British Journal of Pharmacology (1996) 117, 811-816

Binding and competitive inhibition of amine uptake at postsynaptic neurones (transport-P) by tricyclic antidepressants

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1 We have provided evidence for a novel amine uptake process for which prazosin is a substrate in postsynaptic neurones, characterized by a paradoxical increase in accumulation of the radioligand when the concentration of the unlabelled drug is increased above 10^{-7} M. This increase is due to activation of a proton-dependent, vacuolar type-ATPase-linked uptake process which is blocked by desipramine but is resistant to reserpine. We have now examined the effects of tricyclic antidepressants on this uptake system in a cell line derived from hypothalamic peptidergic neurones, known to be innervated by noradrenergic nerve terminals *in vivo*.

2 [^{3}H]-imipramine bound to the cells and was displaced by unlabelled imipramine, desipramine, amitriptyline and nortriptyline. The data fitted a single binding site model. This is the first demonstration of antidepressant binding sites in postsynaptic neurones.

3 There was no increase in the binding of [^{3}H]-imipramine at high concentrations of unlabelled imipramine, suggesting that antidepressants inhibit uptake but are not themselves accumulated by peptidergic gonadotrophin releasing hormone neurones.

4 Accumulation of prazosin was competitively inhibited by antidepressants. Tertiary amines were slightly more potent than secondary amines and the presence of a nitrogen atom in the heterocyclic ring enhanced blocking activity.

5 The affinities of the antidepressants for the uptake process are within the range of plasma concentrations that are observed during therapeutic use of these compounds. Since it is likely that this uptake process has a physiological function, its inhibition by antidepressants may provide a new avenue for investigating the mechanism of action of these compounds.

Keywords: Uptake; biological transport; tricyclic antidepressants; noradrenaline; prazosin

Introduction

Presynaptic nerve terminals possess transporters for reuptake of amine and amino acid neurotransmitters. These carrier molecules are located in the plasma membrane and serve to recapture released neurotransmitters from the extracellular synaptic space into the cytoplasm. Other transporters, located in the membranes of neurosecretory vesicles, then transport the recaptured neurotransmitters from the cytoplasm for storage in the vesicles (Iversen, 1967; Axelrod, 1971; Lester *et al.*, 1994). Neurotransmitters may also be accumulated by transporters into some non-neuronal cells, including myocytes, glia and endothelial cells (Iversen, 1965; Henn & Hamberger, 1971; Hosli & Hosli, 1978; Kimelberge & Pelton, 1983; Pines *et al.*, 1992; Storck *et al.*, 1992; Bouvier *et al.*, 1992; Bryan-Lluka *et al.*, 1992).

In addition to the amine uptake processes in presynaptic nerve terminals and in non-neuronal cells, recent evidence has suggested the existence of a novel uptake process for amines in postsynaptic neurones. Primary hypothalamic cell cultures and a cell line of hypothalamic neurones were found to accumulate amines by a desipramine-blockable process (Al-Damluji & Krsmanovic, 1992; Al-Damluji *et al.*, 1993). These cells accumulate amines which are present in nanomolar concentrations in the extracellular space by an energy-dependent process that is linked to a vacuolar-type ATPase (V-ATPase) and requires an electrochemical gradient of protons for its source of energy (Al-Damluji & Kopin, 1996). Uptake is due to a carrier which is activated by increasing concentrations of prazosin, resulting in a paradoxical increase in the apparent binding of [^{3}H]-prazosin. While it resembles the presynaptic plasma membrane transporters in that it is blocked by desipramine, it differs from these transporters by its independence of sodium and reliance on protons for a source of energy. Uptake of prazosin differs from the vesicular transporters by its insensitivity to reserpine and blockade by antidepressants (Al-Damluji & Kopin, 1996). Thus, this carrier is distinguishable from other neuronal transporters both by its anatomical location and by its functional properties. Uptake of prazosin is insensitive to steroid hormones and is not absolutely dependent on sodium ions (Al-Damluji & Kopin, 1996), which distinguishes it from non-neuronal uptake processes, such as uptake$_2$ in myocytes and the uptake process in pulmonary endothelial cells (Salt, 1972; Bryan-Lluka *et al.*, 1992).

The postsynaptic uptake process is a site of action of drugs which act on α_1-adrenoceptors (Al-Damluji *et al.*, 1993). We suggested that the physiological significance of postsynaptic uptake may be to remove neurotransmitter from the vicinity of postsynaptic receptors, thus preventing desensitization of the receptors and maintaining the responsiveness of postsynaptic neurones to repeated bursts of neurotransmitter released from presynaptic nerve terminals. In contrast, the presynaptic transporters are presumably less effective in removing neurotransmitter from postsynaptic receptors, as they would rely on diffusion of neurotransmitter back across the synapse, against its concentration gradient (Al-Damluji *et al.*, 1993). Clearly, a concentration gradient must exist across the synapse to enable forward diffusion of the transmitter.

Hypothalamic peptidergic neurones are innervated by noradrenergic nerve terminals and noradrenaline plays an important role in regulating the physiological functions of these cells (for review, see Al-Damluji, 1993). Tricyclic antidepressants are known to inhibit the uptake of amines in presynaptic nerve terminals. We have now studied the effects

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of a series of tricyclic antidepressants on this novel amine uptake process in peptidergic gonadotrophin-releasing hormone (GnRH) neurones.

Methods

Uptake assay

Immortalised GT1-1 GnRH neuronal cells were cultured as previously described in detail (Al-Damluji *et al.*, 1993). The cells were grown in culture medium consisting of Dulbecco's modified Eagle's medium (DMEM) and Ham's F-12 (ratio 1:1) with 10% foetal bovine serum in Costar 12-well plates at an initial density of 2×10^6 cells/well. Culture media were changed at 48-h intervals and experiments were carried out after four days in culture. Uptake studies were performed at 37°C, using unlabelled prazosin 10^{-6} M, unless otherwise indicated. The concentration of the radioligand was 2×10^{-9} M. The cells were incubated in the presence of the indicated drugs for 60 min, after which they were washed and cellular radioactivity was extracted and assayed as decribed previously (Al-Damluji *et al.*, 1993). Half-maximal inhibitory concentrations (IC_{50}) values were calculated both graphically and by the 'Allfit' programme which uses a four-parameter logistic equation (De Lean *et al.*, 1978). Similar values were obtained by the two methods. Potency was defined as the inverse of IC_{50}. Relative potencies of compounds were expressed in relation to desipramine, which was assigned a value of 100.

Binding assay

GT1-1 GnRH cells were grown in Costar 12-well plates at an initial density of 2×10^6 cells/well and experiments were carried out after four days in culture. Binding studies were performed at 37°C, using [^{3}H]-imipramine 10^{-9} M, with or without the indicated concentrations of unlabelled compounds. Drugs were dissolved in 'binding medium' consisting of DMEM with 25 mM HEPES (N-[2-hydroxyethyl]piperazine-N'-[2-ethanesulphonic acid]) and 0.5×10^{-3} M sodium ascorbate, pH 7.4. The cells were washed twice with 1 ml binding medium then incubated in 1 ml binding medium containing the indicated drugs for 60 min. At the end of the incubation period, the wells were placed on ice and the cells were washed twice with 1 ml volumes of ice-cold binding medium. The cells were then solubilized with 2 ml of a warm solution of 0.1% sodium dodecyl sulphate and 0.1 M NaOH. Aliquots (50 μl) were removed for protein assay and 10 ml of scintillation liquid (Hydrofluor) was then added to the cell extract, mixed and radioactivity was measured in a beta scintillation counter with an efficiency of 50%. Protein content was measured by the bicinchoninic acid modification of the biuret reaction (Smith *et al.*, 1985) using albumin standards and reagents supplied by Pierce (Rockford, Illinois, U.S.A.). The data are presented both as displacement curves and as Scatchard plots, which were calculated by the 'Ligand' curve-fitting programme (Munson & Rodbard, 1980). The binding affinity (K_D) and maximum binding capacity (B_{max}) were derived by the 'Ligand' programme. In the displacement curves, non-specific binding was defined as the remaining cellular radioactivity in the presence of excess unlabelled imipramine (10^{-4} M). In the Scatchard plots, non-specific binding reflects the optimum fit to the data (Munson & Rodbard, 1980). In four separate experiments, non-specific binding averaged 35% of total counts (Figure 2b).

Materials

[^{3}H]-prazosin (TRK.843; specific activity 73–82 Ci mmol^{-1}; batch numbers 28 and 39) was from Amersham. [^{3}H]-imipramine was from NEN-Dupont (NET-710; specific activity 85.7 Ci mmol^{-1}; lot no. 3043-122). Unlabelled compounds and culture media were from Sigma.

The data are expressed as the means ± s.e.mean. Error bars are not shown when they are smaller than the size of the symbol.

Results

[^{3}H]-prazosin (2×10^{-9} M) was accumulated in GnRH cells in a time-dependent manner, reaching equilibrium at approximately 30 min. In the presence of unlabelled prazosin (10^{-5} M), the GnRH cells, rather than displacing the isotopically labelled compound, accumulated greater amounts of [^{3}H]-prazosin (Figure 1). GnRH cells also accumulated [^{3}H]-imipramine and apparent equilibrium was reached by 30 min. However, addition of unlabelled imipramine 10^{-5} M reduced the amount of [^{3}H]-imipramine associated with the GnRH cells (Figure 1).

In the GnRH cells at equilibrium (60 min), [^{3}H]-prazosin (at 2.6×10^{-9} M) was displaced by unlabelled prazosin in concentrations of 10^{-9} to 10^{-7} M (B_o: 34,088 ± 908 d.p.m.; unlabelled prazosin 10^{-7} M: 25,248 ± 520 d.p.m.). However, at concentrations of unlabelled prazosin greater than 10^{-7} M, there was an increase in the accumulation of [^{3}H]-prazosin (Figure 2; unlabelled prazosin 10^{-6} M: 93,676 ± 2,442 d.p.m.). Imipramine 10^{-5} M reduced the accumulation of [^{3}H]-prazosin 2.6×10^{-9} M (B_o 23,372 ± 594 d.p.m.; Figure 2). Imipramine also abolished the increase of [^{3}H]-prazosin at concentrations of unlabelled prazosin greater than 10^{-7} M; in the presence of imipramine, only displacement of [^{3}H]-prazosin by unlabelled prazosin was seen (Figure 2). Desipramine and amitriptyline had similar effects to imipramine (Figure 3).

Unlabelled imipramine displaced [^{3}H]-imipramine (at 1.7×10^{-9} M) from GnRH cells and there was no increase of the radioligand at high concentrations of unlabelled imipramine (Figure 2). [^{3}H]-imipramine was displaced by unlabelled imipramine (K_D 8.4×10^{-6} M; B_{max} 7.9×10^{-10} mol mg^{-1} protein), desipramine (K_D 4.7×10^{-6} M; B_{max} 5.1×10^{-10} mol mg^{-1}), nortriptyline (K_D 9.7×10^{-6} M; B_{max} 1.2×10^{-9} mol mg^{-1}) and amitriptyline (K_D 1.5×10^{-5} M; B_{max} 1.9×10^{-9} mol mg^{-1}). Scatchard plots revealed that the displacement curves could be fitted to a single site model for each of the four antidepressants (Figure 4).

In GnRH cells, the tricyclic antidepressants inhibited the accumulation of prazosin (10^{-6} M) with the following order of potency: imipramine (IC_{50} 10^{-6} M) > desipramine (1.2×10^{-6} M) > amitriptyline (1.6×10^{-6} M) > nortriptyline

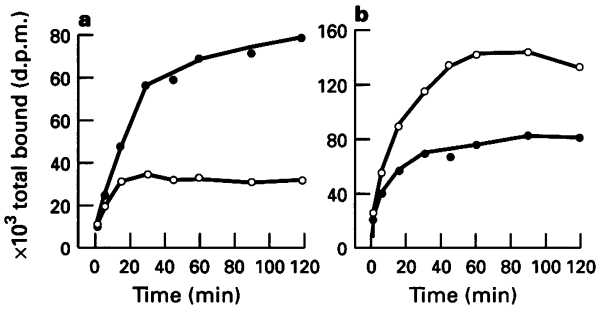

Figure 1 Effects of unlabelled amines on the accumulation of ^{3}H-labelled amines in GT1-1 GnRH cells: comparison of prazosin and imipramine. (a) Time course of the accumulation of prazosin in GT1-1 GnRH cells. The cells were incubated at 37°C with [^{3}H]-prazosin 2×10^{-9} M, with (●) or without (○) unlabelled prazosin 10^{-5} M. (b) Time course of the accumulation of imipramine in GT1-1 GnRH cells. The cells were incubated with [^{3}H]-imipramine 2×10^{-9} M, with (●) or without (○) unlabelled imipramine 10^{-5} M. Addition of unlabelled prazosin caused a greater amount of [^{3}H]-prazosin to be associated with the cells. In contrast, addition of unlabelled imipramine displaced [^{3}H]-imipramine and did not cause an increase in the binding of [^{3}H]-imipramine to the cells.

(2.0×10^{-6} M; Figure 5). The inhibitory effect of imipramine could be reversed by increasing concentrations of prazosin in the range 10^{-6} to 3×10^{-6} M (Figure 5).

Discussion

We found that hypothalamic peptidergic neurones possess specific binding sites for tricyclic antidepressants, and that these compounds competitively inhibit amine accumulation in the cells. These effects of antidepressants at postsynaptic neurones extend our previous findings on desipramine (Al-Damluji *et al.*, 1993), and represent an additional potential site of action of antidepressant compounds.

In the GnRH cells, [^{3}H]-prazosin 2×10^{-9} was displaced by unlabelled prazosin in concentrations of 10^{-9} to 10^{-7} M and the paradoxical increase was evident at higher concentrations of unlabelled prazosin (Figures 1 and 2). In the presence of imipramine, only displacement of [^{3}H]-prazosin by unlabelled prazosin was seen, presumably representing inhibition of binding of [^{3}H]-prazosin to α_1-adrenoceptors in the GnRH cells (Al-Damluji *et al.*, 1993). Imipramine inhibited the accumulation of [^{3}H]-prazosin 2×10^{-9} M (Figure 2), indicating that uptake takes place at these low extracellular concentrations of prazosin. Although imipramine may bind to α_1-adre-

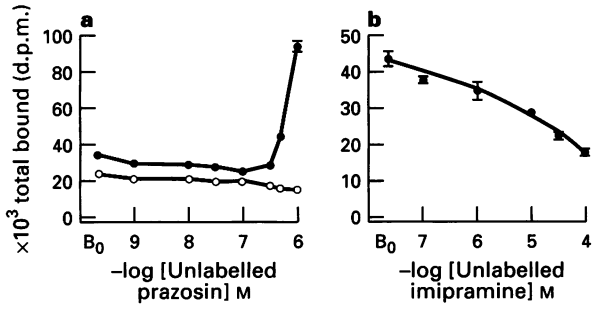

Figure 2 (a) Effect of imipramine on [^{3}H]-prazosin (2.6×10^{-9} M) binding in GT1-1 GnRH cells. In the control experiment (●) displacement of the radioligand at equilibrium (60 min) was observed at unlabelled prazosin concentrations of 10^{-9} to 10^{-7} M. At concentrations of unlabelled prazosin greater than 10^{-7} M, there was a paradoxical increase in the apparent binding of [^{3}H]-prazosin. In the presence of imipramine 10^{-5} M (○), the paradoxical effect was abolished and only displacement was seen. (b) Displacement of [^{3}H]-imipramine 1.7×10^{-9} M (●) by unlabelled imipramine in GT1-1 GnRH cells. Only displacement of the radioligand by the unlabelled compound is seen, indicating the presence of specific binding sites in these cells. The data represent total bound d.p.m.s.

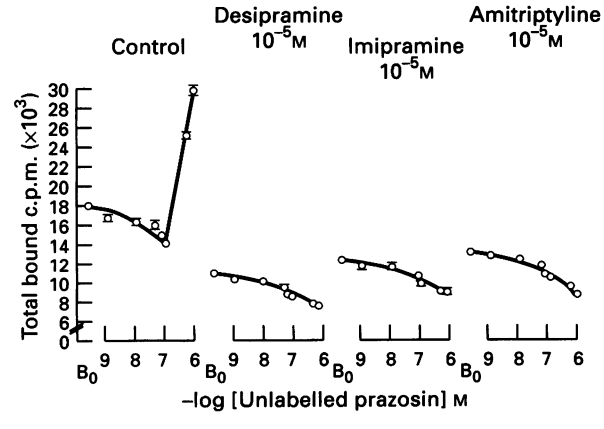

Figure 3 Effects of tricyclic antidepressant compounds on [^{3}H]-prazosin binding in GT1-1 GnRH cells. In the control experiment, displacement of the radioligand at equilibrium (60 min) was observed at unlabelled prazosin concentrations of 10^{-9} to 10^{-7} M. At concentrations of unlabelled prazosin greater than 10^{-7} M, there was a paradoxical increase in the apparent binding of [^{3}H]-prazosin. In the presence of tricyclic compounds, the paradoxical effect was abolished and only displacement was seen.

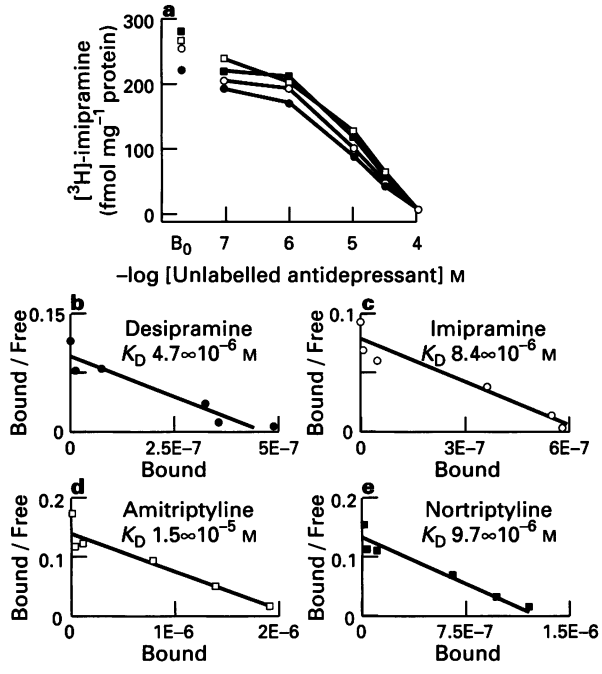

Figure 4 (a) Displacement of [^{3}H]-imipramine 1.6×10^{-9} M by unlabelled tricyclic antidepressants in GT1-1 GnRH cells: (●) desipramine; (○) imipramine; (□) amitriptyline; (■) nortriptyline. The data represent specific binding expressed as fmol [^{3}H]-imipramine mg^{-1} protein. Non-specific binding was defined as the remaining d.p.m. in the presence of unlabelled imipramine 10^{-4} M, and represented 35% of total bound d.p.m. (see Figure 2b). (b, c, d, e) Scatchard plots of the displacement curves in the upper panel. The data fit a single binding site model for tricyclic antidepressants in GnRH cells.

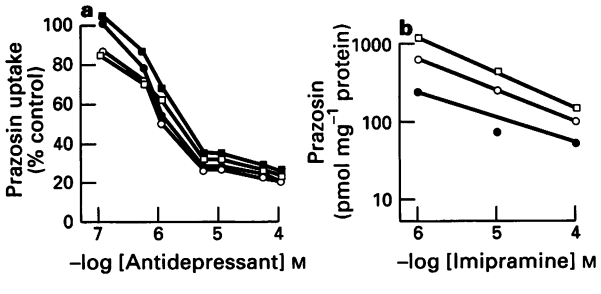

Figure 5 (a) Inhibition of the uptake of prazosin by tricyclic antidepressants in GT1-1 GnRH cells. The cells were incubated with [^{3}H]-prazosin 2×10^{-9} M and unlabelled prazosin 10^{-6} M, with or without the indicated concentrations of the tricyclic antidepressants: (●) desipramine; (○) imipramine; (□) amitryptaline; (■) nortriptyline. (b) Reversal of the inhibitory effect of imipramine by increasing concentrations of the substrate (prazosin): (□) 3×10^{-6} M; (○) 2×10^{-6} M; (●) 10^{-6} M; indicating that the inhibitory effect of imipramine is competitive.

noceptors (U'Prichard *et al.*, 1978), the inhibitory effect of imipramine on the uptake of [^{3}H]-prazosin 2×10^{-9} M is unlikely to represent displacement from α_1-adrenoceptors, as displacement of [^{3}H]-prazosin by unlabelled prazosin is evident in the presence of imipramine (Figures 2 and 3). Imipramine also abolished the paradoxical increase in the binding of [^{3}H]-prazosin at concentrations of unlabelled prazosin greater than 10^{-7} M (Figure 2), confirming that this increase is due to cellular uptake of the radioligand (Al-Damluji *et al.*, 1993). The inhibitory effect of imipramine on the uptake of prazosin 10^{-6} M is unlikely to represent displacement from α_1-adrenoceptors; at a concentration of 10^{-6} M unlabelled prazosin, [^{3}H]-prazosin is completely displaced from α_1-adrenoceptors (Al-Damluji & Kopin, 1996). The action of imipramine therefore represents inhibition of uptake, rather than inhibition of binding to α_1-adrenoceptors.

Prazosin and imipramine appear to be substrates for the same uptake process in GnRH cells. Thus, [^{3}H]-prazosin and [^{3}H]-imipramine were accumulated by GnRH cells by a desipramine-sensitive process (Figures 4 and 5) and imipramine inhibited the uptake of prazosin 10^{-6} M in these cells (Figure 5). Further, the inhibitory effect of imipramine could be reversed by increasing concentrations of prazosin (Figure 5), indicating that blockade of uptake by tricyclic antidepressants is competitive. This competitive action suggests that prazosin and the antidepressants may act on the same transporter molecule in the GnRH cells. In presynaptic noradrenergic nerve terminals, tricyclic antidepressants inhibited the uptake of noradrenaline in a competitive manner (Maxwell *et al.*, 1969; 1974), and the tricyclics and noradrenaline were subsequently shown to act on the same cloned transporter molecule (Giros *et al.*, 1994; Pacholczyk *et al.*, 1991).

Although imipramine and prazosin are likely to act on the same site in the GnRH cells, unlabelled imipramine did not increase the binding of [^{3}H]-imipramine (Figures 1 and 2). Imipramine is believed to bind to presynaptic plasma membrane neuronal amine transporters but is not internalized by these neurones (Graefe & Bonisch, 1989). It seems possible that imipramine may interact with the uptake process in postsynaptic neurones in a similar manner. Imipramine is known to dissolve in lipid bilayers by a process that is not saturable in concentrations up to 7×10^{-4} M (Romer & Bickel, 1979). In contrast, [^{3}H]-imipramine bound to GnRH cells in nanomolar concentrations and was displaced by low concentrations of unlabelled antidepressants (Figure 4). This indicated the presence of specific, saturable antidepressant binding sites which are presumably the transporter molecules that are responsible for the uptake of prazosin in GnRH cells.

It is difficult to compare the potencies of antidepressants in inhibiting transport-P to their potencies in inhibiting other neuronal processes. This is because transport-P differs from other neuronal transporters and receptors in that it is activated by its substrate. Thus, the IC_{50} of imipramine for inhibiting the uptake of prazosin depends on the concentration of prazosin in the extracellular space (Figure 5). Nevertheless, it is possible to compare the concentrations of antidepressants that are observed *in vivo* to the concentrations that were required to inhibit the uptake of prazosin *in vitro*. The recommended therapeutic plasma concentrations for antidepressant treatment with imipramine (0.9–1.8 μM), amitriptyline (0.1–1.0 μM), desipramine (0.4–0.7 μM) and nortriptyline (0.2–0.6 μM; DeVane & Jarecke, 1992; Gram *et al.*, 1984) are within the range of concentrations in which these compounds inhibit the uptake of prazosin 10^{-6} M in GnRH cells (0.1–5.0 μM; Figure 5). In rats, brain concentrations of antidepressants are over 10 fold greater than their concentrations in plasma (DeVane & Jarecke, 1992). Experiments in rats have suggested that the postsynaptic uptake process may exist in hypothalamic peptidergic neurones (Al-Damluji *et al.*, 1993). It therefore seems possible that in the doses used *in vivo*, the antidepressants may influence the function of transport-P.

We examined four compounds which have been in clinical use as antidepressants: a secondary amine and a tertiary amine dihydrodibenzazepine (desipramine and imipramine, respectively) and a secondary amine and a tertiary amine dibenzocycloheptadene (nortriptyline and amitriptyline). The dihydrodibenzazepines differ from the dibenzocycloheptadenes by the presence of a nitrogen atom at the apex of the heterocyclic ring. The nitrogen atom is connected to the side chain by a single bond, which is believed to result in greater flexibility of the side chain (Maxwell & White, 1978). Table 1 summarizes the effects of these structural features on the interaction of these antidepressants with transport-P and with other pharmacological actions of these four antidepressants.

The tertiary amine antidepressants (imipramine and amitriptyline) were 25% more potent than their secondary amine

Table 1 Effects of the presence of a tertiary alkyl amine and of a heterocyclic nitrogen atom on the pharmacological activities of tricyclic antidepressants

Activity	*Tertiary NH_2*	*Heterocyclic N*	*Reference*
Transport-P	Enhances 0.25 fold	Enhances 0.6 fold	a
NA uptake	Reduces 4–15 fold	Enhances 1–3 fold	b,c
DA uptake	No effect	Reduces 0.5–1.9 fold	b
5-HT uptake	Enhances 0.4–3.2 fold	No effect	b,d
Histamine release	Enhances 0.25–0.6 fold	Reduces 0.15–0.5 fold	e
H_1 receptor blockade	Enhances 39–59 fold	Reduces 32–49 fold	f
H_2 receptor blockade	Enhances 1–3 fold	Reduces 0.14–1 fold	g
Muscarinic receptor blockade	Enhances 1–9 fold	Reduces 2–7 fold	f
Nicotinic ion channel	Reduces 1–1.3 fold	No effect	h
α_1-Adrenoceptor blockade	Enhances 1.6–2 fold	Reduces 1–1.4 fold	i
D_1 dopamine receptor blockade	Enhances 1.5–2 fold	Reduces 18–22 fold	j

[a]Present study; [b]Koe, 1976; rat hypothalamic (NA) and striatal (DA and 5-HT) synaptosomes; [c]Pacholczyk *et al.*, 1991; human noradrenaline transporter cDNA; [d]Barker *et al.*, 1994; human and rat 5-HT transporter cDNAs; [e]Frisk-Holmberg & Kleijn, 1972; rat mast cells; [f]Richelson, 1978; mouse neuroblastoma cells; [g]Kanof & Greengard, 1978; guinea-pig brain; [h]Aronstam, 1981; electric organ of *Torpedo ocellata*; [i]U'Prichard *et al.*, 1978; rat brain homogenates; [j]Faedda *et al.*, 1989; rat striatal homogenates.

The table compares the effects of these structural modifications on the inhibition by tricyclic compounds of the uptake or release of the amines prazosin (transport-P), noradrenaline (NA), dopamine (DA), 5-hydroxytryptamine (5-HT) and histamine. It also analyses the effects of these modifications on the blockade by tricyclic compounds of H_1 and H_2 histamine, muscarinic, nicotinic and D_1 dopamine receptors and α_1-adrenoceptors. The analysis is based on the four tricyclic antidepressants that were used in the present study (imipramine, desipramine, amitriptyline and nortriptyline). References are representative of published literature and are not intended to be comprehensive.

analogues (desipramine and nortriptyline) in inhibiting the uptake of prazosin in GnRH cells. In most aspects of their pharmacological activities, tertiary amine tricyclics are more potent than secondary amines (Table 1). As the tertiary amine tricyclics are more hydrophobic (Moffat, 1986), these findings have been assumed to be due to the presence of hydrophobic regions in the vicinity of the amine-binding sites in the transporter and receptor molecules. Exceptions to this rule are inhibition of the uptake of dopamine, in which the configuration of the amine is unimportant, and inhibition of the uptake of noradrenaline, in which secondary amines are more potent than tertiary amines (Table 1). The latter has led to the assumption that the amine-binding site of the noradrenaline transporter may lie within a relatively hydrophilic pocket (Maxwell & White, 1978).

The dihydrodibenzazepines were 60% more potent than their dibenzocycloheptadene analogues, suggesting that the presence of a heterocyclic nitrogen enhances blocking activity. Similar results have been described for inhibition of the uptake of noradrenaline in rat brain and rabbit aorta (Table 1). The presence of a heterocyclic nitrogen has no effect on the potency of these compounds in inhibiting the uptake of 5-hydroxytryptamine, but in most of their other actions, a heterocyclic nitrogen reduces the potency of tricyclic antidepressants (Table 1).

It is difficult to compare the potency of the compounds in blocking uptake to their potency in displacing [^{3}H]-imipramine, because potency of uptake blockade is dependent on the concentration of prazosin. Examination of the relative potencies of the antidepressants in inhibiting the binding of [^{3}H]-imipramine reveals that as for inhibition of the uptake of prazosin, the dihydrodibenzazepines (imipramine and desipramine) were 80–100% more potent than their respective dibenzocycloheptadene analogues (Figure 4). This confirms that the heterocyclic nitrogen increases the potency of these compounds. However, unlike the inhibition of prazosin uptake, secondary amines (desipramine and nortriptyline) were more potent than tertiary amines in displacing [^{3}H]-imipramine (Figure 4). The reason for this discrepancy is unclear.

The four tricyclic compounds which were used in this study are the prototypes of drugs which have antidepressant activity. Their therapeutic efficacy has been established in double-blind, placebo-controlled clinical trials over many years (Morris & Beck, 1974). These compounds and their newer analogues are the main therapeutic agents for the treatment of endogenous depression (Montgomery, 1994). Although several hypotheses have been proposed for the biochemical basis of depression and the mechanism of the therapeutic effect of tricyclic compounds, none is completely compatible with the pharmacological data on the antidepressants (for a recent review, see Leonard, 1993). The hypothesis that has attracted most attention is that depression is caused by a deficiency of amines in the extracellular space, and that tricyclic compounds exert their therapeutic effect by inhibiting the presynaptic uptake of amines, resulting in an increase in extracellular amine concentrations (Schildkraut, 1965). However, objections to this hypothesis have been raised (Anonymous, 1982; Carlsson, 1984; Leonard, 1993). The known pharmacological actions of the antidepressants are believed to explain the various clinical untoward effects of these compounds, but they do not correlate with their therapeutic efficacy (Morris & Beck, 1974; Baldessarini, 1990). Endogenous depression is characterized by derangements of hypothalamic function, including disturbances of pituitary hormone secretion, sleep pattern, appetite and reproductive function. Our finding of a postsynaptic amine uptake site provides a novel alternative for investigating the mechanism of action of tricyclic compounds, particularly regarding their effects on hypothalamic function.

References

AL-DAMLUJI, S. (1993). Adrenergic control of the secretion of anterior pituitary hormones. *Bailliere's Clinical Endocrinology and Metabolism*, vol. 7, part 2, pp. 355–392.

AL-DAMLUJI, S. & KOPIN, I.J. (1996). Functional properties of the uptake of amines in immortalised peptidergic neurones (Transport-P). *Br. J. Pharmacol.*,**117**, 111–118.

AL-DAMLUJI, S. & KRSMANOVIC, L. (1992). High-affinity uptake of noradrenaline by GnRH cells. *Abst. Endocr. Soc.*, **74**, 197.

AL-DAMLUJI, S., KRSMANOVIC, L. & CATT, K.J. (1993). High-affinity uptake of noradrenaline in post-synaptic neurones. *Br. J. Pharmacol.*, **109**, 299–307.

ANONYMOUS (1982). Alpha-2 adrenergic receptors in depression. *Lancet*, **i**, 781.

ARONSTAM, R.S. (1981). Interactions of tricyclic antidepressants with a synaptic ion channel. *Life Sci.*, **28**, 59–64.

AXELROD, J. (1971). Noradrenaline: fate and control of its biosynthesis. *Science*, **173**, 598–606.

BALDESSARINI, R.J. (1990). Drugs and the treatment of psychiatric disorders. In *Pharmacological Basis of Therapeutics*. ed. Gilman, A.G., Rall, T.W., Nies, A.S. & Taylor, P. pp. 405–435. New York: Pergamon Press, Eighth edition.

BARKER, E.L., KIMMEL, H.L. & BLAKELY, R.D. (1994). Chimeric human and rat serotonin transporters reveal domains involved in recognition of transporter ligands. *Mol, Pharmacol.*, **46**, 799–807.

BOUVIER, M., SZATKOWSKI, M., AMATO, A. & ATTWELL, D. (1992). The glial cell glutamate uptake carrier countertransports pH-changing anions. *Nature*, **360**, 471–474.

BRYAN-LLUKA, L.J., WESTWOOD, N.N. & O'DONNELL, S.R. (1992). Vascular uptake of catecholamines in perfused lungs of the rat occurs by the same process as uptake$_1$ in noradrenergic neurones. *Naunyn-Schmied. Arch. Pharmacol.*, **345**, 319–326.

CARLSSON, A. (1984). Current theories on the mode of action of antidepressant drugs. *Adv. Biochem. Psychopharmacol.*, **39**, 213–211.

DE LEAN, A., MUNSON, P.J. & RODBARD, D. (1978). Simultaneous analysis of families of sigmoidal curves: application to bioassay, radioligand assay, and physiological dose-response curves. *Am. J. Physiol.*, **235**, E97–E102.

DEVANE, C.L. & JARECKE, C.R. (1992). Cyclic antidepressants. In *Applied Pharmacokinetics*. ed. Evans, E.E., Schentag, J.J., Jusko, W.J. & Relling, M.V. pp. 33-1 to 33-47. Vancouver: Applied Therapeutics, Inc. Third edition.

FAEDDA, G., KULA, N.S. & BALDESSARINI, R.J. (1989). Pharmacology of binding of ^{3}H-SCH-23390 to D-1 dopaminergic receptor sites in rat striatal tissue. *Biochem. Pharmacol.*, **38**, 473–480.

FRISK-HOLMBERG, M. & KLEIJN, E.V.D. (1972). The relationship between the lipophilic nature of tricyclic neuroleptics and antidepressants, and histamine release. *Eur. J. Pharmacol.*, **18**, 139–147.

GIROS, B., WANG, Y.M., SUTER, S., MCLESKEY, S.B., PIFL, C. & CARON, M.G. (1994). Delineation of discrete domains for substrate, cocaine, and tricyclic antidepressant interactions using chimeric dopamine-norepinephrine transporters. *J. Biol. Chem.*, **269**, 15985–15988.

GRAEFE, K.H. & BONISCH, H. (1989). The transport of amines across axonal membranes of noradrenergic and dopaminergic neurones. In *Handbook Exp. Pharmacol.*, ed. Trendelenburg, U. & Weiner, N. 90 (Part 1), pp. 193–245. Berlin: Springer-Verlag.

GRAM, L.F., KRAGH-SORENSEN, P., KRISTENSEN, C.B., MOLLER, M., PEDERSEN, O.L. & THAYSSEN, P. (1984). Plasma level monitoring of antidepressants: theoretical basis and clinical application. *Adv. Biochem. Psychopharmacol.*, **39**, 399–411.

HENN, F.A. & HAMBERGER, A. (1971). Glial cell function: uptake of transmitter substances. *Proc. Natl. Acad. Sci. U.S.A.*, **68**, 2686–2690.

HOSLI, L. & HOSLI, E. (1978). Action and uptake of neurotransmitters in CNS tissue culture. *Rev. Physiol. Biochem. Pharmacol.*, **81**, 135–188.

IVERSEN, L.L. (1965). The uptake of catechol amines at high perfusion concentrations in the rat isolated heart: a novel catechol amine uptake process. *Br. J. Pharmacol.*, **25,** 18–33.

IVERSEN, L.L. (1967). The Uptake and Storage of noradrenaline in sympathetic nerves. Cambridge: University Press.

KANOF, P.D. & GREENGARD, P. (1978). Brain histamine receptors as targets for antidepressant drugs. *Nature*, **272,** 329–333.

KIMELBERG, H.K. & PELTON, E.W. (1983). High-affinity uptake of [^{3}H]norepinephrine by primary astrocyte cultures and its inhibition by tricyclic antidepressants. *J. Neurochem.*, **40,** 1265–1270.

KOE, B.K. (1976). Molelcular geometry of inhibitors of the uptake of catecholamines and serotonin in synaptosomal preparations of rat brain. *J. Pharmacol. Exp. Ther.*, **199,** 649–661.

LEONARD, B.E. (1993). The comparative pharmacology of new antidepressants. *J. Clin. Psychiatry*, **54,** (suppl.), 3–15.

LESTER, H.A., MAGER, S., QUICK, M.W. & COREY, J.L. (1994). Permeation properties of neurotransmitter transporters. *Annu. Rev. Pharmacol. Toxicol.*, **34,** 219–249.

MAXWELL, R.A., FERRIS, R.M., BURCSU, J., WOODWARD, E.C., TANG, D. & WILLIARD, K. (1974). The phenyl rings of tricyclic antidepressants and related compounds as determinants of the potency of inhibition of the amine pumps in adrenergic neurons of the rabbit aorta and in rat cortical synaptosomes. *J. Pharmacol. Exp. Ther.*, **191,** 418–430.

MAXWELL, R.A., KEENAN, P.D., CHAPLIN, E., ROTH, B. & ECKHARDT, S.B. (1969). Molecular features affecting the potency of tricyclic antidepressants and structurally related compounds as inhibitors of the uptake of tritiated norepinephrine by rabbit aortic strips. *J. Pharmacol. Exp. Ther.*, **166,** 320–329.

MAXWELL, R.A. & WHITE, H.L. (1978). Tricyclic and monoamine oxidase inhibitor antidepressants: structure-activity relationships. In *Handbook of Psychopharmacology*. ed. Iversen, L.L., Iversen, S.D. & Snyder, S.H. London: Plenum Press. Volume **14,** pp. 83–155.

MOFFAT, A.C. (1986). Clarke's isolation and identification of drugs. London: The Pharmaceutical Press.

MONTGOMERY, S.A. (1994). Antidepressants in long-term treatment. *Annu. Rev. Med.*, **45,** 447–457.

MORRIS, J.B. & BECK, A.T. (1974). The efficacy of antidepressant drugs. *Arch. Gen. Psychiatry*, **30,** 667–674.

MUNSON, P.J. & RODBARD, D. (1980). LIGAND: a versatile computerized approach for characterization of ligand-binding systems. *Analyt. Biochem.*, **107,** 220–239.

PACHOLCZYK, T., BLAKELY, R.D. & AMARA, S.G. (1991). Expression-cloning of a cocaine- and antidepressant-sensitive human noradrenaline transporter. *Nature*, **350,** 350–354.

PINES, G., DANBOLT, N.C., BJORAS, M., ZHANG, Y., BENDAHAN, A., EIDIE, L., KEOPSELL, H., STORM-MATHISEN, J., SEBERG, E. & KANNER, B.I. (1992). Cloning and expression of a rat brain L-glutamate transporter. *Nature*, **360,** 464–467.

RICHELSON, E. (1978). Tricyclic antidepressants block histamine H_1 receptors of mouse neuroblastoma cells. *Nature*, **274,** 176–177.

ROMER, J. & BICKEL, M.H. (1979). Interactions of chlorpromazine and imipramine with artificial membranes investigated by equilibrium dialysis, dual-wavelength photometry, and fluorimetry. *Biochem. Pharmacol.*, **28,** 799–805.

SALT, P.J. (1972). Inhibition of noradrenaline uptake in the isolated rat heart by steroids, clonidine and methoxylated phenylethylamines. *Eur. J. Pharmacol.*, **20,** 329–340.

SCHILDKRAUT, J.J. (1965). The catecholamine hypothesis of affective disorders. *Am. J. Psychiatry*, **122,** 509–522.

SMITH, P.K., KROHN, R.I., HERMANSON, G.T., MALLIA, A.K., GARTNER, F.H., PROVENZANO, M.D., FUJIMOTO, E.K., GOEKE, N.M., OLSON, B.J. & KLENK, D.C. (1985). Measurement of protein using bicinchoninic acid. *Anal. Biochem.*, **150,** 76–85.

STORCK, T., SCHULTE, S., HOFMANN, K. & STOFFEL, W. (1992). Structure, expression and functional analysis of a Na^+-dependent glutamate/aspartate transporter from rat brain. *Proc. Natl. Acad. Sci. U.S.A.*, **89,** 10955–10959.

U'PRICHARD, D.C., GREENBERG, D.A. SHEEHAN, P.P. & SNYDER, S.H. (1978). Tricyclic antidepressants: therapeutic properties and affinity for alpha noradrenergic receptor binding sites in the brain. *Science*, **199,** 197–198.

(Received September 5, 1995
Revised October 27, 1995
Accepted October 31, 1995)