE) neuronal pathways in the central nervous system are involved in the mechanism of action of antidepressant drugs and, additionally, they may be involved in the pathogenesis of depression (Schildkraut 1965; Blier and de Montigny 1994; Delgado et al. 1990, 1993). However, antidepressant therapies that activate 5-HT or NE pathways have a latency of two to three weeks in response and only about 70–80% of patients are responders (Roose et al. 1986; Thase and Rush 1995). Clinical studies have indicated that combination therapy with drugs that inhibit 5-HT and NE uptake and thereby enhance activity of both neuronal systems may have improved therapeutic efficacy and possibly a faster onset of activity compared to drugs that inhibit only one monoamine uptake system (Weilburg et al. 1989; Nelson et al. 1991; Seth et al.

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1992). The tertiary amine containing tricyclic antidepressants such as imipramine and amitriptyline inhibit both NE and 5-HT uptake *in vitro* to variable degrees and therefore may be considered dual uptake inhibitors. However, *in vivo* imipramine and amitriptyline are rapidly metabolized to secondary amines that are potent and selective NE uptake inhibitors and no longer inhibit uptake of both monoamines (Wong et al. 1995). Furthermore, the tricyclic antidepressants are also antagonists at a number of neuronal receptors including muscarinic, α-adrenergic and histamine $H_1$ receptors, which causes significant side effects (Wong et al. 1995). Studies comparing the efficacy of tricyclic antidepressants with selective 5-HT reuptake inhibitors (SSRI) suggest that tricyclic antidepressants may be slightly more efficacious (Nelson 1998; Anderson 2000) but, arguably, compounds with adequate dual uptake inhibition have not been thoroughly evaluated clinically. In order to test the hypothesis that agents endowed with the ability to block both 5-HT and NE uptake would have supplemental efficacy in the treatment of depression, compounds such as duloxetine (Wong et al. 1993; Wong 1998) and venlafaxine (Muth et al. 1986; for review see Artigas 1995) with combined 5-HT and NE uptake inhibitor (SNRI) properties in a single molecule have been developed. Duloxetine and venlafaxine have both been shown to be clinically effective antidepressants (Berk et al. 1997; Mendels et al. 1993; Schweizer et al. 1994).

Duloxetine potently inhibits 5-HT and NE uptake processes *in vitro* and *in vivo* at similar doses (Kasamo et al. 1996; Béïque et al. 1998; Wong et al. 1993; Wong 1998). However, venlafaxine has been shown to display relatively low affinity for NE uptake into rat synaptosomes compared to 5-HT uptake and for the human NE transporter versus the 5-HT transporter (Bolden-Watson and Richelson 1993; Béïque et al. 1998; Tatsumi et al. 1997). *In vivo*, intravenously administered venlafaxine was about 10 times more potent at prolonging recovery of firing activity of dorsal hippocampal neurons after microiontophoretically applied 5-HT than NE (Béïque et al. 1998). In addition, venlafaxine after intravenous administration was about 3-fold more active at suppressing firing activity of 5-HT neurons in the dorsal raphe compared to NE neurons in locus coeruleus. Thus, it has been pointed out that there is a discrepancy between *in vitro* and *in vivo* potencies of venlafaxine for the effects on the noradrenergic neurons (Béïque et al. 1999). To further address this issue, this study compared the affinity of the two compounds for human and rat 5-HT, NE, and DA transporters and blockade of 5-HT and NE uptake into rat synaptosomes. The ability of the two drugs to block 5-HT and NE transporters *ex vivo* and block the monoamine depletion induced by 5-HT and NE transporter-specific neurotoxins was also investigated. Finally, the selectivity of duloxetine and venlafaxine was investigated by determining the inhibition of radioligand binding to a number of neuronal receptors.

## METHODS

### Transporter Binding

Membranes from HEK 293, MDCK and HEK293 cell lines transfected with human 5-HT, NE and DA transporters, respectively, were obtained from Receptor Biology, Inc. (Beltsville, MD). All assays were performed in triplicate in a final volume of 0.8 ml containing each of the following: 0.2 ml of various concentrations of drug in buffer (50 mM Tris Cl pH 7.4, 150 mM NaCl, and 5 mM KCl for 5-HT and NE transporters; 50 mM Tris Cl, pH 7.4, and 100 mM NaCl for the DA transporter); 0.2 ml of [$^3$H]-paroxetine (0.2 nM, 25 Ci/mmol, New England Nuclear), [$^3$H]-nisoxetine (1.0 nM, 86 Ci/mmol, New England Nuclear) or [$^3$H]-Win35,428 (1.0 nM, 86 Ci/mmol, New England Nuclear) in buffer for the human 5-HT, NE and DA transporters, respectively; 0.2 ml membrane (10.3 μg, 16.9 μg or 6.2 μg protein, respectively) and 0.2 ml buffer. After incubation, 37°C for 40 minutes for the 5-HT transporter and 25°C for 30 minutes for NE and DA transporters, the binding was terminated by rapid vacuum filtration over Whatman GF/B filters (presoaked in 0.5% polyethylenimine) and the filters were washed four times with cold 50 mM Tris Cl buffer, pH 7.4. The filters were then placed in vials containing liquid scintillation fluid and radioactivity was measured by liquid scintillation spectrometry. Non-specific binding was determined by including separate samples of 1 μM duloxetine, 10 μM desipramine or 10 μM nomifensine, for 5-HT, NE and DA transporters, respectively.

For binding to rat monoamine transporters, rat frontal cortical membranes were prepared by ABS, Inc. (Wilmington, DE). Briefly, male Sprague-Dawley rats were killed by decapitation, the brains rapidly removed and the frontal cortex was dissected over ice. The tissue was homogenized in 40 volumes of ice chilled 50 mM Tris-Cl, pH 7.4, and centrifuged at 39,800 × g for 10 minutes and the supernatant decanted. This process was repeated twice and the resulting homogenate was incubated at 30°C for 20 minutes, then centrifuged as before. The final pellets were immediately frozen at −80°C. After thawing, aliquots of the membranes containing 50 μg protein were incubated with various concentrations of drugs, either [$^3$H]-paroxetine (0.2 nM) or [$^3$H]-nisoxetine (1 nM), respectively, in 50 mM Tris Cl, pH 7.4, 150 mM NaCl (300 mM NaCl for nisoxetine) and 5 mM KCl buffer. After a 30-min incubation at room temperature, the reaction mixtures were filtered under vacuum with a cell harvester fitted with a GF/B filter (Brandel, Gaithersburg, MD). The filters were then

placed in vials containing liquid scintillation fluid and radioactivity was measured by liquid scintillation spectrometry. Samples containing 1 μM duloxetine and 10 μM desipramine were included to assess non-specific binding to the serotonin and norepinephrine transporter, respectively.

### Monoamine Uptake into Synaptosomes

Uptake of monoamines was determined according to method of Wong et al. (1993). Briefly, the uptake of [$^3$H]-5-HT (20 nM, 21.8 Ci/mmol, New England Nuclear), [$^3$H]-NE (20 nM, 17.2 Ci/mmol, New England Nuclear), and [$^3$H]-DA (20 nM, 45 Ci/mmol, New England Nuclear) was determined in synaptosomes from cerebral cortex, hypothalamus, and striatum, respectively, of rat (Harlan Sprague-Dawley, Cumberland, IN). Accumulation of radioactivity at 4°C represented non-specific uptake and was subtracted from each sample. The uptake of 5-HT into platelets from humans was conducted according to the method of Wong et al. (1993).

### *Ex vivo* Uptake

*Ex vivo* uptake was determined in groups of five Sprague-Dawley male rats (Harlan Sprague-Dawley) weighing 100–150 gm according to the method of Wong et al. (1993). Briefly, the rats were treated with specified doses of drugs either by the subcutaneous (s.c.) or oral route for one hour and then euthanized by decapitation. Uptake of [$^3$H]-5-HT, [$^3$H]-NE and [$^3$H]-DA in whole homogenates of cerebral cortex, hypothalamus and striatum was determined as previously described.

### *Ex vivo* Binding

Sprague-Dawley male rats in groups of five weighing about 100–150 gm were administered s.c. either saline vehicle or varying doses of drugs and the rats were euthanized one hour after the injection. The cerebral cortices were quickly dissected and frozen on dry ice. After weighing, the tissue was homogenized in 10 volumes of 50 mM Tris Cl buffer, pH 7.4, and frozen overnight at −80° C. After thawing, the whole homogenates were preincubated at 37° C for 30 minutes. *Ex vivo* binding of [$^3$H]-paroxetine and [$^3$H]-nisoxetine to the 5-HT and NE transporters, respectively, was evaluated as previously described.

### Blockade of p-chloramphetamine (p-CA) and 6-hydroxydopamine (6-OHDA) effects

The 5-HT selective neurotoxin p-chloramphetamine hydrochloride (p-CA) was dissolved in distilled $H_2O$ for i.p. administration. Male Sprague-Dawley rats in groups of five weighing 180–230 grams were injected with p-CA (10 mg/kg i.p.) two hours before being killed by cervical dislocation. Vehicle, duloxetine, or venlafaxine were injected one hour prior to p-CA. The tissues were quickly removed, dissected, frozen on dry ice and stored at –70°C until assayed. 6-Hydroxydopamine hydrobromide (6-OHDA), a catecholaminergic neurotoxin, was dissolved in 0.1% ascorbic acid. Duloxetine or venlafaxine were injected i.p. at various doses one hour prior to intracerebroventricular injection of 6-OHDA (50 μg/rat) and the rats were killed one week later. Whole brain 5-HT concentrations in the p-CA study and NE concentrations in the hypothalamus for the 6-OHDA study were measured using high pressure liquid chromatography with electrochemical detection (HPLC-EC) as previously described (Fuller and Perry 1989).

### Determination of Binding to Neuronal Receptors and Monoamine Oxidase Activity

Binding to dopamine $D_2$, opiate, muscarinic, histamine $H_1$, $\alpha_1$-adrenergic and $\alpha_2$-adrenergic receptors was determined using [$^3$H]-spiperone, [$^3$H]-naloxone, [$^3$H]-quinuclidinyl benzylate (QNB), [$^3$H]-pyrilamine, [$^3$H]-WB4101 and [$^3$H]-clonidine, respectively, as previously described (Wong et al. 1993). The binding to human 5-HT receptor subtypes was determined according to published methods (5-$HT_{1A}$ and 5-$HT_{1E}$, Zgombick et al. 1991; 5-$HT_{1B}$ and 5-$HT_{1D}$, Weinshank et al. 1992; 5-$HT_{1F}$, Adham et al. 1993; 5-$HT_{2A}$, 5-$HT_{2B}$, 5-$HT_{2C}$, Wainscott et al. 1996; 5-$HT_6$, Boess et al. 1997; 5-$HT_7$, Bard et al. 1993). Inhibition of binding to 5-$HT_4$ and other neuronal receptors was provided by NovaScreen (Hanover, MD). Monoamine oxidase (MAO) activity *in vitro* was measured with rat brain mitochondria as the enzyme source with [$^{14}$C]-5-HT (100 μM, 3.93 mCi/mmol, New England Nuclear) or [$^{14}$C]-phenylethylamine (12.5 μM, 3.6 mCi/mmol, New England Nuclear) as substrate for MAO type A or MAO type B, respectively (Fuller et al. 1970).

### Data Analysis

Inhibition curves for *in vitro* studies were analyzed by nonlinear least-squares curve fitting to obtain $IC_{50}$ values. The $K_i$ values were calculated from $IC_{50}$ and $K_d$ values according to the method of Cheng and Prusoff (1973). The $K_m$ values for 5-HT, NE and DA uptake were 8for the 5-HT, NE and DA transporters were 0.18, 2.5 and 22.8 nM, respectively. Statistical analyses for *in vivo* studies were done by analysis of variance using Tukey's Honestly Significant Difference method ($p < .05$) based on the mean square error.

### Drugs

Duloxetine, (+)-N-methyl-3-(1-naphthalenyloxy)-3-(2-thienyl)propamine HCl or oxalate) and venlafaxine (1-[2-

(dimethyl-amino)-1-(4-methoxyphenyl)-ethyl] cyclohexanol hydrochloride) were provided by the Lilly Research Laboratories, Indianapolis IN. 6-Hydroxydopamine hydrobromide and p-CA HCl, were purchased from Sigma Chemical Co. (St Louis, MO). All other chemicals were reagent grade.

## RESULTS

Duloxetine and venlafaxine dose-dependently inhibited binding of the serotonin transporter radioligand [$^3$H]-paroxetine to membranes from cells transfected with the human 5-HT transporter (Fig. 1). The $K_i$ values for duloxetine and venlafaxine were 0.8 ± 0.04 and 82 ± 3 nM, respectively (Table 1). Duloxetine and venlafaxine inhibited binding of the NE transporter ligand [$^3$H]-nisoxetine to membranes from cells transfected with the human NE transporter with $K_i$ values of 7.5 ± 0.3 and 2480 ± 43 nM, respectively (Fig. 2, Table 1). The binding of [$^3$H]-Win35,428 to the human dopamine transporter was inhibited by duloxetine and venlafaxine with $K_i$ values of 240 ± 23 and 7647 ± 793 nM (Table 1). Duloxetine inhibited [$^3$H]-5-HT uptake into human platelets with a $K_i$ value of 0.20 ± 0.04 nM, in close agreement to the affinity for clonal human 5-HT transporters (Wong et al. 1993).

The binding of [$^3$H]-paroxetine to rat cerebral cortical membranes was inhibited by duloxetine and venlafaxine with $K_i$ values of 0.5 ± 0.1 (Wong et al. 1993) and 138 ± 21 nM, respectively, whereas the binding of [$^3$H]-nisoxetine was inhibited with $K_i$ values of 3.6 ± 0.3 and 3187 ± 186 nM, respectively (Table 1.) The $K_i$ values for inhibition of uptake of [$^3$H]-5-HT into rat cerebral cortical synaptosomes was 4.6 ± 1.1 (Wong et al. 1993) and 77 ± 2 nM for duloxetine and venlafaxine, respectively

**Table 1.** Inhibition of Monoamine Uptake and Transporter Binding In Vitro by Duloxetine and Venlafaxine

| | Duloxetine | Venlafaxine |
|---|---|---|
| **Measurement** | **$K_i$, nM** | |
| [$^3$H]-Paroxetine binding—human | 0.8±0.04 | 82±3 |
| [$^3$H]-Nisoxetine binding—human | 7.5±0.3 | 2480±43 |
| [$^3$H]-Win35428 binding—human | 240±23 | 7647±793 |
| [$^3$H]-Paroxetine binding—rat | 0.5±0.1* | 138±21 |
| [$^3$H]-Nisoxetine binding—rat | 3.6±0.3 | 3187±186 |
| [$^3$H]-5-HT uptake synaptosomes | 4.6±1.1* | 77±2 |
| [$^3$H]-NE uptake synaptosomes | 16±2.9* | 538±43 |
| [$^3$H]-DA uptake synaptosomes | 369±38* | 6371±1366 |
| [$^3$H]-5-HT uptake platelets—human | 0.20±0.04* | — |

Synaptosomal uptake of [$^3$H]-5-HT, [$^3$H]-NE, and [$^3$H]-DA was in synaptosomes from rat cerebral cortex, hypothalamus, and striatum, respectively. All values determined from three or more independent experiments with at least 6 concentrations of drug in triplicate. *Data from Wong et al. (1993).

(Table 1). Duloxetine and venlafaxine inhibited uptake of [$^3$H]-NE into hypothalamic synaptosomes with $K_i$ values of 16 ± 2.9 (Wong et al. 1993) and 538 ± 43 nM , respectively, and with $K_i$ values of 369 ± 38 (Wong et al. 1993) and 6371 ± 1366 for [$^3$H]-DA uptake in rat striatal synaptosomes, respectively (Table 1).

Duloxetine inhibited binding to human 5-$HT_6$ and 5-$HT_{2A}$ receptor subtypes with $K_i$ values of 419 ± 89 and 504 ± 87 nM, respectively and had affinity of >900 nM for the other 5-HT receptor subtypes examined (Table 2). Similarly, venlafaxine had relatively low affinity for the 5-HT receptor subtypes examined (Table 2). Neither compound had significant affinity for muscarinic, dopamine $D_2$, $\alpha_1$-, $\alpha_2$-adrenergic or histamine $H_1$ receptors and did not appreciably inhibit monoamine oxidase type A or B (Table 2). Further, duloxetine and venlafax-

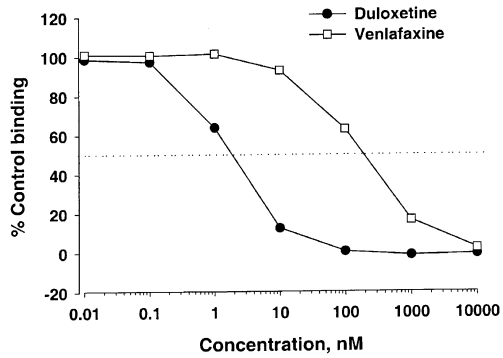


**Figure 1.** Concentration-dependent inhibition of [$^3$H]-paroxetine binding to human 5-HT transporters by duloxetine and venlafaxine. Binding was determined by incubation of [$^3$H]-paroxetine (0.2 nM) with membranes from cells transfected with human 5-HT transporters and with various concentrations of drugs for 40 minutes at 37°C. Data are expressed as % control specific binding.

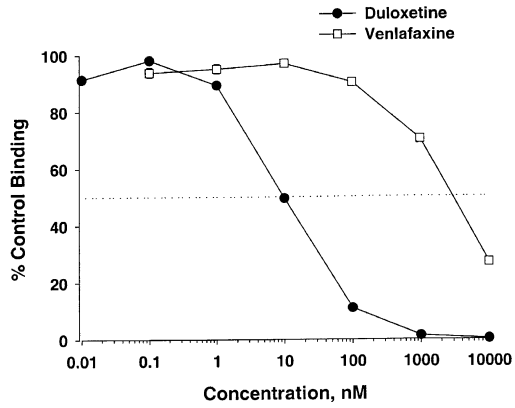


**Figure 2.** Concentration-dependent inhibition of [$^3$H]-nisoxetine binding to human norepinephrine transporters by duloxetine and venlafaxine. Binding was determined by incubation of [$^3$H]-nisoxetine (1 nM) with membranes from cells transfected with human NE transporters and with various concentrations of drugs for 30 minutes at 25°C. Data are expressed as % control specific binding.

**Table 2.** Affinity of Duloxetine and Venlafaxine for Human 5-HT Receptor Subtypes, Other Neuronal Receptors and Blockade of Monoamine Oxidase Activity

| Receptor subtype | Duloxetine | Venalfaxine |
|---|---|---|
| 5-$HT_{1A}$ | >5000 | >10000 |
| 5-$HT_{1B}$ | 3959±810 | >10000 |
| 5-$HT_{1D}$ | >3000 | >10000 |
| 5-$HT_{1E}$ | 3733±618 | >10000 |
| 5-$HT_{1F}$ | 4447±30 | >10000 |
| 5-$HT_{2A}$ | 504±87 | 2230±723 |
| 5-$HT_{2B}$ | 2100±206 | >10000 |
| 5-$HT_{2C}$ | 916±190 | 2004±808 |
| 5-$HT_4$ | >1000** | >1000** |
| 5-$HT_6$ | 419±89 | 2792±431 |
| 5-$HT_7$ | 2261±115 | >10000 |
| Muscarinic-nonselective | 3000* | >10000[a] |
| Dopamine $D_2$ | 14000 | >10000[a] |
| $\alpha_1$-adrenergic | 8300* | >10000[a] |
| $\alpha_2$-adrenergic | 8600 | >10000[a] |
| Histamine $H_1$ | 2300* | >10000[a] |
| Monoamine oxidase A | 87000[#] | >100000# |
| Monoamine oxidase B | 18000[#] | >100000# |

**All 5-HT receptor subtypes were human except for 5-$HT_4$ which was from guinea pig striatum. * = Data in $IC_{50}$, nM units from Wong et al., 1993; [a] = Muth et al. (1986), [#] = data in $IC_{50}$, nM units.

ineneither at 1 μM concentration did not significantly inhibited radioligand binding to 52 other neurotransmitter receptors, ion channel receptors, second messenger receptors, additional neurotransmitter transporters or brain/gut peptide receptors (Table 3).

The ability of duloxetine and venlafaxine to penetrate into the brain after systemic administration and interact with monoamine transporters was investigated using *ex vivo* binding to transporters and *in vivo* blockade of transporter-dependent neurotoxins. Duloxetine and venlafaxine penetrated into the brain and dose-dependently inhibited *ex vivo* binding of [$^3$H]-paroxetine to rat cerebral cortical homogenates with $ED_{50}$ values of 0.03 and 2.0 mg/kg s.c., respectively (Table 4, Fig. 3, Panel A). The *ex vivo* binding of [$^3$H]-nisoxetine was inhibited in a dose dependent manner with $ED_{50}$ values of 0.7 and 54 mg/kg s.c., respectively, for duloxetine and venlafaxine (Fig. 3, Panel B, Table 4). Duloxetine inhibited 5-HT *ex vivo* uptake with $ED_{50}$ values of 12.2 ± 1.6 mg/kg p.o. and 2.0 mg/kg s.c., respectively, and NE *ex vivo* uptake with $ED_{50}$ values of 14.6 ± 2.3 p.o. and 3 mg/kg s.c., respectively (Wong et al. 1993). Thus, duloxetine was 66 and 77 times more potent at inhibiting 5-HT and NE transporters than venlafaxine. The depletion of 5-HT in rat brain induced by p-CA was antagonized dose-dependently by duloxetine and venlafaxine with $ED_{50}$ values of 2.3 and 5.9 mg/kg i.p., respectively (Table 4, Fig. 4, Panel A). The depletion of norepinephrine levels in rat hypothalamus induced by 6-OHDA was dose-dependently blocked by duloxetine and venlafaxine with $ED_{50}$ values of 12 and 94 mg/kg i.p., respectively (Table 4, Fig. 4, Panel B).

The NE/5-HT selectivity ratio of duloxetine for human transporters was 9.4 and 7.2 for rat transporters, respectively (Table 5). The NE/5-HT selectivity ratio of venlafaxine was 30 and 23 for human and rat transporters, respectively. The ratio for blockade of 5-HT and NE uptake processes in rat synaptosomes was 3.5 and 7 for duloxetine and venlafaxine, respectively. *In vivo*, the NE/5-HT selectivity ratio for *ex vivo* transporter binding was 23 and 27 for duloxetine and venlafaxine, respectively. The ratio for blockade of 6-OHDA/p-CA was 5.2 and 16 for duloxetine and venlafaxine, respectively.

## DISCUSSION

In the present study the ability of duloxetine and venlafaxine to block 5-HT and NE transporters *in vitro* and *in vivo* was compared. Duloxetine potently inhibited binding to the human 5-HT transporters with a $K_i$ value of 0.8 nM, whereas venlafaxine was 106 times less potent. Duloxetine also potently inhibited binding to the human NE transporter with a $K_i$ value of 7.5 nM and venlafaxine inhibited binding to the human NE transporter with a $K_i$ value of 2480 nM. Thus, venlafaxine inhibited binding to the NE transporter with 331 times lower affinity than duloxetine. Duloxetine and venlafaxine inhibited binding to the rat 5-HT and NE transporters with similar affinities to that of human transporters. The $K_i$ values of duloxetine for inhibition of 5-HT and NE transporters are in agreement with previously reported values in rat tissue (Béïque et al. 1998; Wong et al. 1993) and for venlafaxine in human and rodent tissue (Tatsumi et al. 1997; Béïque et al. 1998). The selectivity ratios of duloxetine and venlafaxine for NE/5-HT blockade of human transporters were 9.4 and 30, respectively. Similar selectivity ratios of 7.2 and 23 for rat NE and 5-HT transporters, respectively, were determined. Relative to their affinity for 5-HT transporters, neither drug had high affinity for the human dopamine transporter.

The dual uptake inhibitors also inhibited *in vitro* uptake of monoamines in synaptosomes of rat brain. Duloxetine inhibited 5-HT, NE and DA uptake with $K_i$ values of 4.6, 16 and 369 nM, respectively, in good agreement with values obtained with transporter binding in rat tissue. Venlafaxine inhibited uptake with 17, 34 and 17-fold lower potency, respectively. The NE/5-HT selectivity ratios of duloxetine and venlafaxine for rat synaptosomal uptake was 3.5 and 7, respectively.

Non-selective interaction of uptake inhibitors with neuronal receptors may increase their side effect potential and new drugs in this class have been designed to have low affinity for these receptors (Wong et al. 1995).

**Table 3.** Duloxetine and Venlafaxine Have Low Affinity (>1 μM) for Other Neuronal Receptors*

| Receptor (Species) | Receptor (Species) |
|---|---|
| Neurotransmitter receptors | |
| Adenosine, A1 (R) | Glutamate, NMDA,Glycine(Stry-insen.) (R) |
| Adenosine, A2 (B) | Glycine, Strychnine-sensitive (R) |
| Adrenergic, α1A (R) | Histamine, H2 (GP) |
| Adrenergic, α1B (R) | Melatonin (C) |
| Adrenergic, α2A (H) | Muscarinic, M1 (H) |
| Adrenergic, α2B (M) | Muscarinic, M2 (H) |
| Adrenergic, β1 (R) | Nicotinic (a-bungarotoxin insens) (R) |
| Adrenergic, β2 (R) | Opiate, Delta 1 (R) |
| Dopamine, D1 (R) | Opiate, Kappa 1 (GP) |
| GABA A, Agonist Site (B) | Opiate, Mu (R) |
| GABA A, Benzodiazepine, Cent. (B) | Opiate, Non-selective (R) |
| Glutamate, AMPA Site (R) | Sigma 1 (GP) |
| Glutamate, Kainate Site (R) | Sigma 2 (GP) |
| Glutamate, MK-801 Site (R) | |
| Ion channels | |
| Calcium Channel, Type L (R) | Glutamate, NMDA, PCP (R) |
| Calcium Channel, Type N | Potassium Channel, ATP-Sens. (R) |
| GABA, Chloride, TBOB Site (R) | Potassium Channel, $Ca^{++}$ Act., Volt Sens. (R) |
| Glutamte, Chloride Site (R) | Sodium, Site 1 (R) |
| Glutamate, MK-801 Site (R) | Siodium, Site 2 (R) |
| Second messengers | |
| Adenylate Cyclase, Forskolin (R) | NOS (Neuronal-Binding) (R) |
| Inositol Triphosphate (R) | Protein Kinase C, PDBu (M) |
| Transporters | |
| Choline transporter (R) | Adenosine transporter (R) |
| GABA transporter (R) | |
| Brain/gut peptides | |
| Cholecystokinin, CCK1 (CCKA) (M) | Neurokinin, NK3 (R) |
| Cholecystokinin, CCK2 (CCKB) (M) | Neuropeptide, NPY1 (H) |
| Neurokinin, NK1 (R) | Neurotensin (R) |
| Neurokinin, NK2 (NKA) (H) | Somatostatin, Non-selective (R) |

*<50% inhibition of receptors by 1 μM concentration of duloxetine or venlafaxine.
Abbreviations: R, rat; M, mouse; GP, guinea pig; B, bovine; H; human.

Duloxetine and venlafaxine demonstrated remarkably low levels of interaction with a number of neuronal receptors, suggesting that they would be free from side effects due to cholinergic, histaminic, dopaminergic, and adrenergic blockade.

Duloxetine and venlafaxine also blocked uptake transporters *in vivo* in rats. Duloxetine blocked *ex vivo* 5-HT and NE transporter binding with $ED_{50}$ values of 0.03 and 0.7 mg/kg s.c. Venlafaxine was 67 and 77-fold less potent than duloxetine at inhibiting 5-HT and NE *ex vivo* transporter binding. Duloxetine with comparable potency blocked *ex vivo* uptake of 5-HT and NE into brain homogenates after s.c. and oral administration, although higher doses were required to block *ex vivo* uptake than *ex vivo* transporter binding, consistent with lower affinity at uptake blockade than inhibition of transporter binding. Duloxetine blocked transporter-dependent depletion of 5-HT concentrations in rat brain by p-CA with an $ED_{50}$ value of 2.3 mg/kg i.p., demonstrating *in vivo* blockade of the 5-HT transporter (Fuller et al. 1994). Similarly, venlafaxine blocked p-CA–induced 5-HT depletion, but was 2.6 times less potent. Blockade of NE transporter-dependent 6-OHDA-induced depletion of NE concentrations in rat hypothalamus was used to evaluate blockade of the NE transporter *in vivo* (Fuller et al. 1994). Duloxetine and venlafaxine blocked 6-OHDA–induced NE depletion with $ED_{50}$ values of 12 and 94 mg/ kg i.p., respectively. The selectivity ratio for blocking NE/5-HT transporter-dependent effects *in vivo* was 5.2 and 16-fold, respectively. Thus, both drugs penetrate into the brain and inhibit both 5-HT and NE uptake processes, although considerably higher doses of venlafaxine were needed to block the NE transporter.

Thus, duloxetine compared to venlafaxine had higher affinity and more potent blockade of 5-HT and NE transporters *in vitro* and *in vivo*. The results are in general agreement with previously reported results on the two compounds. For example, venlafaxine was found to have 10-fold higher potency for prolonging suppression of dorsal hippocampal $CA_3$ neuronal firing by microiontophoretically applied 5-HT versus NE

**Table 4.** Blockade of Ex Vivo Transporter Binding, Ex Vivo and In Vivo Monoamine Uptake by Duloxetine and Venlafaxine

| | Duloxetine | Venlafaxine |
|---|---|---|
| **Measurement** | **$ED_{50}$, mg/kg (route)** | |
| [$^3$H]-Paroxetine ex vivo binding | 0.03(s.c.) | 2.0(s.c.) |
| [$^3$H]-Nisoxetine ex vivo binding | 0.70(s.c.) | 54(s.c.) |
| [$^3$H]-5-HT ex vivo uptake | 12.2 ± 1.6(p.o.)* 2.0 (s.c.)* | ND |
| [$^3$H]-NE ex vivo uptake | 14.6 ± 2.3(p.o.)* 3(s.c.)* | ND |
| [$^3$H]-DA ex vivo uptake | >40(p.o.)* | ND |
| 5-HT depletion by p-CA in rat brain | 2.3 (i.p.)** | 5.9 (i.p.) |
| NE depletion by 6-OHDA in rat hypothalamus | 12 (i.p.)** | 94 (i.p.) |

*Data from Wong et al., 1993; **=data from Fuller et al., 1994, ND = not determined.

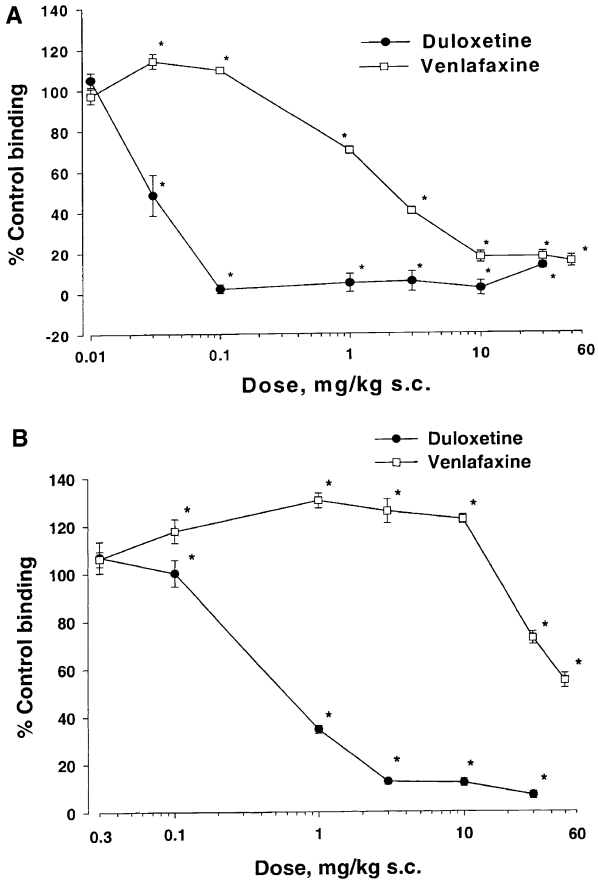


**Figure 3.** Dose dependent inhibition of *ex vivo* binding of [$^3$H]-paroxetine **(Panel A)** and [$^3$H]-nisoxetine **(Panel B)** to whole homogenates from frontal cortex of rats treated with vehicle or various doses of duloxetine or venlafaxine. Rats in groups of five were administered vehicle or drugs s.c. for one hour prior to euthanasia. Data are expressed as % vehicle specific binding ± standard error of the mean (SEM). *=$p<.05$.

(Béïque et al. 1998). Venlafaxine was also 3-fold more potent at suppressing spontaneous firing activity of 5-HT neurons in the dorsal raphe compared to suppressing NE neuronal activity in locus coeruleus (Béïque et al. 1999), although venlafaxine did not completely suppress firing in the locus coeruleus, unlike other NE uptake inhibitors including duloxetine (Kasamo et al. 1996). In the same electrophysiological paradigm, duloxetine was 4.8-fold more potent at inhibiting spontaneously firing activity in the dorsal raphe 5-HT neurons than inhibiting NE neurons in the locus coeruleus (Kasamo et al. 1996). The data presented in this paper demonstrate at least a 16-fold difference for *in vivo* potency of venlafaxine to block 5-HT and NE transporter-specific neurotoxins, whereas there was only 5-fold difference for duloxetine. The discrepancy between the affinity of venlafaxine for blocking 5-HT and NE transporters versus the potency of suppression of firing activity of cell bodies may be related to the different routes of administration, formation of active metabolites and/or sensitivity of firing of neurons. Overall, venlafaxine was consistently more potent *in vivo* than would be predicted from *in vitro* binding data, possibly due to relatively low protein binding or other bioavailability attributes (Troy et al. 1996).

Results from microdialysis studies indicate that duloxetine increases extracellular 5-HT and NE levels in frontal cortex and hypothalamus (Gobert et al. 1997; Kihara and Ikeda 1995; Engleman et al. 1995) in a similar dose range to doses that block both 5-HT and NE transporters, consistent with uptake blockade increasing extracellular levels of the neurotransmitters. Selective blockade of 5-HT and NE autoreceptors markedly augmented the duloxetine-induced increase of extracellular levels of 5-HT and NE, respectively, suggesting that increased levels of monoamines activated inhibitory autoreceptors (Engleman et al. 1996; Millan et al. 1998; Gobert et al. 1997). This is supported by the previously mentioned reports of inhibition of spontaneous firing activity of dorsal raphe 5-HT and locus ceruleus NE neurons by duloxetine (Kasamo et al. 1996). Duloxetine was active in several rodent behavior models of NE uptake inhibition including reserpine-induced hypothermia and tetrabenazine-induced ptosis and models for augmentation of 5-HT responses such as 5-hydroxytryptophan-induced head movements (Katoh et al. 1995). Duloxetine and venlafaxine also produced effects in the rat forced swim test consistent with blockade of multiple neurotransmitter systems (Reneric and Lucki 1998).

In summary, both duloxetine and venlafaxine inhibit 5-HT and NE uptake processes and transporter binding *in vitro* and *in vivo*. However, duloxetine has 100-fold or higher affinity for human and rat 5-HT transporters and at least 300-fold higher affinity for NE transporters

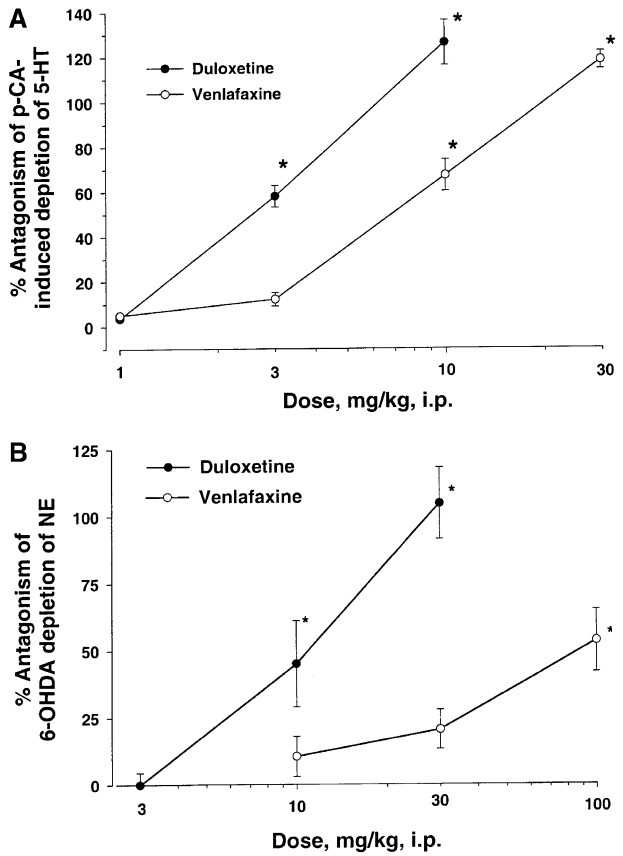


**Figure 4.** Blockade of p-chloramphetamine-induced depletion of brain 5-HT concentrations **(Panel A)** and 6-hydroxydopamine-induced depletion of hypothalamic norepinephrine (NE) concentrations **(Panel B)** by duloxetine and venlafaxine. p-Chlorampetamine (p-CA, 10 mg/kg i.p.) was administered two hours before euthanasia and one hour after vehicle or various i.p. doses of duloxetine or venlafaxine. 6-hydroxydopamine (6-OHDA) was injected intracerebroventricularly (50 μg/rat) one hour after injection of vehicle or various i.p. doses of duloxetine or venlafaxine. The rats were euthanized one week after the 6-OHDA injection. The levels of 5-HT in whole brain and NE in hypothalamus were determined by HPLC-EC. Data are expressed as % antagonism ± SEM of the reduction in monoamine levels by the neurotoxin for five rats per group. The concentration of 5-HT in brain of control- and p-CA–treated rats was 2.42 ± 0.10 and 1.11 ± 0.06 nmol/gm, respectively. The concentration of NE in hypothalamus of control- and 6-OHDA–treated rats was 5.75 ± 0.31 and 2.70 ± 0.45 nmol/gm, respectively. *= $p < .05$.

*in vitro* compared to venlafaxine. *In vivo*, duloxetine blocks 5-HT and NE transporter dependent monoamine depletion by neurotoxins with 2.5 and 7.8-fold higher potency than venlafaxine. Overall, these data are consistent with microdialysis data indicating that duloxetine increases 5-HT and NE extracellular levels at similar doses (Engleman et al. 1995). Clinical testing of these compounds is required to determine if the supplemental addition of blockade of NE as well as 5-HT uptake inhibition enhances the efficacy and decreases the delay in onset of antidepressant activity compared to SSRIs.

**Table 5.** Selectivity Ratios of Duloxetine and Venlafaxine for NE and 5-HT Transporters *in vitro* and *in vivo*

| | Duloxetine | Venlafaxine |
|---|---|---|
| **Measurement** | **Ratio NE/5-HT** | |
| NE/5-HT human transporters in vitro | 9.4 | 30 |
| NE/5-HT rat transporters in vitro | 7.2 | 23 |
| NE/5-HT uptake rat synaptosomes in vitro | 3.5 | 7 |
| NE/5-HT rat transporters ex vivo | 23 | 27 |
| NE/5-HT rat ex vivo uptake (s.c.) | 1.5 | ND |
| 6-OHDA/p-CA antagonism | 5.2 | 16 |

Data from Table 1 and Table 4, ND = not determined.

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