Br. J. Pharmac. (1986), **89**, 673–683

Activation and desensitization of presynaptic α_2-adrenoceptors after inhibition of neuronal uptake by antidepressant drugs in the rat vas deferens

J.A. García-Sevilla[1] & J.K. Zubieta

Department of Pharmacology, Faculty of Medicine, University of the Basque Country, Leioa, Vizcaya, Spain

1 The isolated field-stimulated vas deferens of the rat (0.1 Hz, 3 ms, 30–40 V) was used to study the relationship between the *in vivo* inhibition of neuronal uptake of noradrenaline (NA) by cyclic antidepressant drugs and the subsequent activation/desensitization of presynaptic α_2-adrenoceptors. Receptor activation was indirectly measured by quantifying the ability of each drug to inhibit basal twitch responses after their acute administration. Receptor desensitization was also indirectly measured by quantifying the ability of the drugs to reduce the inhibitory effects of selective α_2-adrenoceptor agonists on the electrically-induced twitch responses after their long-term administration.

2 The acute *in vivo* administration of desipramine and other antidepressants (0.5–10 mg kg^{-1}; i.p.; 2 h) resulted in dose-dependent inhibitions of the basal twitch responses which were rapidly reversed to control values by idazoxan (10^{-5} M). *In vitro*, desipramine and other antidepressants also inhibited in a concentration-dependent manner (10^{-9}–10^{-5} M) the twitch responses. In rats pretreated 12 h earlier with reserpine (1 mg kg^{-1}; i.p.) or oxypertine (4 mg kg^{-1}; i.p.), desipramine (10 mg kg^{-1}; 2 h) did not induce inhibition of the basal twitch responses or it induced a smaller effect, respectively.

3 For the various antidepressants the degree of inhibition of the basal twitch responses (desipramine > protriptyline > nortriptyline > maprotiline = imipramine > amitriptyline > viloxazine > iprindole ➤ zimelidine) was highly correlated ($r = 0.914$) with the potency for blockade of [^{3}H]-NA uptake into rat brain synaptosomes.

4 Clonidine and xylazine inhibited in a concentration-dependent manner (10^{-9}–10^{-6} M) the twitch responses. The long-term (7–14 days) administration of antidepressants or cocaine (10 mg kg^{-1}, i.p.) resulted in significant decreases in sensitivity to clonidine or xylazine. Short-term (3 days) treatment with desipramine did not reduce the sensitivity to clonidine.

5 The results indicate that the acute *in vivo* inhibition of NA neuronal uptake by antidepressants leads to the activation (through endogenous NA) of presynaptic inhibitory α_2-adrenoceptors which results in inhibition of the twitch responses. In contrast, prolonged *in vivo* inhibition of NA reuptake is followed by a slow desensitization process of the same receptors which results in a reduction of sensitivity to clonidine.

Introduction

Inhibition of neuronal uptake is an early and major biochemical effect by which most cyclic antidepressant drugs appear to initiate their therapeutic actions (Schildkraut, 1965; Richelson & Pfenning, 1984). However, there is no temporal relationship between the onset (weeks) of clinical action of these drugs and their effects (minutes) on neurotransmitter reuptake systems (Oswald *et al.*, 1972; Manias & Taylor, 1983). This lack of temporal relationship suggests that in addition to inhibition of neuronal uptake, other molecular processes are involved in the mechanism of action of cyclic antidepressant drugs.

Presynaptic inhibitory α_2-adrenoceptors play an important physiological role in the regulation of transmitter release from noradrenergic nerve terminals (Langer, 1981; Starke, 1981). Biochemical and functional studies have suggested that endogenous depression is related to α_2-adrenoceptor supersen-

[1] Author for correspondence: Departamento de Farmacología y Terapéutica, Facultad de Medicina, Universidad del País Vasco, Leioa, Vizcaya, Spain.

sitivity (García-Sevilla *et al.*, 1981; 1986) and desensitization of these inhibitory receptors also has been involved in the mechanism of action of cyclic antidepressant drugs (Crews & Smith, 1978; 1980). Thus, long-term but not acute administration of cyclic antidepressant drugs has been associated with a decrease in the number or sensitivity of central and peripheral α_2-adrenoceptors both in animals (Svensson & Usdin, 1978; Smith *et al.*, 1981; Pilc & Vetulani, 1982; Smith & Hollingsworth, 1984; Finberg & Tal, 1985) and man (García-Sevilla *et al.*, 1981; Charney *et al.*, 1983).

Since inhibition of neuronal uptake of noradrenaline (NA) by cyclic antidepressant drugs results in the accumulation of this neurotransmitter in the synaptic cleft, the sensitivity of presynaptic inhibitory α_2-adrenoceptors could be indirectly modulated by these drugs. The isolated field-stimulated vas deferens of the rat was used as a model system to study the possible relationship between the inhibition of neuronal uptake of NA and the subsequent activation/desensitization of presynaptic α_2-adrenoceptors. This simple model was chosen because in addition to a high concentration of endogenous NA (Zieher & Jaim-Etcheverry, 1971) and a powerful reuptake mechanism for this neurotransmitter (Raisman *et al.*, 1982), the motor transmission in the vas deferens, independently of the adrenergic or non-adrenergic nature of the nerve terminals (Sneddon *et al.*, 1982; Farmer, 1985), is modulated by inhibitory presynaptic α_2-adrenoceptors (Drew, 1977; Illes & Dörge, 1985).

Using this peripheral model recent experimental work has focused on the desensitization process of presynaptic α_2-adrenoceptors that follows the long-term administration of antidepressant drugs (Finberg & Tal, 1985; Doxey *et al.*, 1985b). However, the possible early activation of these inhibitory receptors after the acute *in vivo* administration of cyclic antidepressant drugs has not been explored in detail (Svensson & Usdin, 1978; Sugrue, 1980; Doxey *et al.*, 1985b). *In vitro* these drugs activate presynaptic inhibitory α_2-adrenoceptors probably as a consequence of inhibition of neuronal uptake (Lotti *et al.*, 1981). The present study clearly demonstrates that cyclic antidepressant drugs, through inhibition of neuronal uptake, induce effects on presynaptic α_2-adrenoceptors that are opposite, depending on the duration of treatment. Thus, after the acute *in vivo* administration the receptors are more readily activated during trains of nerve impulses and this is followed by a slow desensitization process with long-term administration. This dual modulation of presynaptic α_2-adrenoceptors by cyclic antidepressant drugs could have relevant therapeutic implications.

Preliminary reports of this work were given at meetings of the Spanish and British Pharmacological Societies (Jaca Meeting, December 1984 and Edinburgh Meeting, September 1985, respectively) (García-Sevilla & Zubieta, 1985).

Methods

Preparation of vas deferens in vitro

Male Sprague-Dawley rats (280–330 g) were used. The animals received a standard diet with water freely available and were housed at 22 ± 2°C with a 12 h light/dark cycle. The rats were killed by cervical dislocation. The entire vasa deferentia were removed and carefully cleaned. The tissues were set up in 25 ml organ baths in Krebs bicarbonate medium containing (in mM): NaCl 112, KCl 4.7, $CaCl_2$ 2.5, KH_2PO_4 1.1, $MgSO_4$ 1.2, $NaHCO_3$ 25.0 and glucose 11.1. The solution was maintained at 31 ± 0.5°C and gassed with 95% O_2–5% CO_2. Tissues were placed under an initial resting tension of 0.5 g, and were washed every 10 min over a period of 30 min.

Tissues were continuously field-stimulated via two ring platinum electrodes mounted in parallel by use of a Cibertec model CS-14 stimulator. A square wave pulse of 3 ms duration and supramaximal voltage (30–40 V) at a frequency of 0.1 Hz, resulted in constant isometric tension changes (UF-1 transducer) which were recorded as individual contractile responses (twitches) of the vas deferens on a OmniScribe recorder. The preparation was stimulated for 20–30 min to allow for equilibration of the twitch responses before starting the experiments.

Experimental protocols, treatments and calculation of results

The acute *in vivo* inhibitory effects induced by various cyclic antidepressant drugs on the electrically-induced basal twitch responses were first investigated. Rats were treated with saline or various cyclic antidepressant drugs (0.5–10 mg kg^{-1}; i.p. or s.c.) for 0.5–48 h. After these periods of time, tissues were prepared and stimulated as above. When constant basal twitch responses were obtained, a maximal concentration of idazoxan (10^{-5} M) was added directly to the organ bath and the resulting enhancement of contraction was taken as the maximal twitch response of the preparation. The inhibitory effects induced by the cyclic antidepressant drugs on the basal twitch responses were measured (mg of developed tension) and expressed as % of maximal twitch (idazoxan) for comparisons.

In some experiments, rats were pretreated for 12 h with reserpine (1 mg kg^{-1} i.p.) or oxypertine (4 mg kg^{-1}, i.p.) before the acute *in vivo* effect of desipramine (10 mg kg^{-1}, i.p., 2 h) was assessed as described. In these experiments, vasa deferentia from control

(saline) and reserpine or oxypertine pretreated rats were also taken for biochemical determination of tissue NA content which was estimated by a h.p.l.c. method with electrochemical detection (Wagner *et al.*, 1982).

The acute *in vitro* inhibitory effects of representative cyclic antidepressant drugs on basal twitch responses were also investigated. For these experiments, cumulative concentration-response curves for desipramine (10^{-9}–10^{-6} M), maprotiline (10^{-8}–10^{-5} M) and zimelidine (10^{-7}–10^{-5} M) were obtained by geometric increases (× 3.3) of molar concentration of the drugs until a maximal inhibitory response was reached. IC_{50} values (IC_{50} being defined as the nM concentration of the drug necessary to inhibit the twitch response by 50%) were calculated by linear regression analysis of the $\log_{10}$ concentration-response curve by use of a computer programme.

Finally, the sensitivity of presynaptic α_2-adrenoceptors after the long-term administration of cyclic antidepressant drugs was investigated in the field-stimulated vas deferens. Rats were treated with saline or various cyclic antidepressant drugs (10 mg kg^{-1}, i.p.) every 24 h for 14 days and then were killed 24 h after the last injection. After this period of time, tissues were prepared and stimulated as above. Cumulative concentration-response curves for the inhibition of the twitch responses were obtained as described previously with clonidine (10^{-9}–10^{-7} M) or xylazine (10^{-8}–10^{-5} M) used as agonists. Comparisons of affinities were based on pD_2 values (pD_2 being defined as the negative $\log_{10}$ of the molar concentration of the agonist necessary to produce a response 50% of the maximal inhibition) which were calculated by linear regression analysis of the $\log_{10}$ concentration-response curve by use of a computer programme.

In another series of experiments, rats were treated with desipramine (10 mg kg^{-1}, i.p.) for 3, 7 and 14 days or for 14 days followed by discontinuation of treatment for 5–10 days. After these periods of treatment, pD_2 values for clonidine were determined as described above. In some experiments, rats were also treated with desipramine (10 mg kg^{-1}, i.p.) for 14 days and then were killed 2–24 h after the last injection for serial evaluation of basal twitch responses (mg of developed tension) and comparison with those obtained after the acute *in vivo* administration of the drug.

Statistics

Student's two-tailed *t*-test was used for the statistical evaluations. Correlation coefficients were calculated by the least squares method. $P = 0.05$ was chosen as the level of significance.

Drugs

The following drugs were used: desipramine HCl (USV laboratories, New York, U.S.A.); amitriptyline HCl and protriptyline HCl (Merck, Sharp & Dohme, Rahway, U.S.A.); maprotiline HCl and imipramine HCl (Ciba-Geigy, Barcelona, Spain); reserpine (Serpasol, Ciba-Geigy, Barcelona, Spain); viloxazine HCl (ICI-Farma, Barcelona, Spain); iprindole HCl (Wyeth Laboratories, Philadelphia, U.S.A.); zimelidine HCl (Astra Läkemedel, Södertajle, Sweden); oxypertine HCl (Sterling-Winthrop, New York, U.S.A.); idazoxan HCl (Reckitt & Colman, Hull); clonidine HCl (Boehringer Ingelheim, Barcelona, Spain); xylazine HCl (Bayer AG, Barcelona, Spain); cocaine HCl (Depósito de Estupefacientes, Ministerio de Sanidad ŷ Consumo, Madrid, Spain). All drugs were dissolved in glass-distilled water.

Results

Characterization of the inhibitory effect induced by cyclic antidepressant drugs upon the electrically-induced twitch responses

In control preparations basal twitch responses elicited by field stimulation for 20–30 min developed a constant tension of 850 ± 69 mg ($n = 10$). The addition to the bath of a maximal concentration of idazoxan (10^{-5} M), a selective α_2-adrenoceptor antagonist, intially enhanced the twitch responses of the vas deferens (20 ± 2%, $P < 0.05$) which declined rapidly to a final steady-state of 867 ± 60 mg not different from basal twitch responses (Figure 1, Table 1). These results indicated that the feed-back mechanism mediated through α_2-adrenoceptors was operative.

Under these experimental conditions, the acute *in vivo* administration of desipramine, maprotiline and zimelidine (10 mg kg^{-1}, i.p.or s.c., 2 h), three representative cyclic antidepressant drugs with respect to their potencies in inhibiting the neuronal uptake of NA (Richelson & Pfenning, 1984), resulted in different degrees of inhibition of the basal twitch responses (94 ± 1%, $P < 0.001$; 43 ± 4%, $P < 0.01$; and 6 ± 4%, NS; respectively) which were rapidly reversed by idazoxan (10^{-5} M) to steady-state twitch values not different from basal twitch responses of control preparations (Figure 1, Table 1). Therefore, the enhancement of contraction induced by idazoxan (10^{-5} M) was taken as the assumed basal twitch response of that particular vas deferens (i.e. each experimental preparation also was its own control) (Table 1). The inhibitory effect induced by desipramine (10 mg kg^{-1}, i.p., 2 h) was reversed by idazoxan in a concentration-dependent manner (10^{-9}–10^{-4} M) and the maximum potentiation of the

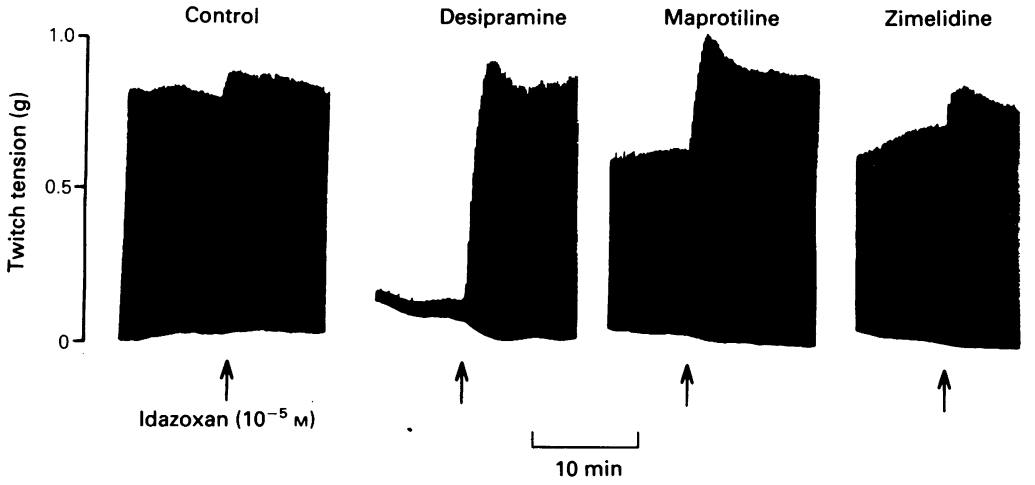

Figure 1 Recordings of the inhibition of the twitch response of the rat vas deferens induced by desipramine, maprotiline and zimelidine (10 mg kg^{-1}, i.p., 2 h) and their reversal by idazoxan (10^{-5} M). Ordinates: g of twitch tension developed.

Table 1 Effect of acute administration of antidepressants on basal twitch responses elicited by field-stimulation of the vas deferens

Drug	*Basal twitch* (mg)	*Maximal twitch* (mg)	*Basal twitch (% maximal)*
Control	850 ± 69	867 ± 60	97 ± 2
Desipramine	53 ± 5***	854 ± 66	6 ± 1***
Protriptyline	95 ± 10***	727 ± 82	13 ± 2***
Nortriptyline	258 ± 86***	620 ± 159	42 ± 7***
Maprotiline	481 ± 64**	837 ± 93	57 ± 4**
Imipramine	427 ± 79**	731 ± 97	57 ± 5**
Amitriptyline	423 ± 105**	678 ± 145	60 ± 3**
Viloxazine	609 ± 82*	958 ± 98	63 ± 3*
Iprindole	561 ± 63*	812 ± 92	69 ± 4*
Zimelidine	916 ± 93	981 ± 108	94 ± 4

Values are mean ± s.e. mean of 4–10 experiments. Doses of drugs: 10 mg kg^{-1}, i.p. Tissues removed 2 h after the injection. Maximal twitch induced by idazoxan (10^{-5} M).
*$P<0.05$, **$P<0.01$, ***$P<0.001$ when compared with control basal twitch (t test).

twitch was achieved with 10^{-5} M of the antagonist. Similar results were obtained in the presence of prazosin (10^{-6} M), indicating that the weak α_1-adrenoceptor antagonist properties of idazoxan (10^{-5} M) were not involved in this mechanism (data not shown). This rapid and complete reversal of the twitch responses by idazoxan indicated that the inhibitory effect induced by the cyclic antidepressant drugs was an α_2-adrenoceptor-mediated mechanism.

The inhibitory effect of a single dose of desipramine or maprotiline (10 mg kg^{-1}, i.p.) showed a distinctive time course with maximal twitch inhibitions between 0.5 and 5 h, followed by a slow recovery of the twitch responses towards control values by 12–48 h (Figure 2). In contrast, zimelidine (10 mg kg^{-1}, i.p.) did not induce any significant inhibitory effect upon the twitch responses at the same time intervals (Figure 2). The inhibitory effect induced by desipramine also was dose-dependent (0.5–10 mg kg^{-1}, i.p.) (Figure 3) and the 10 mg kg^{-1} dose that induced an almost complete supression of the twitch responses was taken as the standard dose in all subsequent experiments with the various cyclic antidepressant drugs. It should be mentioned that in rats treated with a dose of de-

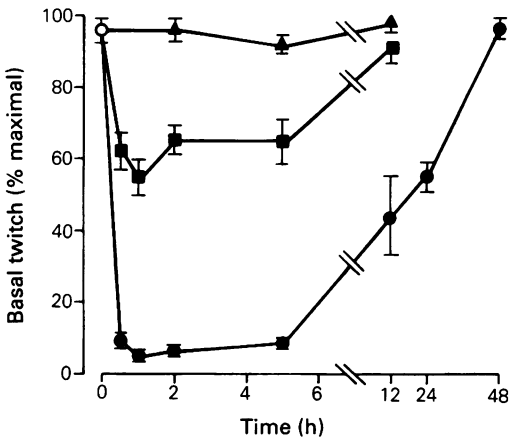

Figure 2 Time course for the inhibition of the twitch response of the rat vas deferens induced by desipramine (●), maprotiline (■) and zimelidine (▲). Abscissae: time in h after drugs (10 mg kg^{-1}, i.p.). Ordinates: inhibition of basal twitch expressed as % of maximal twitch induced by idazoxan (10^{-5} M). The size of maximal twitch was similar in all experiments (range: 760 ± 41 mg to 922 ± 102 mg). Data are mean of 10 experiments for saline control (○) and 4–9 experiments for each time interval after drugs; vertical lines show s.e. mean.

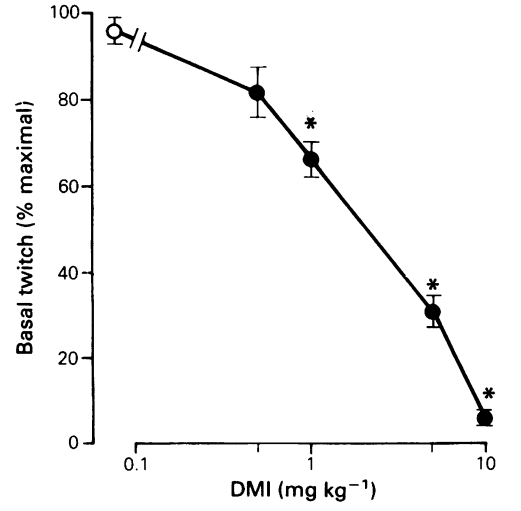

Figure 3 Dose-response curve for the inhibition of the twitch response of the rat vas deferens induced by desipramine (DMI). Abscissae: dose of DMI (mg kg^{-1}, i.p.). Ordinates: inhibition of basal twitch expressed as % of maximal twitch induced by idazoxan (10^{-5} M). Tissues removed 2 h after saline (○) or after each dose of DMI (●). Data are mean of 10 experiments for saline control and 4–5 experiments for each dose of DMI; vertical lines show s.e. mean. *$P < 0.001$ when compared with saline control (t test).

sipramine (0.1 mg kg^{-1}, i.p., 2 h) which did not inhibit the basal twitch response and presumably did not inhibit NA uptake, idazoxan (10^{-5} M) markedly enhanced the response (much more than in controls) which declined rapidly to reach a final steady-state similar to that obtained in control preparations (data not shown). This marked initial effect of idazoxan, similar to that reported *in vivo* by Doxey *et al.* (1985a), was not observed in rats treated with higher doses of desipramine, including the 0.5 mg kg^{-1} dose which only slightly inhibited the basal twitch response (Figure 3).

In rats pretreated with reserpine (1 mg kg^{-1}, i.p., 12 h) the NA content of the vas deferens was reduced by 95% of control values and both the basal and maximal (idazoxan) twitch responses elicited by field stimulation were smaller than in control preparations (43 ± 5%, $P < 0.005$ and 27 ± 3%, $P < 0.05$, respectively) (Table 2). Under these experimental conditions, desipramine (10 mg kg^{-1}; i.p., 2 h) did not induce a significant inhibition (14 ± 3% of respective control, $P > 0.05$) of the basal twitch responses but idazoxan still moderately increased the twitch (Table 2). In contrast, pretreatment with oxypertine (4 mg kg^{-1}, i.p., 12 h) did not reduce the NA content of the vas deferens (13%; $P > 0.05$) and under these conditions desipramine induced a significant inhibition (69 ± 6% of respective control; $P < 0.001$) of the basal twitch responses, although it was smaller than that induced in control preparations (94 ± 1% inhibition) (Table 2).

In vitro and as expected (Lotti *et al.*, 1981) desipramine also inhibited in a concentration-dependent manner (10^{-9}–10^{-6} M) the twitch responses (IC_{50} = 16.6 ± 6.5 nM; n = 6) with a maximal inhibition of 77 ± 4% with respect to basal twitches. Similarly, maprotiline (IC_{50} = 100 ± 4 nM; n = 5) and to a lesser extent zimelidine (IC_{10} = 780 ± 97 nM; n = 3) also inhibited the twitch responses with maximal inhibitions of basal twitches of 54 ± 4% and 15 ± 3%, respectively. For the three cyclic antidepressant drugs the maximal inhibitions obtained *in vitro* were similar to those obtained after the *in vivo* administration (Table 1). However, for the *in vitro* effects the preparation required a prolonged exposure to the drug (45–60 min for each concentration) to develop fully the inhibitory effect.

Activation of presynaptic α_2-adrenoceptors by cyclic antidepressant drugs

The activation of presynaptic α_2-adrenoceptors after the acute *in vivo* administration of cyclic antidepressant drugs was measured by quantifying the ability of each drug to inhibit the electrically-induced basal twitch responses of the vas deferens.

The acute *in vivo* administration of various cyclic antidepressant drugs (10 mg kg^{-1}, i.p., 2 h) resulted in

Table 2 Effect of desipramine on the basal twitch responses elicited by field-stimulation of the vas deferens from rats pretreated with reserpine or oxypertine

Pretreatment	*Group*	*Noradrenaline content* ($\mu g\,g^{-1}$)	*Basal twitch* (mg)	*Maximal twitch* (mg)	*Basal twitch* (% maximal)
Saline	Control	11.77 ± 0.30	850 ± 69	867 ± 60	97 ± 2
	Desipramine		53 ± 5*	854 ± 66	6 ± 1*
Reserpine	Control	0.60 ± 0.06*	483 ± 56†	636 ± 78†	77 ± 2†
	Desipramine		322 ± 47	491 ± 73	66 ± 4
Oxypertine	Control	10.24 ± 1.63	959 ± 126	1100 ± 133	87 ± 4
	Desipramine		253 ± 63*	944 ± 73	27 ± 6*

Values are mean ± s.e.mean of 4–8 experiments. Doses of drugs: reserpine, 1 mg kg^{-1} i.p. for 12 h; oxypertine, 4 mg kg^{-1} i.p. for 12 h; desipramine, 10 mg kg^{-1} i.p. Tissues removed 2 h after desipramine. Maximal twitch induced by idazoxan (10^{-5} M).
*$P < 0.001$ when compared with the corresponding control (t test).
†At least $P < 0.05$ when compared with the corresponding saline control (t test).

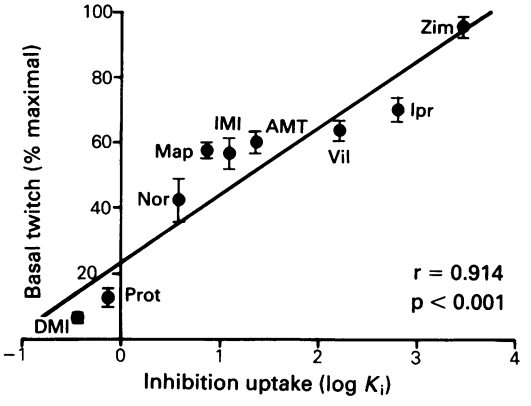

Figure 4 Correlation between inhibition of noradrenaline (NA) neuronal uptake and inhibition of the twitch response of the rat vas deferens induced by cyclic antidepressant drugs. Abscissae: $\log_{10}$ inhibitor constants (K_i) for blockade of [^{3}H]-NA uptake into rat brain synaptosomes (Richelson & Pfenning, 1984). Ordinates: inhibition of basal twitch expressed as % of maximal twitch induced by idazoxan (10^{-5} M). Doses of drugs: 10 mg kg^{-1}, i.p. Tissues removed 2 h after drug administration. Data are mean of 4–10 experiments for each drug; vertical lines show s.e.mean. The line represents the regression of the correlation which was calculated by the least squares method. The coefficient of correlation is $r = 0.914$ ($t = 5.96$, 07, $P < 0.001$, two-tailed t test). DMI: desipramine; Prot: protriptyline; Nor: nortriptyline; Map: maprotiline; IMI: imipramine; AMT: amitriptyline; Vil: vilozaxine; Ipr: iprindole; Zim: zimelidine.

marked different degrees of inhibition of the basal twitch responses which were rapidly reversed to control values by idazoxan (10^{-5} M) (Table 1). The rank order of potency in inhibiting the basal twitch responses was found to be (twitch remaining, % maximal): desipramine (6 ± 1%) > protriptyline (13 ± 2%) > nortriptyline (42 ± 7%) > maprotiline (57 ± 4%) = imipramine (57 ± 4%) > amitriptyline (60 ± 3%) > viloxazine (63 ± 3%) > iprindole (69 ± 4%) >> zimelidine (94 ± 4%) (Table 1). Moreover, there was a highly significant correlation ($r = 0.914$; $P < 0.001$) between the potencies ($\log_{10} K_i$, inhibitor constants) of these drugs for blockade of [^{3}H]-NA uptake into rat brain synaptosomes (Richelson & Pfenning, 1984) and the inhibitions of the basal twitch responses induced by the same drugs in the rat vas deferens (Figure 4). A similar correlation between the two variables was obtained ($r = 0.903$; $P < 0.001$) when the potencies ($\log_{10} K_i$) of cyclic antidepressant drugs for inhibition of [^{3}H]-NA uptake were taken from another independent study (Hyttel, 1982).

These data indicate that the acute *in vivo* inhibition of neuronal uptake of NA by cyclic antidepressant drugs activates (through endogenous NA) presynaptic inhibitory α_2-adrenoceptors which results in inhibitions of basal twitch responses and that the degree of receptor activation is related to the potency of the drug to block the reuptake mechanism.

Desensitization of presynaptic α_2-adrenoceptors by cyclic antidepressant drugs

The desensitization of presynaptic α_2-adrenoceptors after the long-term administration of cyclic antidepressant drugs was measured by quantitating the ability of each drug to reduce the inhibitory effect of the selective α_2-adrenoceptor agonist clonidine on the electrically-induced twitch responses of the vas deferens.

Table 3 Effect of long-term administration of antidepressants (14 days) and cocaine (21 days) on clonidine-sensitivity in the field-stimulated vas deferens

	pD_2 i	Δ
Control	8.19 ± 0.17(8)	–
Desipramine	7.04 ± 0.10(4)***	14.1
Protriptyline	7.70 ± 0.08(4)*	3.1
Nortriptyline	7.74 ± 0.09(5)*	2.8
Maptrotiline	7.21 ± 0.27(3)*	9.5
Viloxazine	7.61 ± 0.07(5)**	3.8
Iprindole	7.48 ± 0.06(5)**	5.1
Zimelidine	7.35 ± 0.06(3)**	6.9
Cocaine	7.59 ± 0.10(5)*	4.0

Values are mean ± s.e.mean of the number of experiments indicated in parentheses. Doses of drugs: 10 mg kg^{-1} day^{-1} i.p. Tissues removed 24 h after the last injection.
$pD_2 = -\log_{10} EC_{50}$ (M) and Δ indicates changes in sensitivity. *$P<0.05$, **$P<0.01$; ***$P<0.001$ when compared with control value (t test).
The various chronic treatments did not change significantly the size of basal twitches (control: 813 ± 92 mg; desipramine: 1190 ± 256 mg; protriptyline: 834 ± 155 mg; nortriptyline: 871 ± 94 mg; maprotiline: 929 ± 157 mg; viloxazine: 707 ± 80 mg; iprindole: 792 ± 102 mg; zimelidine: 807 ± 48 mg; cocaine: 965 ± 138 mg).

Table 4 Effect of desipramine treatment and then its discontinuation on clonidine sensitivity in the field-stimulated vas deferens

	Days of treatment	pD_2	Δ
Control	–	8.19 ± 0.17(8)	–
Desipramine	3	7.93 ± 0.09(6)	1.8
	7	7.64 ± 0.05(5)*–	3.5
	14	7.04 ± 0.10(4)**	14.1
	Days after discontinuation		
Desipramine	5	7.16 ± 0.10(4)**	10.7
(for 14 days)	10	8.01 ± 0.12(3)	1.5

Values are mean ± s.e. of the number of experiments indicated in parentheses. Dose of desipramine: 10 mg kg^{-1} day^{-1} i.p. $pD_2 = -\log_{10}$ EC_{50} (M) and Δ indicates changes in sensitivity. *$P<0.01$, **$P<0.001$ when compared with control value (t test).

In control preparations clonidine inhibited in a concentration-dependent manner (10^{-9}–10^{-7} M) the twitch responses ($pD_2 = 8.19 \pm 0.17$; $n = 8$). The long-term administration of various cyclic antidepressant drugs (10 mg kg^{-1}, i.p., 14 days) resulted in significant decreases of clonidine sensitivity with parallel shifts to the right of the concentration-effect curves (Table 3). The long-term administration of cocaine, an inhibitor of neuronal uptake of NA which is not an antidepressant, also resulted in a significant decrease of clonidine effectiveness (Table 3). It should be noted that the various chronic treatments did not modify significantly basal twitch responses, indicating that the decreases of clonidine-sensitivity were not related to the size of basal twitches (Table 3). However, chronic desipramine showed a tendency to increase basal twitch responses although they did not reach statistical significance when compared with control basal twitches (Table 3). Chronic desipramine (10 mg kg^{-1}, i.p., 14 days) also decreased the sensitivity to xylazine, another presynaptic α_2-adrenoceptor agonist (control, $pD_2 = 6.86 \pm 0.24$; $n = 5$; chronic desipramine, $pD_2 = 5.94 \pm 0.34$; $n = 4$; $P<0.05$). For the various cyclic antidepressant drugs the rank order of potency in decreasing the sensitivity to clonidine was found to be (times reduced): desipramine (14.1) > maprotiline (9.5) > zimelidine (6.9) > iprindole (5.1) > viloxazine (3.8) > protriptyline (3.1) > nortriptyline (2.8) (Table 3). This rank order of potency was different from that observed for the same drugs after their acute *in vivo* administration (Table 1). There was no significant correlation between the potencies ($\log_{10} K_i$, inhibitor constants) of these drugs for blockade of [^{3}H]-NA uptake into rat brain synaptosomes (Richelson & Pfenning, 1984) and the decreases in sensitivity to clonidine after their long-term administration; although a tendency for a negative correlation was found ($r = -0.268$; $P > 0.05$).

Treatment for 3 days with desipramine (10 mg kg^{-1}, i.p.) did not reduce significantly the sensitivity to clonidine. After 7 days of treatment the effectiveness of clonidine was reduced 3.5 fold ($P<0.01$) and after 14 days by 14 fold ($P<0.001$) (Table 4). After this latter period of treatment and 5 days after discontinuation of desipramine, the sensitivity to clonidine was found still significantly reduced (11 fold, $P<0.001$) reaching control values 10 days after discontinuation of treatment (Table 4).

In rats treated for 14 days with desipramine (10 mg kg^{-1}, i.p.) the inhibitory effect induced by the drug upon the basal twitch responses was less than after its acute *in vivo* administration (Table 5). Thus, basal twitch responses obtained 2–5 h after the last dose of desipramine were smaller (for 5 h: 41 ± 5%; $n = 4$; $P<0.05$) than in control preparations, but they were much higher than those obtained after the acute

Table 5 Acute and chronic effects of desipramine (DMI) on basal twitch responses elicited by field stimulation of the vas deferens

	Time (h)	*Basal twitch* (mg)	*Maximal twitch* (mg)	*Basal twitch* (% maximal)
Acute saline	–	850 ± 69	867 ± 60	97 ± 2
Acute DMI	2	53 ± 5***	854 ± 66	6 ± 1***
	5	53 ± 13***	700 ± 141	8 ± 1***
	12	301 ± 39***	712 ± 98	44 ± 11***
	24	512 ± 98**	922 ± 142	55 ± 4***
Chronic saline	–	812 ± 22***	879 ± 93	93 ± 2
Chronic DMI	2	490 ± 125*	1673 ± 195**	30 ± 8***
	5	462 ± 107**	1453 ± 261	31 ± 6***
	12	1357 ± 310	2020 ± 295**	65 ± 10*
	24	1258 ± 162*	1710 ± 183**	75 ± 11

Values are means ± s.e.mean of 4–10 experiments. Dose of DMI: 10 mg kg^{-1} i.p. Tissues removed at the indicated h after 1 injection (acute) or after 14 injections (chronic). Maximal twitch induced by idazoxan (10^{-5} M).
*$P<0.05$, **$P<0.02$, ***$P<0.001$ when compared with the corresponding saline control (t test).

administration of the cyclic antidepressant drug (Table 5). In contrast, 12–24 h after the last dose of chronic desipramine, basal twitch responses tended to be higher than in control preparations (for 24 h: 55 ± 7%; $n = 4$; $P<0.05$). This series of experiments confirmed the tendency of chronic desipramine to increase basal twitch responses. Moreover, after the long-term administration of desipramine and independently of the time interval after the last dose, idazoxan (10^{-5} M) markedly enhanced the basal twitch responses of the vas deferens (range: 65–130%; $n = 4$; $P<0.02$) (Table 5).

Discussion

Twitch responses elicited by field stimulation of the isolated vas deferens of the rat were reduced after the acute *in vivo* pretreatment of the rats with a wide variety of cyclic antidepressant drugs, markedly different reductions occurring with different drugs. These reductions of responses compared with untreated controls were rapidly removed by idazoxan, a highly selective α_2-adrenoceptor antagonist (Doxey *et al.*, 1983), indicating that they were mediated through an α_2-adrenoceptor mechanism. These results confirm and extend recent *in vivo* findings with desipramine in the pithed rat (Doxey *et al.*, 1985b).

For the cyclic antidepressant drugs there was a highly significant correlation ($r = 0.914$; $P<0.001$) between their respective potencies (over a range of 4 $\log_{10}$ units) for blockade of [^{3}H]-NA uptake into rat brain synaptosomes (Richelson & Pfenning, 1984) and the magnitude of the inhibitory effects upon the basal twitch responses induced by the same drugs after the acute *in vivo* administration. Since cyclic antidepressant drugs are equally potent in inhibiting neuronal uptake of NA in brain and peripheral tissues (Raisman *et al.*, 1982), the observed correlation strongly suggested that the degree of presynaptic α_2-adrenoceptor activation which results in inhibition of the twitch was indirectly mediated through inhibition of NA neuronal uptake and that NA was the activating agonist. In the rat vas deferens both NA and dopamine are released during nerve stimulation (Bell *et al.*, 1984). However, the inhibitory effects induced by cyclic antidepressant drugs upon the basal twitch responses did not correlate with the potencies of the drugs to inhibit [^{3}H]-dopamine uptake ($r = 0.225$) (Richelson & Pfenning, 1984) suggesting that dopamine was not the agonist involved in the inhibitory effects. These inhibitory effects also did not correlate with the potencies of the drugs in inhibiting [^{3}H]-5-hydroxytryptamine (5-HT) uptake ($r = 0.032$) (Richelson & Pfenning, 1984).

Desipramine induced a dose-dependent reduction of the basal twitch responses and the onset of action and the time course of this inhibitory effect were in agreement with the *in vivo* inhibition of the reuptake mechanism (Manias & Taylor, 1983) and the pharmacokinetic characteristics ($t_{1/2}$ about 16 h) (Nagy, 1980; Barkai *et al.*, 1984) reported for this drug in the rat. Also the *in vitro* results with desipramine, maprotiline and zimelidine are in general agreement with those of Marshall *et al.* (1977) and Lotti *et al.* (1981) who suggested that *in vitro* cocaine and cyclic antidepressant drugs, through inhibition of neuronal uptake, activate presynaptic α_2-adrenoceptors. Treatment with reserpine reduced the NA content of the vas deferens by 95% and almost abolished the inhibitory effect induced by desipramine, yet idazoxan moderate-

ly increased the twitch response. These results indicated that endogenous NA mediates the inhibition of the twitch and suggested that the residual effects of desipramine and idazoxan could be due to the existence of a small reserpine-resistant NA pool as described for other transmitters (Niddam *et al.*, 1985; Kuhn *et al.*, 1985). However some postsynaptic potentiating effect of idazoxan cannot be ruled out. Oxypertine, a reserpine-like drug (Andén & Fuxe, 1971), did not reduce the total content of NA or the basal twitch but attenuated the inhibitory effect of desipramine upon the twitch responses. It has been suggested that oxypertine, in contrast to reserpine, depletes only a small pool of newly synthesized transmitter (Curle *et al.*, 1985) which could explain both the unchanged total content of NA and the reduced effect of desipramine upon the twitch responses in the oxypertine-treated rats. These results suggested that the small NA pool readily available for release was also involved in mediating the inhibitory effect of desipramine.

The chronic experiments with a wide variety of cyclic antidepressant drugs confirm and extend previous findings with desipramine (Finberg & Tal, 1985; Doxey *et al.*, 1985b) and clearly indicate that prolonged inhibition of NA neuronal uptake, in sharp contrast to the acute inhibition, is followed by desensitization of presynaptic α_2-adrenoceptors which results in decreases in sensitivity to selective α_2-adrenoceptor agonists. Moreover, after the long-term administration of desipramine the basal twitch responses of the vas deferens showed a tendency to be higher than in controls as mentioned by Lotti *et al.* (1981) which could further suggest the existence of a less efficient feed-back autoinhibition as a consequence of the α_2-adrenoceptor desensitization. However, this possibility was not in agreement with the fact that after chronic desipramine, idazoxan markedly potentiated the height of the twitch, although some postsynaptic effect of idazoxan could be involved in this mechanism.

After the long-term administration of cyclic antidepressant drugs and cocaine there was no significant correlation ($r = -0.268$) between the potencies of the drugs for blockade of [^{3}H]-NA uptake into rat brain synaptosomes (Richelson & Pfenning, 1984) and the degree of desensitization of presynaptic α_2-adrenoceptors (i.e. reduced sensitivity to clonidine). This lack of correlation suggests that prolonged inhibition of NA neuronal uptake, independently of the potency of the drug on the uptake system, always results in α_2-adrenoceptor desensitization and that time is the important variable in this respect. A persistent increase in the concentration of the agonist NA in the synaptic cleft would finally lead to presynaptic α_2-adrenoceptor desensitization. In this context, desensitization of presynaptic α_2-adrenoceptors after the *in vitro* exposure to NA has been demonstrated in various functional preparations (Langer & Dubocovich, 1977; Ball *et al.*, 1982).

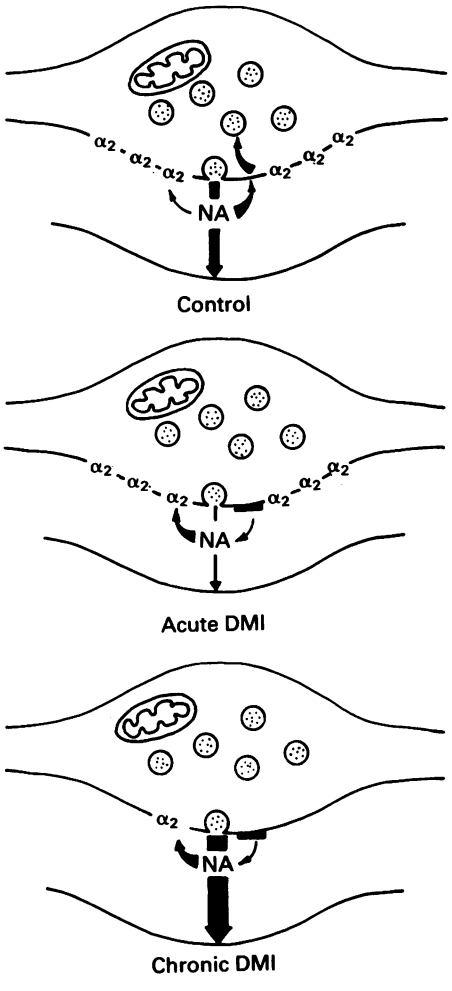

Figure 5 Simplified model of the acute and chronic effects of desipramine (DMI) on adrenergic nerve function. Modified from Crews & Smith (1980).

The main conclusions from this study have been incorporated in the simplified model depicted in Figure 5 for the prototype drug, desipramine. Neuronal uptake functions normally to remove NA from the synaptic cleft and during repetitive nerve stimulation the amount of NA that is released per nerve impulse is under the control of presynaptic inhibitory α_2-adrenoceptors. The acute inhibition of NA neuronal uptake by desipramine would lead, through activation of the inhibitory receptor, to a decreased neurotransmitter release. In contrast,

prolonged inhibition of NA neuronal uptake which results in desensitization of the same receptor would lead to an enhancement of NA release after the chronic administration of desipramine. Since most cyclic antidepressant drugs are more potent at blocking uptake of NA than uptake of other neurotransmitters (Richelson & Pfenning, 1984) the above conclusions appear to be of general value. The proposed mechanism of action of cyclic antidepressant drugs on adrenergic nerve function is in agreement (chronic effects) but also modifies (acute effects) a previously postulated model (Crews & Smith, 1980). In the present modified model (Figure 5) it is postulated that there is a reduction (as opposed to no change; Crews & Smith, 1980) in NA release after the acute administration of desipramine. Thus, the acute effects of cyclic antidepressant drugs are in fact opposite to the chronic effects and probably with negative therapeutic implications. The proposed dual modulation of presynaptic inhibitory α_2-adrenoceptors might explain, if similar receptor changes operate in the CNS (see Sugrue, 1980 for central changes in NA turnover), the delayed onset of action of cyclic antidepressant drugs.

This study was supported by CAICYT Grant No. 1018/81. The authors wish to thank Ms F. Pi for the h.p.l.c. assay of noradrenaline, and the various pharmaceutical firms that generously supplied the drugs used in the study.

References

ANDEN, N.-E. & FUXE, K. (1971). The influence of benzquinamide, oxypertine and prenylamine on monoamine levels and on monoamine effects in the spinal cord. *Acta pharmac. tox.*, **30**, 225–237.

BALL, N., DANKS, J.L., DORUDI, S. & NASMYTH, P.A. (1982). Desensitization by noradrenaline of responses to stimulation of pre- and postsynaptic adrenoceptors. *Br. J. Pharmac.*, **76**, 201–210.

BARKAI, A.I., SUCKOW, R.F. & COOPER, T.B. (1984). Imipramine and its metabolites: Relationship to cerebral catecholamines in rats in vivo. *J. Pharmac. exp. Ther.*, **230**, 330–335.

BELL, C., GILLESPIE, J.S. & MACRAE, J.M. (1984). Release of noradrenaline and dopamine by nerve stimulation in the guinea-pig and rat vas deferens. *Br. J. Pharmac.*, **81**, 563–569.

CHARNEY, D.S., HENINGER, G.R. & STERNBERG, D.E. (1983). Alpha-2 adrenergic receptor sensitivity and the mechanism of action of antidepressant therapy. The effect of long-term amitriptyline treatment. *Br. J. Psychiat.*, **142**, 265–275.

CREWS, F.T. & SMITH, C.B. (1978). Presynaptic alpha-receptor subsensitivity after long-term antidepressant treatment. *Science.*, **202**, 322–324.

CREWS, F.T. & SMITH, C.B. (1980). Potentiation of responses to field-stimulation of isolated rat left atria during chronic tricyclic antidepressant administration. *J. Pharmac. exp. Ther.*, **215**, 143–149.

CURLE, P.F., GEDDES, C. & PALOMO, T. (1985). The effect of oxypertine on dopamine, dopac and HVA in the rat striatum. *Br. J. Pharmac.*, **84**, 197P.

DOXEY, J.C., ROACH, A.G. & SMITH, C.F.C. (1983). Studies on RX 781094: a selective, potent and specific antagonist of α_2-adrenoceptors. *Br. J. Pharmac.*, **78**, 489–505.

DOXEY, J.C., LANE, A.C., ROACH, A.G., SMITH, C.F.C. & WALTER, D.S. (1985a). Selective α_2-adrenoceptor agonists and antagonists. In *Pharmacology of Adrenoceptors.* ed. Szabadi, E., Bradshaw, C.M. & Nahorski, S.R. pp.13–22. London: MacMillan Press Ltd.

DOXEY, J.C., ROACH, A.G. & SAMUEL, J. (1985b). Effects of desipramine on stimulation-induced contractions of the vas deferens of rats pretreated either chronically with desipramine or acutely with idazoxan. *Clin. Science.*, **68**, Suppl. 10, 155s–159s.

DREW, G.M. (1977). Pharmacological characterization of the presynaptic α_2-adrenoceptor in the rat vas deferens. *Eur. J. Pharmac.*, **42**, 123–130.

FARMER, S.G. (1985). Noradrenaline and ATP – Cotransmitters? *Trends Pharmac. Sci.*, **6**, 10–11.

FINBERG, J.P.M. & TAL, A. (1985). Reduced peripheral presynaptic adrenoceptor sensitivity following chronic antidepressant treatment in rats. *Br. J. Pharmac.*, **84**, 609–617.

GARCÍA-SEVILLA, J.A., ZIS, A.P., HOLLINGSWORTH, P.J., GREDEN, J.F. & SMITH, C.B. (1981). Platelet α_2-adrenergic receptors in major depressive disorder. *Arch. gen. Psychiat.*, **38**, 1327–1333.

GARCÍA-SEVILLA, J.A. & ZUBIETA, J.K. (1985). Activation and desensitization of presynaptic α_2-adrenoceptors after inhibition of neuronal uptake. *Br. J. Pharmac.*, **86**, 580P.

GARCÍA-SEVILLA, J.A., GUIMON, J., GARCÍA-VALLEJO, P. & FUSTER, M.J. (1986). Biochemical and functional evidence of supersensitive platelet α_2-adrenoceptors in major affective disorder. Effect of long-term lithium treatment. *Arch. gen. Psychiat.*, **43**, 51–57.

HYTTEL, J. (1982). Citalopram - Pharmacological profile of a specific serotonin uptake inhibitor with antidepressant activity. Progr. *Neuro-Psychopharmac. & Biol. Psychiat.*, **6**, 277–295.

ILLES, P. & DÖRGE, L. (1985). Mechanism of α_2-adrenergic inhibition of neuroeffector transmission in the mouse vas deferens. *Naunyn-Schmiedebergs Arch. Pharmac.*, **328**, 241–247.

KUHN, D.M., WOLF, W.A. & YOUDIM, M.B.H. (1985). 5-Hydroxytryptamine release in vivo from a cytoplasmic pool: Studies on the 5-HT behavioural syndrome in reserpinized rats. *Br. J. Pharmac.*, **84**, 121–129.

LANGER, S.Z. (1981). Presynaptic regulation of the release of catecholamines. *Pharmac. Rev.*, **32**, 337–362.

LANGER, S.Z. & DUBOCOVICH, M.L. (1977). Subsensitivity of presynaptic α_2-adrenoceptors after exposure to noradrenaline. *Eur, J. Pharmac.*, **41**, 87–88.

LOTTI, V.J., CHANG, R.S.L. & KLING, P. (1981). Pre- and postsynaptic adrenergic activation by norepinephrine reuptake inhibitors in the field-stimulated rat vas deferens. *Life Sci.*, **29**, 633–639.

MANIAS, B. & TAYLOR, D.A. (1983). Inhibition of in vitro amine uptake into rat brain synaptosomes after in vivo

administration of antidepressants. *Eur. J. Pharmac.*, **95,** 305–309.

MARSHALL, I., NASMYTH, P.A. & SHEPPERSON, N.B. (1977). Pre-synaptic α_2-adrenoceptors and the inhibition by uptake blocking agents of the twitch response of the mouse vas deferens. *Br. J. Pharmac.*, **59,** 511P.

NAGY, A. (1980). On the kinetics of imipramine and related antidepressants. *Acta Psychiat. Scand.*, **61,** Suppl. 280, 147–156.

NIDDAM, R., ARBILLA, S., SCATTON, B., DENNIS, T. & LANGER, S.Z. (1985). Amphetamine induced release of endogenous dopamine in vitro is not reduced following pretreatment with reserpine. *Naunyn-Schmiedebergs Arch. Pharmac.*, **329,** 123–127.

OSWALD, J., BREZINOVA, V. & DUNLEAVY, D.L.F. (1972). On the slowness of action of tricyclic antidepressant drugs. *Br. J. Psychiat.*, **120,** 673–677.

PILC, A. & VETULANI, J. (1982). Attenuation by chronic imipramine treatment of [^{3}H]clonidine binding to cortical membranes and of clonidine-induced hypothermia: The influence of central chemosympathectomy. *Brain Res.*, **238,** 499–504.

RAISMAN, R., SETTE, M., PIMOULE, C., BRILEY, M. & LANGER, S.Z. (1982). High-affinity [^{3}H]desipramine binding in the peripheral and central nervous system: a specific site associated with the neuronal uptake of noradrenaline. *Eur. J. Pharmac.*, **78,** 345–351.

RICHELSON, E. & PFENNING, M. (1984). Blockade by antidepressants and related compounds of biogenic amine uptake into rat brain synaptosomes: most antidepressants selectively block norepinephrine uptake. *Eur. J. Pharmac.*, **104,** 277–286.

SCHILDKRAUT, J.J. (1965). The catecholamine hypothesis of affective disorders: A review of supporting evidence. *Am. J. Psychiat.*, **122,** 509–522.

SMITH, C.B., GARCÍA-SEVILLA, J.A. & HOLLINGSWORTH, P.J. (1981). α_2-Adrenoreceptors in rat brain are decreased after long-term tricyclic antidepressant drug treatment. *Brain Res.*, **210,** 413–418.

SMITH, C.B. & HOLLINGSWORTH, P.J. (1984). α_2-Adrenoceptors and antidepressant treatments. In *Proceedings IUPHAR 9th International Congress of Pharmacology*, vol. 3. pp. 131–136. London: MacMillan Press Ltd.

SNEDDON, P., WESTFALL, D.P. & FEDAN, J.S. (1982). Cotransmitters in the motor nerves of the guinea-pig vas deferens: electrophysiological evidence. *Science.*, **218,** 693–695.

STARKE, K. (1981). Presynaptic receptors. *A. Rev. Pharmac. Tox.*, **21,** 7–30.

SUGRUE, M.F. (1980). Changes in rat brain monoamine turnover following chronic antidepressant administration. *Life Sci.*, **26,** 423–429.

SVENSSON, T.H. & USDIN, T. (1978). Feedback inhibition of brain noradrenaline neurons by tricyclic antidepressants: α-receptor mediation. *Science*, **202,** 1089–1091.

WAGNER, J., VITALI, P., PALFREYMAN, M.G., ZRAIKA, M. & HUOT, S. (1982). Simultaneous determination of 3,4-dihydroxyphenylalanine, 5-hydroxytryptophan, dopamine, 4-hydroxy-3-methoxyphenylalanine, norepinephrine, 3,4-dihydroxyphenylacetic acid, homovanillic acid, serotonin, and 5-hydroxyindolacetic acid in rat cerebrospinal fluid and brain by high-performance liquid chromatography with electrochemical detection. *J. Neurochem.*, **38,** 1241–1254.

ZIEHER, L.M. & JAIM-ETCHEVERRY, G. (1971). Regional variations in the distribution of noradrenaline along the rat vas deferens. *J. Pharm. Pharmac.*, **23,** 61–62.

(Received March 8, 1986.
Revised June 30, 1986.
Accepted August 21, 1986.)