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Short communication

Affinities of venlafaxine and various reuptake inhibitors for the serotonin and norepinephrine transporters

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Abstract

In vitro radioligand binding studies were carried out in rat brain membranes to assess the affinity of various reuptake inhibitors for the serotonin (5-hydroxytryptamine, 5-HT) and the norepinephrine transporters using the selective ligands [^{3}H]cyanoimipramine and [^{3}H]nisoxetine, respectively. The selective 5-HT reuptake inhibitors paroxetine, indalpine and fluvoxamine displayed a high affinity for the 5-HT transporter, whereas the norepinephrine reuptake inhibitor desipramine had a high affinity for the norepinephrine transporter. Duloxetine, a dual 5-HT and norepinephrine reuptake inhibitor, displayed a high affinity for both the 5-HT and the norepinephrine transporters. Interestingly, venlafaxine, a dual 5-HT and norepinephrine reuptake inhibitor, displayed only a moderate affinity for the 5-HT transporter (K_i = 74 nM) and a very low affinity for the norepinephrine transporter (K_i = 1.26 μM). The relatively low affinities of venlafaxine contrast with its potent in vivo 5-HT and norepinephrine reuptake blocking properties. These results raise the possibility that the in vivo effects on the 5-HT and norepinephrine reuptake observed with venlafaxine may not be mediated solely by its binding to the [^{3}H]cyanoimipramine and [^{3}H]nisoxetine binding sites.

Keywords: Antidepressant; 5-HT (5-hydroxytryptamine, 5-HT) transporter; Norepinephrine transporter; Duloxetine; Binding

1. Introduction

Several lines of evidence point towards the involvement of the serotonergic (5-hydroxytryptamine; 5-HT) and norepinephrine systems in the mechanism of action of antidepressant treatments (Blier and De Montigny, 1994). Hence, a new class of drugs known as dual 5-HT and norepinephrine reuptake inhibitors has been developed. For instance, venlafaxine blocks in vitro the synaptosomal uptake of both [^{3}H]5-HT and [^{3}H]norepinephrine (Muth et al., 1986; Bolden-Watson and Richelson, 1993). Clinical studies are now suggesting that not only venlafaxine displays antidepressant activity, but that it (i) would also be more efficacious than fluoxetine for the treatment of major depression (Dierick et al., 1996), (ii) would be effective in the treatment of resistant depression (Nierenberg et al., 1994; De Montigny et al., 1996) and, finally, (iii) would show an early onset of action (Rickels, 1991). However, while venlafaxine shows an appreciable potency for inhibiting both 5-HT and norepinephrine reuptakes in vivo (Béïque et al., 1996), its potency to block in vitro the uptake of these monoamines is relatively low when compared to other reuptake inhibitors such as paroxetine for 5-HT uptake or desipramine for norepinephrine uptake (Bolden-Watson and Richelson, 1993). This latter observation, combined with the potentially unique therapeutic profile of venlafaxine, thus underscores the importance of a more accurate understanding of its pharmacological actions.

The present study was thus undertaken to directly determine the affinities of venlafaxine for both the 5-HT and norepinephrine transporters. To this end, the selective radioligands [^{3}H]cyanoimipramine and [^{3}H]nisoxetine, that bind selectively to the 5-HT and norepinephrine transporters, respectively (Burkard, 1980; Dumbrille-Ross and Tang, 1983; Tejani-Butt et al., 1990), were used. In order to provide a direct within study comparison, the affinities for the 5-HT and norepinephrine transporters of other monoamine reuptake inhibitors have also been determined, as well as those of duloxetine, an antidepressant candidate, which is also a dual 5-HT and norepinephrine reuptake inhibitor (Wong et al., 1993; Kasamo et al., 1996).

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2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats (250–275 g) were used.

2.2. Radioligand binding procedures

Binding assays were performed on total brain minus cerebellum using procedures modified from Burkard (1980) and Dumbrille-Ross and Tang (1983) for [^{3}H]cyanoimipramine binding and from Tejani-Butt et al. (1990) for [^{3}H]nisoxetine binding. Brains were homogenized in 50 vol Tris–HCl buffer (50 mM, pH: 7.4). Following centrifugation (40 000 $\times g$, 10 min at 4°C), the pellet was washed (3 $\times$) by resuspension and centrifugation. For [^{3}H]cyanoimipramine binding, the final pellet was resuspended in 50 vol Tris–HCl buffer (50 mM, pH: 7.4) containing 120 mM NaCl and 5 mM KCl and immediately frozen at -70°C until used. For [^{3}H]nisoxetine binding, it was resuspended in 300 mM NaCl and 5 mM KCl.

2.3. Saturation experiments

Saturation experiments were conducted in a final volume of 250 μl containing 8 different concentrations (0.003 to 4 nM) of either [^{3}H]cyanoimipramine or [^{3}H]nisoxetine and $\sim$250 μg of proteins. Non-specific binding for [^{3}H]cyanoimipramine was determined with 10 μM of paroxetine, while that for [^{3}H]nisoxetine with 10 μM of mazindol. The incubation period, 1 h at 23°C for [^{3}H]cyanoimipramine or 4 h at 4°C for [^{3}H]nisoxetine, was terminated by the addition of 4 ml of ice-cold buffer to each tube. The membranes were rinsed 3 times with the same buffer. The contents were filtered under vacuum through Whatman GF/B glass fibre filters pre-soaked for 1 h in 0.3% polyethylenimine, using a Brandel cell harvester.

2.4. Competition assay

For the in vitro determination of K_i values, aliquots of membrane suspensions ($\sim$250 μg protein) were incubated for 1 h at 23°C with 1 nM [^{3}H]cyanoimipramine or for 4 h at 4°C with 2 nM of [^{3}H]nisoxetine and with 19 to 22 concentrations (range 10^{-13}–10^{-5} M) of test molecules in a final volume of 250 μl. After incubation, 4 ml of ice-cold buffer were added and rapidly filtered, as described for the saturation experiments. The filters were subsequently washed (3 times), dried and placed in scintillation vials with 5 ml scintillation cocktail. Radioactivity contained on the individual filters was determined by liquid scintillation spectrometry (Beckman LS 6000SE).

2.5. Statistics

Binding data were analysed by nonlinear regression analysis using the 'Receptor Fit Competition' program (Lundon Software, Chagrin Falls, OH).

3. Results

3.1. Saturation studies

Nonlinear regression analysis of the saturation curves obtained following the incubation with different concentrations of [^{3}H]cyanoimipramine and [^{3}H]nisoxetine with rat brain homogenates indicated that each of these two radioligands labels a single class of saturable binding sites with a Hill coefficient close to unity. [^{3}H]Cyanoimipramine binding yielded a K_d of 0.61 ± 0.13 nM and a B_{max} of 225.5 ± 31.8 fmol/mg proteins whereas for [^{3}H]nisoxetine a K_d of 0.76 ± 0.042 nM and a B_{max} of 63.60 ± 5.91 fmol/mg protein were obtained.

3.2. Competition studies

Desipramine, duloxetine, fluoxetine, fluvoxamine, indalpine, paroxetine and venlafaxine were tested for their ability to displace [^{3}H]cyanoimipramine or [^{3}H]nisoxetine from the 5-HT and norepinephrine transporters, respectively. The competition curves are shown in Fig. 1. All

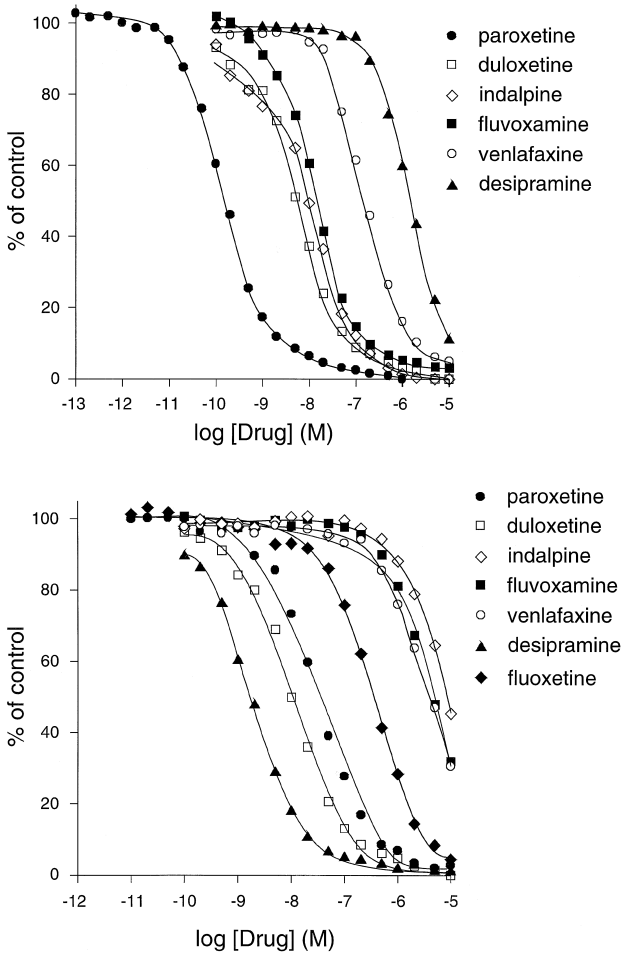

Fig. 1. Competition curves showing the displacement of [^{3}H]cyanoimipramine (1 nM) (top) and [^{3}H]nisoxetine (2 nM) (bottom) binding by reuptake inhibitors in rat brain (total brain minus cerebellum) membranes. The data are expressed as a percentage of specific binding. All competition curves yielded Hill coefficients close to unity.

Table 1
Inhibition, expressed as K_i values (±S.E.M.), of [^{3}H]cyanoimipramine and [^{3}H]nisoxetine binding to rat brain homogenates by different monoamine reuptake inhibitors

	K_i (nM)		Selectivity ratio
	[^{3}H]cyanoimipramine	[^{3}H]nisoxetine	
Duloxetine	1.8±0.1	3±0.3	1.7
Venlafaxine	74±1.9	1260±144	17
Fluvoxamine	6.7±0.2	1361±36	203
Paroxetine	0.04±0.004	11.8±0.6	294
Indalpine	5±0.3	2758±245	557
Desipramine	575±19	0.55±0.04	0.0009
Fluoxetine	ND	119±4	

tested compounds yielded monophasic competition curves with Hill coefficients close to unity for both radioligands used. The K_i values, as well as the selectivity ratios (5-HT/norepinephrine), are shown in Table 1. Nineteen to 22 concentrations (10^{-13} to 10^{-5} M) of unlabelled compounds were used for each value, expressed as mean ± S.E.M. from three experiments performed in triplicate.

4. Discussion

In the present study, duloxetine displayed a high and nearly equivalent affinity for both transporters, while venlafaxine, surprisingly, had only a moderate affinity for the 5-HT transporter and a low affinity for the norepinephrine transporter.

[^{3}H]Cyanoimipramine has been shown to bind to a site that is associated with the 5-HT transporter, and the reported K_d values are in keeping with the one reported in the present study (Burkard, 1980; Dumbrille-Ross and Tang, 1983). Similarly, [^{3}H]nisoxetine labels a site known to be associated with the norepinephrine transporter, and the observed binding parameters are in keeping with those of previous studies (Tejani-Butt et al., 1990; Gehlert et al., 1995). However, regarding the competition studies with [^{3}H]nisoxetine, all the K_i values obtained with the compounds used in the present study are lower than those reported by Cheetham et al. (1996). Nonetheless, the order of affinity of the common compounds for the [^{3}H]nisoxetine binding site is similar in the present study with that of Cheetham et al. (i.e., desipramine ≫ paroxetine > fluoxetine > fluvoxamine).

Duloxetine is a dual 5-HT/norepinephrine reuptake inhibitor (Wong et al., 1993; Kasamo et al., 1996). Wong et al. (1993) have reported the affinities of duloxetine for both the 5-HT and norepinephrine transporters using [^{3}H]paroxetine and [^{3}H]tomoxetine, respectively. Interestingly, although the radioligands used in that latter study were different from those used in the present one, the reported K_i are in the same range as those reported herein, but with slightly different 5-HT/norepinephrine selectivity ratios (4 vs. 1.7), both studies showing a preferential affinity of duloxetine for the 5-HT reuptake site. This preferential action of duloxetine on the 5-HT transporter has also been observed functionally in vivo, using an electrophysiological paradigm and also in ex vivo uptake studies carried out on hippocampal slices (Kasamo et al., 1996).

The affinity of venlafaxine for the [^{3}H]imipramine binding site has been previously assessed by Muth et al. (1986), and the reported K_i value falls into the same range as that found in the present study for [^{3}H]cyanoimipramine (i.e., 90 in the former vs. 74 nM in the present study). A most striking discrepancy emerges from the direct comparison of the affinities of venlafaxine for both transporters, with its functional uptake blocking potencies when done in relation with other reuptake inhibitors. For instance, in the present study, venlafaxine displays a near 2000-fold lesser affinity than paroxetine for the 5-HT transporter whereas, in vitro, venlafaxine inhibits [^{3}H]5-HT uptake with only a 53 times lesser potency than paroxetine (Bolden-Watson and Richelson, 1993). The discrepancy is even more important when an in vivo electrophysiological paradigm is used for assessing 5-HT reuptake inhibition in the hippocampus and in the dorsal raphe nucleus, as venlafaxine and paroxetine are equipotent in inhibiting 5-HT reuptake (Béïque et al., 1996).

Similarly, the very low affinity of venlafaxine for the norepinephrine transporter which is 2000 times less than that of desipramine contrasts with the fact that, in vitro, venlafaxine is only 344 times less potent than desipramine to block [^{3}H]norepinephrine uptake (Bolden-Watson and Richelson, 1993). Similarly to what was observed with 5-HT reuptake inhibition, the discrepancy is even more manifest when an in vivo electrophysiological paradigm is used as an index of norepinephrine reuptake inhibition, as venlafaxine was only slightly (~3 times) less potent than desipramine to block norepinephrine reuptake (Béïque et al., 1996).

5. Summary

The present study provides novel data on the binding affinities of duloxetine and venlafaxine for the 5-HT and norepinephrine transporters. Duloxetine binds with high affinity to both transporters in keeping with previous biochemical and electrophysiological studies. However, the binding profile of venlafaxine to the two transporters reported herein would be predictive of a much weaker blockade of both the 5-HT, and norepinephrine reuptake process by venlafaxine than that reported in functional studies (in vivo electrophysiological and in vitro uptake studies), as evidenced by the direct comparison with other reuptake inhibitors. This latter assertion thus raises the possibility that the effects observed in functional studies

with venlafaxine could be mediated via a mechanism distinct from a sole binding to the [^{3}H]cyanoimipramine and [^{3}H]nisoxetine binding sites. These important and crucial aspects of discrepancy and considerations, combined with the seemingly unique clinical profile of this drug, thus warrants further studies in order to clarify these issues.

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