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Reduced peripheral presynaptic adrenoceptor sensitivity following chronic antidepressant treatment in rats

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1 Responses of the isolated vas deferens of the rat to clonidine (inhibition of contractions to field stimulation at 0.1 Hz) and noradrenaline (contraction of longitudinal muscle) were determined after one or 21 daily injections of the animals with desmethylimipramine, clorgyline, selegiline or tranylcypromine.

2 Desmethylimipramine (10 mg kg^{-1}) and clorgyline (2 mg kg^{-1}) increased the clonidine EC_{50} in the isolated vas deferens after 21 but not after one daily injection(s). Tranylcypromine (5 mg kg^{-1}) increased clonidine EC_{50} after both one and 21 injections and selegiline (1 mg kg^{-1}) did not affect clonidine EC_{50} after either one or 21 injections.

3 Only desmethylimipramine had a significant effect on noradrenaline responsiveness, producing an inconsistent decrease in EC_{50} with a consistent increase in maximum contractile response after 21 but not after one daily injection(s).

4 Clorgyline (10^{-5}M) increased the contractile response of the isolated vas deferens to field stimulation and antagonized the inhibitory effect of clonidine when added directly to the isolated tissue preparation.

5 Neither clorgyline (5 mg kg^{-1}), selegiline (1 mg kg^{-1}) nor tranylcypromine (2.5 mg kg^{-1}) affected significantly the inhibitory response to clonidine (1 μg kg^{-1}) on contractions of *in situ* sympathetically stimulated vas deferens in pithed rats.

6 These results show that down-regulation of α_2-presynaptic adrenoceptors by chronic treatment with desmethylimipramine and clorgyline occurs in peripheral organs as well as in the central nervous system.

Introduction

Chronic treatment of rats with both tricyclic and monoamine oxidase (MAO) inhibitor antidepressant drugs has been shown to cause down-regulation of several types of central neurotransmitter receptors, including β- and α_2-adrenoceptors. Reduction in α_2-adrenoceptor sensitivity has been demonstrated using ligand binding (Smith *et al.*, 1981; Cohen *et al.*, 1982a), behavioural (Spyraki & Fibiger, 1980), electrophysiological (Svensson & Usdin, 1978) and biochemical (Sugrue, 1981) techniques. Central α_2-adrenoceptors are situated both presynaptically (Dubocovich, 1979) and postsynaptically (Langer, 1980). Presynaptic α_2-adrenoceptors are inhibitory on noradrenaline release (Dubocovich, 1979), and their down-regulation would lead to increased noradrenaline release from the neurones if they play an inhibitory role under physiological conditions. Evidence for and against such a role has been presented (Kalsner, 1984).

Drugs which inhibit active neuronal amine uptake (e.g. desmethylimipramine) make more noradrenaline available at both pre- and postsynaptic adrenoceptors, which may lead to compensatory down-regulation of these receptors. Monoamine oxidase inhibitors increase tissue levels of noradrenaline, dopamine and 5-hydroxytryptamine (Waldmeier *et al.*, 1981) and increase both vesicular and free noradrenaline content within the neurone (Fillenz & Stanford, 1981). The cytoplasmic free noradrenaline may leave the neurone by active or passive efflux, and may be localised in the vicinity of the presynaptic receptors, leading to their

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down-regulation (Cohen *et al.*, 1980). Reduction in presynaptic α_2-adrenoceptor number has been suggested as the initial event during chronic MAO inhibition, which leads subsequently to increased noradrenaline release and down-regulation of postsynaptic β- and α-adrenoceptors (Cohen *et al.*, 1982b). This suggestion is reinforced by the observation that combination of desmethylimipramine with yohimbine treatment leads to rapid down-regulation of cortical β-adrenoceptors in the rat brain (Scott & Crews, 1983).

Central and peripheral noradrenergic nerves have similar processes of amine uptake, storage and release and both have inhibitory α_2-presynaptic autoreceptors. Establishment of a peripheral model for α_2-adrenoceptor down-regulation would have certain advantages over study of these events in the CNS: peripheral amine release can be more easily measured, and a simple neuronal system can be investigated without the multiple inputs received by central monoaminergic neurones. Employing this principle, Crews & Smith (1978) demonstrated that the postsynaptic effects of sympathetic stimulation, and release of noradrenaline, were enhanced in isolated atria of rats treated chronically with desmethylimipramine.

The present work describes a study of the effect of chronic treatment with a tricyclic antidepressant and MAO inhibitor drugs on presynaptic and postsynaptic adrenoceptor function in the rat vas deferens, with the aim of establishing such a model system. The MAO inhibitor drugs used were representatives of the groups of agents which cause non-selective inhibition of MAO types A and B (tranylcypromine), selective inhibition of MAO type A (clorgyline) and selective inhibition of MAO type B (selegiline). Sympathetic nerves are believed to contain mainly the A type of the enzyme (Goridis & Neff, 1971a; Jarrott, 1971) which shows selectivity for noradrenaline as substrate (Goridis & Neff, 1971b). Selective inhibition of MAO type A, but not of type B, is associated with antidepressant effect in man (Murphy *et al.*, 1981). A preliminary account of some of these results was given at meetings of the Collegium Internationale Neuro-Psychopharmacologicum Jerusalem, (1982) and the British Pharmacological Society (Finberg & Tal, 1983).

Methods

Animals and injections

Rats (male, Sprague-Dawley derived) were kept 5 per cage and fed rat pellets and tap water *ad libitum*. Drugs were dissolved in 0.9% w/v NaCl, injected i.p. in a volume of 1.0 ml kg^{-1}, and animals were killed 24 h after 1 or 21 daily injections of the antidepressant drugs. Animals in chronic treatment experiments weighed 150–180 g at the start of the experiment, and 250–280 g after 21 days treatment. Animals used in acute experiments weighed 250–280 g.

Preparation of vas deferens in vitro

Both vasa deferentia were dissected from close to the junction with the bladder to within 2–3 mm of the epididymis and suspended in 15 ml organ baths. The tissue was bathed in Krebs solution of the following composition (mmol l^{-1}): NaCl 118, KCl 4.7, $MgCl_2$ 1.2, NaH_2PO_4 1.2, $CaCl_2$ 2.5, $NaHCO_3$ 25 and glucose 11, which was gassed with 95% O_2, 5% CO_2 and maintained at 37°C. Longitudinal tension was measured with Statham UC2 isometric transducers coupled to a Brush-Gould recorder. The tissue was initially weighted with 1.2 g tension, after which it normally relaxed over 10–20 min to a tension of 500–600 mg. If the tension fell further it was adjusted to this value.

Following an equilibration period of 40 min, electrical field stimulation of supramaximal current strength (200 mA, 1 ms, 0.1 Hz) was applied via ring and hook electrodes using a Grass S44 stimulator. The preparation was stimulated for 30 min to allow for equilibration of contractions, and a single cumulative dose-responsive curve to clonidine was then obtained. Stepwise increments of clonidine concentration were given every 4.5 min (the time required for each dose to produce maximum effect) until complete inhibition of contractions occurred. The bath fluid was then changed, and several further washes given (normally 3 to 4) until contractions returned to control tension (approximately 20 to 30 min). The stimulator was then turned off, and a non-cumulative dose-response curve to noradrenaline performed, using a contact period of 30 s and a between-dose interval of 4.5 min. The results were plotted graphically, and EC_{50} values for clonidine and noradrenaline calculated for each individual experiment. Preliminary experiments showed that noradrenaline responses were not affected by prior exposure to clonidine using this protocol.

Experiments were also performed in which the acute effect of the antidepressant drugs *in vitro* on contractility was investigated. Tissues were prepared and stimulated as above, and the drugs added directly to the organ bath as described below. When the effect of clorgyline (10^{-5}M) on the dose-response curve to clonidine was determined, an initial control dose-response curve was determined as above; following wash-out of the clonidine and return of contractile response to field-stimulation to normal levels, clorgyline (10^{-5}M) was added to the system, and a second dose-response curve to clonidine determined 20 min later (in the presence of clorgyline). In control experiments, a second clonidine dose-response curve was not significantly different from the first.

Contractile responses of vas deferens in vivo

Rats (250 g body weight) were anaesthetized with halothane (5% in air), the trachea cannulated, and pithed through the orbit. Artificial respiration was instituted using a Harvard rodent respirator (2.2 ml per stroke, 50 strokes min^{-1}), the jugular vein catheterized for intravenous injection of drugs, and blood pressure measured from a carotid artery, using a Statham P 23 Db pressure transducer and Brush-Gould recorder. Contractions of the left vas deferens in response to sympathetic stimulation were obtained as described by Doxey & Everitt (1977). Following equilibration of responses to supramaximal electrical stimulation (280 mA, 50 μs, 6 Hz for 3 s every 1 min), a single sub-maximal dose of clonidine (1 μg kg^{-1}) was injected intravenously and the degree of inhibition of contractile response determined. Following the return of the contractile response to control values, the drug (or saline in control experiments) was injected intravenously, and the response to clonidine redetermined.

Determination of monoamine oxidase activity

Homogenates of hepatic tissue were incubated with [^{14}C]-5-hydroxytryptamine (5-HT) (1 mM) or [^{14}C)-2-phenylethylamine (20 μM) and oxidised metabolites separated on small Amberlite CG50H ion-exchange columns as described by Tipton & Youdim (1976).

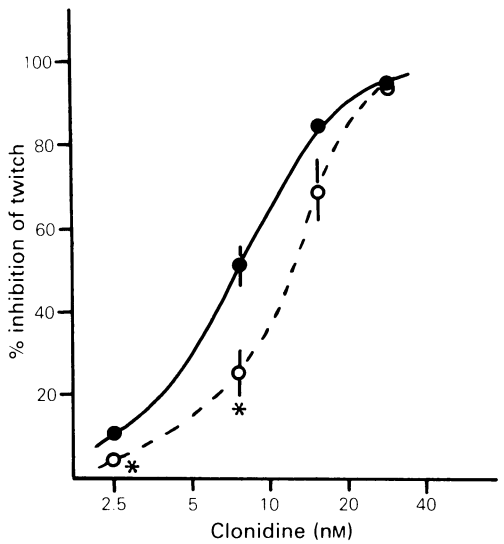

Figure 1 Inhibition of contractions to field-stimulation of isolated vas deferens of the rat by clonidine: (●) control; (○) in presence of clorgyline, 10^{-5}M. *$P < 0.05$ for difference from control value (Wilcoxon rank sum test). Mean values shown ± standard error (n = 5).

Incubation time (37°C) was chosen so that not more than 10% of added substrate was metabolised. The reaction was terminated by addition of tranylcypromine (10^{-4}M); blank samples were included in which tranylcypromine was added before the substrate. Protein concentrations were estimated by the method of Lowry *et al.* (1951) and enzyme activity calculated as nmol substrate metabolized h^{-1} mg^{-1} protein.

Drugs

Desmethylimipramine was obtained from Ciba-Geigy, clorgyline from May & Baker, (±)-tranylcypromine from Smith, Kline & French, clonidine from Boehringer-Ingelheim, (−)-noradrenaline from Sigma and selegiline from Dr J. Knoll, Semmelweiss University of Medicine, Budapest, Hungary. The radioactive compounds, [^{14}C]-5-hydroxytryptamine creatinine sulphate and [^{14}C]-phenylethylamine hydrochloride were obtained from Amersham and New England Nuclear respectively.

Results

Effects of clorgyline, tranylcypromine, selegiline and desmethylimipramine added directly to field-stimulated vas deferens preparation

In order to understand the effects of chronic treatment with these drugs on pharmacological responses of the isolated vas deferens, it was first of all necessary to examine their effects when added acutely to the isolated tissue.

When the vas deferens was field stimulated at a rate of 0.1 Hz, addition of desmethylimipramine, at concentrations of 10^{-8}M or more, caused a gradual decline in contraction. This effect of desmethylimipramine has been described in detail by Lotti *et al.* (1981). Clorgyline enhanced contractile response to field stimulation at a concentration of 10^{-5}M (+28.6 ±6.5%, n = 5), although at 10^{-6}M it produced no detectable effect. The enhancement of contractions started immediately on addition of the clorgyline to the organ bath, and reached a maximum over 10 to 15 min. Selegiline also enhanced the contractile response to field stimulation (0.1 Hz) at concentrations of 10^{-5}M and above. Enhancement of contractions by selegiline also commenced immediately on addition of the drug to the organ bath, but reached a maximum level within 2 to 3 min. Both clorgyline and selegiline reversed the depression of the contractile response to field stimulation caused by clonidine. Clorgyline (10^{-5}M) produced a rightward, parallel shift in the log dose-response curve to clonidine (Figure 1). Higher concentrations of the clorgyline were not studied,

since they would not be attained following systemic administration of the drug. It should be noted that the response to clonidine was expressed as percentage inhibition of contraction, and since clorgyline enhanced contraction (at 10^{-5}M) the change in absolute response to clonidine was slight. Increase in contractile response following clorgyline was also seen in the presence of propranolol (0.1 μM) and prazosin (24 nM).

Tranylcypromine (10^{-6} to 10^{-5}M) caused a decline in the contractile response to field stimulation in some tissues, while in other experiments it enhanced contractility. The inhibitory effect of tranylcypromine on contraction could be blocked by yohimbine (0.26 μM). The effects of clorgyline, selegiline and tranylcypromine on the contractile response of the vas deferens to field stimulation were reversed on washing the drugs out of the organ bath.

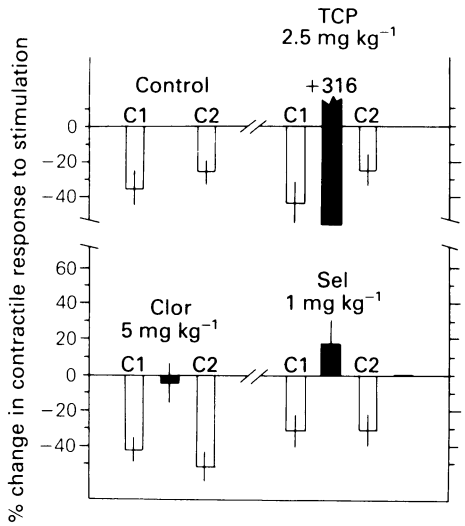

Figure 2 Effect of MAO inhibitors and clonidine on contractions of *in situ* vas deferens of pithed rats following stimulation of lower lumbar sympathetic nerves. Inhibition of contractions (open columns) by two doses of clonidine (1 μg kg^{-1}, i.v.) injected before (C 1) or 20 min after (C 2) intravenous injection of tranylcypromine (TCP), clorgyline (Clor), selegiline (Sel) or an equivalent volume of saline (Control). Peak reduction in contractions immediately after clonidine injection expressed as percentage of contraction preceding clonidine. Change in contractile response to sympathetic stimulation produced by MAO inhibitor drugs alone (peak effect) shown in filled columns. These changes were brief, and contractions returned to control levels within 10 min following injection of MAO inhibitors. Mean values ± standard error presented except for response to TCP, for which maximum and minimum values indicated (n = 5 animals per MAO inhibitor or control group).

Acute effects of clorgyline, tranylcypromine and selegiline on response of vas deferens in situ *to sympathetic stimulation and clonidine in pithed rats*

Effects of the drugs in pithed rats are showed in Figure 2. When injected intravenously, clorgyline, at doses up to 5 mg kg^{-1}, caused only minor, statistically non-significant changes in contractile response of *in situ* vas deferens to sympathetic nerve stimulation. At a dose of 5 mg kg^{-1}, clorgyline also did not modify the inhibitory effect of a single submaximal dose of clonidine on contractions of the vas deferens to sympathetic stimulation. Selegiline caused potentiation of contractile responses to sympathetic stimulation at a dose of 1 mg kg^{-1} in 3 out of 5 preparations, but also did not modify the inhibitory response to clonidine. Tranylcypromine (2.5 mg kg^{-1}) had a highly variable effect on the response of the vas deferens to sympathetic stimulation, causing in some preparations inhibition, in others marked enhancement of contractions, but also did not significantly reduce the inhibitory response to clonidine. Yohimbine (1.5 mg kg^{-1}) consistently potentiated the response of the vas deferens to sympathetic stimulation, and blocked the inhibitory response to clonidine (data not shown).

The effect of acute and chronic treatment of rats with desmethylimipramine and monoamine oxidase inhibitors on responses of isolated vas deferens to clonidine and noradrenaline

The results of these studies are presented in Tables 1 and 2. There was no significant change in strength of contractile response of the isolated vas deferens to electrical field stimulation (0.1 Hz) following chronic treatment with desmethylimipramine or the MAO inhibitors 24 h after 1 or 21 daily doses of the drugs. However, there was also a high degree of variability in this parameter, with individual tissues developing between 600 and 2,000 mg tension.

Desmethylimipramine One day after a single dose (10 mg kg^{-1}, i.p.) of this drug there was no detectable alteration in responsiveness of isolated vas deferens to either clonidine-induced inhibition of contractile response to field stimulation, or of contractile response of the organ to noradrenaline (Table 1). When animals were killed 1 h after a single dose of desmethylimipramine, contractile responses to field stimulation could not be elicited in the isolated vas deferens, and the EC_{50} of the preparation to noradrenaline was reduced (Table 2).

The vas deferens from rats killed 24 h after the last of 21 daily doses of desmethylimipramine (10 mg kg^{-1}) showed increased EC_{50} values for inhibition of contractile response to field stimulation by

Table 1 Presynaptic and postsynaptic α-adrenoceptor agonist responses of isolated vas deferens from rats treated acutely and chronically with desmethylimipramine (DMI) and monoamine oxidase (MAO) inhibitors

Drug treatment	*Clonidine* EC_{50}(nM)	*Noradrenaline* EC_{50}(μM)	Maximal response (mg tension)
Control	(8) 6.8 ± 0.29***	3.08 ± 0.34	1565 ± 132
DMI 21 days	(8) 16.0 ± 1.76	2.72 ± 0.33	2134 ± 148*
Control	(5) 4.5 ± 0.43	1.52 ± 0.36	2175 ± 177
DMI 1 day	(5) 6.5 ± 0.21	1.67 ± 0.33	2029 ± 180
Control	(8) 6.8 ± 0.29	3.08 ± 0.34	1565 ± 132
Clorgyline 21 days	(10) 17.8 ± 1.43***	3.12 ± 0.41	1714 ± 102
Control	(5) 4.5 ± 0.43	1.52 ± 0.36	2175 ± 177
Clorgyline 1 day	(5) 4.8 ± 0.67	1.77 ± 0.32	2509 ± 88
Control	(5) 6.06 ± 0.44	2.80 ± 0.67	3092 ± 169
Tranylcypromine 21 days	(5) 10.81 ± 0.91***	3.21 ± 0.54	3550 ± 198
Control	(5) 5.38 ± 0.40	2.69 ± 0.37	2535 ± 643
Tranylcypromine 1 day	(5) 11.1 ± 1.29***	1.95 ± 0.30	1776 ± 82
Control	(5) 6.81 ± 0.70	2.40 ± 0.60	2299 ± 86
Selegiline 21 days	(5) 5.33 ± 0.72	1.95 ± 0.28	1885 ± 150

Figures in parentheses refer to numbers of animals per group. Mean data given ± s.e.mean. Doses of drugs: clorgyline, 2 mg kg^{-1}; tranylcypromine 5 mg kg^{-1}; selegiline, 1 mg kg^{-1}; DMI, 10 mg kg^{-1}. Tissues removed 24 h after 1 injection (1 day) or 21 daily injections (21 days).
* $P < 0.05$ for difference from control value (*t*-test); ** $P < 0.01$ for difference from control value; *** $P < 0.001$ for difference from control value.

clonidine. This effect has been observed in 3 separate groups of which one representative group is presented in Table 1. The EC_{50} value for noradrenaline-induced contractions of the vas deferens was lower following chronic treatment with desmethylimipramine, but this effect reached statistical significance in only one of the 3 experiments. Maximal response to noradrenaline was significantly enhanced in each of the 3 experiments.

In a separate experiment, dose-response curves to

Table 2 Effect of cocaine and propranolol on responsiveness to noradrenaline of vas deferens from rats treated acutely and chronically with desmethylimipramine (DMI).

		Noradrenaline EC^{50} (μM)		Maximal response (mg tension)	
		Basal	*Cocaine + propranolol*	*Basal*	*Cocaine + propranolol*
Control		3.45 ± 0.78	0.21 ± 0.04	1712 ± 153	1867 ± 210
DMI 1 h	(6)	0.4 ± 0.11**	0.25 ± 0.04	1720 ± 139	1900 ± 251
Control	(6)	2.6 ± 0.69	0.31 ± 0.08	1767 ± 130	1870 ± 353
DMI 21 days	(6)	2.43 ± 0.36	0.30 ± 0.05	2742 ± 220**	3117 ± 344*

Figures in parentheses refer to number of animals per group. Mean data shown ± s.e.mean. Vas deferens removed from rats either 1 h (DMI 1 h) or 24 h after last of 21 daily injections (DMI 21 days) of DMI. Control rats treated similarly with saline. Dose-response curves to noradrenaline measured in basal conditions, and in presence of cocaine (30 μM) and propranolol (1 μM).
* $P < 0.05$, ** $P < 0.01$ for difference from control (*t* test).

noradrenaline were carried out in the presence of cocaine and propranolol, in order to see whether the observed effects of noradrenaline in rats treated chronically with desmethylimipramine were due to inhibition of amine uptake by high residual concentrations of the drug, or selective reduction in inhibitory β-adrenoceptor sensitivity. In these experiments, in which only noradrenaline responses were determined, a dose-response curve was obtained as above and then repeated in the presence of cocaine (30 μM) and propranolol (1 μM). Control experiments showed that a second dose-response curve to noradrenaline (in the absence of cocaine) was identical to the first. Addition of cocaine and propranolol produced the expected reduction in EC_{50} to noradrenaline, with a slight, non-significant, increase in maximal response. In vasa deferentia removed from rats 1 h after desmethylimipramine injection, or following chronic treatment, cocaine-propranolol produced a reduction in EC_{50} to the same value as produced in controls. In chronically treated rats, the maximum response to moradrenaline was greater than in controls both in the presence and the absence of cocaine and propranolol (Table 2).

Clorgyline There was no change in responsiveness of the vas deferens to clonidine or noradrenaline 24 h after a single dose (2 mg kg^{-1}) of clorgyline. However, when tissues were removed from animals killed 24 h after the last of 21 daily doses of clorgyline, EC_{50} values for clonidine were significantly elevated, with no significant change in tissue responsiveness to noradrenaline.

Selegiline There was no significant change in responsiveness of the isolated vas deferens to either clonidine or noradrenaline following 21 daily doses of selegiline (1 mg kg^{-1}) see Table 1.

Table 3 Hepatic monoamine oxidase (MAO) activity in rats treated chronically with clorgyline and selegiline

		Enzyme activity (nmol h^{-1} mg^{-1} protein)	
		MAO-A	*MAO-B*
Control	(5)	131 ± 12.2	11.8 ± 1.8
Clorgyline (2 mg kg^{-1} daily for 21 days)	(5)	18 ± 5.8	2.81 ± 0.83
Selegiline	(5)	69.3 ± 7.1	2.24 ± 0.39

Number of animals in parentheses; mean activity ± s.e.mean.
MAO-A activity determined using [^{14}C]-5-HT (1 mM) as substrate; MAO-B activity determined using [^{14}C]-phenylethylamine (20 μM) as substrate.

Tranylcypromine Treatment of rats with either 1 or 21 daily doses (5 mg kg^{-1}) of tranylcypromine produced a significant elevation of the EC_{50} value for clonidine to a similar extent. There was no significant change in EC_{50} or maximal response to noradrenaline following either acute or chronic treatment with tranylcypromine (Table 1).

Monoamine oxidase activities in tissues of rats injected chronically with clorgyline and selegiline

Chronic treatment with clorgyline produced a marked reduction in both type A and type B MAO activities in liver, to 14 and 24% respectively of control activities. Chronic treatment with selegiline produced a selective inhibition of MAO type B activity, to 19% of control values, while MAO type A activity fell to 52% control (Table 3).

Discussion

Both the tricyclic antidepressant and MAO inhibitor drugs used here had presynaptic effects in the vas deferens. Desmethylimipramine has been reported by others to inhibit contractions of the isolated field-stimulated vas deferens preparation from mice (Harper & Hughes, 1979) and rats (Lotti *et al.*, 1981). This inhibitory effect of desmethylimipramine was converted to a potentiation after treatment with yohimbine, and was reduced by pretreatment with reserpine (Lotti *et al.*, 1981), showing that it is probably due to potentiation of the effect of neuronally-released noradrenaline on α_2-presynaptic receptors. This action of desmethylimipramine is seen at low concentrations, of an order which may be present in tissues removed from animals previously injected with the drug. Since contractions of the tissue in response to field stimulation and exogenous noradrenaline were normal 24 h but not 1 h after desmethylimipramine injection, the 24 h 'wash-out' period appears to be adequate to allow most of the drug to be cleared from the tissues. Desmethylimipramine tissue concentrations, however, are increased following 21 daily repeated injections (Vetulani *et al.*, 1976). The reduction in presynaptic α-adrenoceptor sensitivity observed following chronic desmethylimipramine treatment may, therefore, be underestimated in the present experiments. Further work will be required to determine whether a significant presynaptic effect of desmethylimipramine remains in tissues removed from animals treated chronically with the drug. Reduction in the presynaptic effect of clonidine by chronic desmethylimipramine treatment is unlikely to be due to a direct receptor blocking effect of this drug, which has only very weak antagonistic effects at α_2-adrenoceptors (Maggi *et al.*, 1980). Desmethylimipramine has,

however, been shown to reduce the inhibitory effect of clonidine on stimulation-evoked [^{3}H]-noradrenaline overflow when added acutely to the *in vitro* system of superfused rat cerebral cortex slices (Pelayo *et al.*, 1980). Such a direct interaction between clonidine and desmethylimipramine is not believed to occur in the vas deferens system, because of the 24 h 'wash-out' period used, as described above.

The potentiation of contractile response to field stimulation produced by clorgyline (10^{-5}M) *in vitro* was accompanied by a slight α_2-adrenoceptor blocking effect, which may or may not explain the potentiation. Since neither potentiation of contraction of sympathetically stimulated vas deferens, nor antagonism of the clonidine effect was seen in the pithed rat preparation, it is unlikely that an α_2-adrenoceptor antagonistic effect of clorgyline played a role in the altered α_2-adrenoceptor sensitivity seen in chronically treated animals. This direct α_2-adrenoceptor antagonistic effect was not seen when vas deferens were removed 24 h after a single dose of clorgyline, and appears to be a response seen only following direct addition of the drug to the isolated tissue. The dose of clorgyline used produced substantial inhibition of both MAO types (A and B) whereas selegiline, at the dose used in this experiment, produced a more selective inhibition of MAO type B. Reduction in α_2-presynaptic adrenoceptor sensitivity, therefore, is not associated with inhibition of MAO type B, although the present data do not show that this effect can be produced by selective inhibition of MAO type A. The results of this study, however, are comparable with results of receptor binding studies showing that chronic treatment with clorgyline but not selegiline is effective in down-regulating the number of central α_2-adrenoceptors (Cohen *et al.*, 1982b).

The ability of selegiline to potentiate the effects of sympathetic stimulation in isolated vas deferens was also observed by Knoll (1976). Selegiline has sympathomimetic properties (Simpson, 1978) which may be responsible for this effect. Tranylcypromine also possesses intrinsic amphetamine-like properties, and is an effective inhibitor of neuronal amine uptake (Hendley & Snyder, 1968). The variable effects of tranylcypromine, its ability both to potentiate and inhibit contractile responses to sympathetic stimulation, may be explained by its dual effects of releasing noradrenaline and blocking neuronal re-uptake. The complex pharmacology of tranylcypromine may explain its ability to reduce α_2-presynaptic receptor sensitivity on both acute and chronic treatment, and this response may not be typical of MAO inhibitors in general. Despite the complex effects of tranylcypromine, it was considered important to study the action of this drug on receptor modulation, in view of its proven efficacy as an antidepressant drug.

Of the antidepressant drugs used in this study, only desmethylimipramine modified responsiveness of the vas deferens to noradrenaline on chronic treatment. The increased post-synaptic effect of noradrenaline following chronic desmethylimipramine (i.e. increased maximal contraction) was still present in cocaine-treated tissues, and, therefore, does not appear to be due to inhibition of neuronal noradrenaline uptake by residual drug. The use of cocaine, however, has certain limitations (Lew & Angus, 1983). The increased postsynaptic response to noradrenaline following desmethylimipramine treatment may be contrasted with the observations of Wetzel *et al.*, (1981) who obtained decreased binding of the α_1-adrenoceptor ligand [^{3}H]-WB4101, in the rat vas deferens following chronic treatment with desmethylimipramine (13.6 mg kg^{-1} day^{-1} administered in the drinking water). Density of α_1-adrenoceptors (B_{max}) was reduced by 21% with no change in the affinity constant. Thus chronic treatment with desmethylimipramine causes reduction in post-synaptic α_1-receptor number with increased physiological responsiveness to noradrenaline. This situation may be compared with that following surgical denervation of the vas deferens, which also results in increased physiological response to exogenous noradrenaline and a 40% reduction in B_{max} for [^{3}H]-WB4101 binding (Hata *et al.*, 1981). In the case of chronic denervation, the increase in sensitivity to exogenous noradrenaline caused by blockade of neuronal uptake apparently predominates over the reduction in sensitivity resulting from reduced receptor number. The present finding of an increased maximal response to noradrenaline in the cocaine-treated organ, following chronic treatment with desmethylimipramine, is indicative of an enhanced post-receptor mechanism.

The results of chronic treatment with antidepressant drugs on α_1-adrenoceptor function in other systems are highly variable. Electrophysiological and behavioural experiments show enhanced sensitivity to noradrenaline in the CNS, while most results with α_1-adrenoceptor ligands show no change in receptor number following a variety of antidepressant drugs (for review see Sugrue, 1983). Chronic treatment with clorgyline, however, did reduce the density of α_1-adrenoceptors estimated by [^{3}H]-WB4101 binding in the cerebral cortex (Cohen *et al.*, 1982b).

The use of the vas deferens to estimate receptor sensitivity presents certain difficulties. Responsiveness of the vas deferens to noradrenaline shows considerable variability between animals (Ambache & Zar, 1971). Nevertheless, this study has demonstrated certain adaptive changes in peripheral adrenoceptors following chronic antidepressant treatment, which bear similarities to changes seen in the CNS, i.e. reduction in presynaptic α_2-adrenoceptor sensitivity by inhibitors of MAO type A but not type B, and by a tricyclic antidepressant. Effects of drug treatment on

neurotransmission in the CNS differ from those in the periphery because of differences in synaptic cleft dimensions, receptor distribution and type, efficiency of uptake processes to alter synaptic cleft neurotransmitter concentration and other factors. While not providing an exact model of CNS conditions, the peripheral tissue system described here reproduces the effect of reduced presynaptic receptor sensitivity in conditions which are amenable to further study. In particular, the relationship between altered presynaptic receptor sensitivity and noradrenaline release by sympathetic stimulation will be of interest in this model, and study of this situation may shed further light on similar processes occurring in the CNS. In addition, the effect of chronically-administered antidepressant drugs on the sympathetic nervous system requires further clarification. One factor which must be borne in mind is that antidepressant drugs could affect sympathetically-innervated tissues by altering central sympathetic outflow. Since Sax & Westfall (1981) have demonstrated reduced α_2-presynaptic adrenoceptor sensitivity in animals treated chronically with a ganglion-blocking drug, this possible effect will require evaluation in the present model.

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