Br. J. Pharmac. (1980), **68**, 241–250

EFFECT OF MIANSERIN ON NORADRENERGIC TRANSMISSION IN THE RAT ANOCOCCYGEUS MUSCLE

SHEILA A. DOGGRELL

Department of Pharmacology and Clinical Pharmacology, School of Medicine, University of Auckland, Private Bag, Auckland, New Zealand

1 The effects of mianserin on the accumulation of (−)-[^{3}H]-noradrenaline and on contractile responses to field stimulation, exogenously applied (−)-noradrenaline, and to tyramine were studied in the rat anococcygeus muscle.

2 Mianserin (10^{-9} to 10^{-5} M) and nortriptyline (10^{-9} to 10^{-5} M) inhibited the accumulation of (−)-[^{3}H]-noradrenaline. In this aspect mianserin had a similar potency to nortriptyline, the most potent tricyclic antidepressant in inhibiting noradrenaline accumulation in this tissue.

3 Mianserin 10^{-9} or 10^{-8} M alone had no effect on contractile responses to field stimulation and to (−)-noradrenaline but inhibited the responses to tyramine. The responses to field stimulation at low frequencies and to (−)-noradrenaline were potentiated by 10^{-7} and 10^{-6} M mianserin. It is suggested that the inhibitory effect mianserin has on neuronal accumulation is primarily responsible for these effects. Mianserin 10^{-5} M inhibited responses to field stimulation and to (−)-noradrenaline.

4 In the presence of nortriptyline (10^{-6} M), the contractile responses to field stimulations were potentiated by mianserin (10^{-8}, 10^{-7} and 10^{-6} M), 10^{-8} M being the most potent in this aspect. Mianserin 10^{-8}, 10^{-7}, 10^{-6} and 10^{-5} M had a similar inhibitory effect on responses to (−)-noradrenaline. In the absence of neuronal uptake, the potentiating effect of mianserin on responses to field stimulation may be due to antagonism at presynaptic α-adrenoceptors. In the presence of 10^{-6} M nortriptyline, 10^{-5} M mianserin abolished responses to field stimulation.

5 Following incubation of the tissue in the presence of 6-hydroxydopamine (10^{-3} M for 3 h), mianserin (10^{-7}, 10^{-6} and 10^{-5} M) nortriptyline (10^{-7} and 10^{-6} M) and phentolamine (5×10^{-8} and 5×10^{-7} M) inhibited contractile responses to (−)-noradrenaline. This illustrates the ability of these agents to inhibit the responses to noradrenaline at a postsynaptic site. The inhibitory effect was dose-related with nortriptyline and phentolamine; this illustrates the ability of these agents to antagonize postsynaptic α-adrenoceptors. The inhibitory effect observed with mianserin was not dose-related. This suggests that in addition to its reported ability to antagonize postsynaptic α-adrenoceptors, mianserin may have another post-synaptic action at the level of, or distal to, the α-adrenoceptor.

6 These results illustrate that, in the rat anococcygeus muscle, mianserin is a potent inhibitor of noradrenaline accumulation and may be an antagonist at presynaptic α-adrenoceptors. Mianserin also inhibits the responses to exogenously applied noradrenaline in this tissue by an action or actions at the level of, or distal to, the postsynaptic α-adrenoceptor.

Introduction

Mianserin, 1,2,3,4,10,14b-hexahydro-2-methyldibenzo-[*c*,*f*]pyrazino [1,2-α]azapine, is clinically an effective antidepressant agent (reviewed by Brogden, Heel, Speight & Avery, 1978) which differs from tricyclic antidepressants not only chemically but in several other aspects. Mianserin lacks anticholinergic and cardiotoxic effects (reviewed by Peet & Behagel, 1978; Brogden *et al.*, 1978) and has unusual effects on monoamine metabolism (Leonard, 1978). Thus mianserin, but not tricyclic antidepressants, increases the turnover of noradrenaline in the rat brain. Furthermore, it seems likely that the effects of mianserin on noradrenergic transmission are quantitatively different from those observed with tricyclic antidepressants.

Tricyclic antidepressants are potent inhibitors of the neuronal uptake of noradrenaline both *in vivo* and *in vitro* (reviewed by Biel & Bopp, 1974). Mianserin does inhibit the neuronal uptake of noradrenaline *in vitro* but is generally less potent than the tricyclic antidepressants (Raiteri, Angelini & Bertollini, 1976;

0007-1188/80/020241-10 $01.00

Baumann & Maitre, 1977; Goodlet, Mireylees & Sugrue, 1977; Harper & Hughes, 1977; de Paulis, Kelder Ross & Stjernström, 1978). Furthermore, it has been demonstrated that mianserin is effective, but less potent than imipramine, both *in vivo* and *in vitro* in inhibiting the neuronal uptake system (rat brain, Baumann & Maitre, 1977; rabbit brain, Goodlet *et al.*, 1977).

In addition to its effects on noradrenaline uptake, mianserin influences noradrenergic transmission at the level of the α-adrenoceptor. Mianserin has a similar potency to phentolamine as an antagonist at pre- and postsynaptic α-adrenoceptors (Borowski, Ehrl & Starke, 1976; Doxey, Everitt & Metcalf, 1978). In this aspect it seems likely that mianserin is more potent than the tricyclic agents. High concentrations of the tricyclic antidepressants are necessary to antagonize the post-synaptic α-adrenoceptors (Sturmann, 1971; Doggrell & Woodruff, 1977). Similarly the relatively high potency mianserin has as an antagonist at presynaptic α-adrenoceptors is not shared by desipramine (Baumann & Maitre, 1977).

In the present paper, the effects of mianserin on the accumulation of (−)-[^{3}H]-noradrenaline in the rat anococcygeus muscle are described. The effects of mianserin on contractile responses to field stimulation and to exogenously applied (−)-noradrenaline and tyramine in this tissue have also been studied.

Methods

Mature male Wistar rats were killed by a blow at the base of the skull and exsanguinated. Anococcygeus muscles were dissected as described by Gillespie (1972). All experiments were performed in the presence of a modified Krebs solution of the following composition (mM): NaCl 116, KCl 5.4, $CaCl_2$ 2.5, $MgCl_2$ 1.2, NaH_2PO_4 1.2, $NaHCO_3$ 22.0, D-glucose, 11.2 and Na_2EDTA 0.04, equilibrated with 5% CO_2 in O_2, at 37°C.

Determination of (−)-[^{3}H]-noradrenaline accumulation

Each anococcygeus muscle was mounted on a wire frame under 0.2 to 0.5 g tension in 10 ml Krebs solution. The tissues were equilibrated for 15 min and 5×10^{-8} M (−)-[^{3}H]-noradrenaline was then added for 10 min. The muscles were blotted and transferred to 10 ml of drug-free Krebs solution for a final 10 min wash after which they were blotted and weighed. Each muscle was placed in a test tube with 1 ml of 'Protosolve' (NaOH 120 g in one litre of methanol). When the tissue had dissolved, 10 ml of a toluene-based scintillation fluor and 0.5 ml of glacial acetic acid were added. The tritium in the tissue and medium was determined by liquid scintillation spectrometry. Quenching was corrected for by the channels ratio method.

When studying the effect of either mianserin or nortriptyline on noradrenaline accumulation, different concentrations of these drugs were added to the Krebs solution 5 min before the incubation with (−)-[^{3}H]-noradrenaline. Tissue:medium ratios were calculated.

Recording of contractile responses

Individual anococcygeus muscles were mounted under 0.5 g tension in 5 ml organ baths containing Krebs solution. The tissues were allowed to recover for 30 min, the resting tension being maintained throughout. Tissues were placed between two platinum electrodes, and were stimulated to contract using biphasic pulses of 1 ms duration and supramaximal voltage. Dose-response curves for agonists were determined non-cumulatively. Stimulation or exposure to agonist was continued for 30 s or, if a maximum response was not reached in that time, until a maximum response was obtained. The tissues were then allowed to recover fully, with a minimum period of 5 min, before further stimulation or addition of agonist occurred. Contractile responses were recorded isometrically with force displacement transducers (Grass model FT03.C) connected to a polygraph (Grass model 79B).

When studying the effect of mianserin alone on responses or in the presence of nortriptyline, the drugs were present in the Krebs solution from the beginning of the recovery period. For the 6-hydroxydopamine experiments, the isolated muscles were incubated in the presence of 10^{-3} M 6-hydroxydopamine for 3 h after which they were washed by overflow for 30 min in Krebs solution. When the effects of miaserin, nortriptyline or phentolamine on contractile responses following 6-hydroxydopamine incubation were studied, the drugs were present in the Krebs solution from the beginning of the wash period.

The values obtained, under different conditions, were compared by Student's paired t test and were considered to be significantly different when $P < 0.05$.

When the maximum responses (g), in the presence and absence of drugs, were not significantly different, responses were calculated as a percentage of the maximum response of the individual dose-response curve (i.e., normalized). pD_2 values (negative logarithm of molar concentration of agonist producing 50% of the maximum response) were determined by regression line analysis (over the range 20 to 80% of the maximum response) by a computer (Wang 600). The ability of drugs to potentiate or inhibit responses is expressed as the dose-ratio (the antilog of the difference between the pD_2 values in the presence and absence of drugs). pA_2 values (the negative

logarithm of molar concentration of antagonist which causes a twofold shift of the dose-response curve for the agonist) were determined using the formula $pA_2 = pA_x + \log (x - 1)$, where pA_x is the negative logarithm of the molar concentration of antagonist and x is the dose-ratio (inhibition).

When the maximum responses, in the presence and absence of drugs were significantly different, responses were calculated as a percentage of the maximum response of the control dose-response curve. In this case, it was not possible to determine pD_2 or pA_2 values.

(−)-[^{3}H]-noradrenaline with a specific activity of 2.2 Ci/mmol was obtained from the New England Nuclear Corporation. The other drugs used were phentolamine mesylate* (Ciba), nortriptyline hydrochloride* (Eli Lilly and Co. Ltd), mianserin hydrochloride* (Organon), and 6-hydroxydopamine hydrochloride, (−)noradrenaline bitartrate and tyramine hydrochloride (Sigma Chemicals Ltd). Compounds indicated with an asterisk were generously donated by the companies shown.

Results

Effects of mianserin and nortriptyline on noradrenaline accumulation

Following a 10 min incubation in the presence of 5×10^{-8} M (−)-[^{3}H]-noradrenaline, the rat anococcygeus muscle accumulated noradrenaline giving a tissue:medium ratio of approximately 7:1 (Table 1). This accumulation was inhibited by 10^{-9} M mianserin and 10^{-9} M nortriptyline to a similar degree; 30% and 33%, respectively (Table 1). Higher concentration of these agents also reduced the accumulation: 10^{-8} and 10^{-7} M nortriptyline had a greater inhibitory effect than 10^{-8} and 10^{-7} M mianserin, respectively (Table 1). At 10^{-6} and 10^{-5} M nortriptyline and mianserin were equipotent (Table 1).

Contractility studies

Mianserin had no effect on the resting tone of the rat anococcygeus muscle.

The effect of mianserin alone in Krebs solution Low concentrations of mianserin (10^{-9} and 10^{-8} M) had no effect on contractile responses to field stimulation; this included the magnitude of the maximal response (Table 2). The effect of 10^{-7} M mianserin on the magnitude of the maximal response to field stimulation was tested in 4 preparations; a smaller maximum response was observed in 3 preparations and a larger maximum response seen in the other preparation; 10^{-7} M mianserin potentiated the responses to field stimulation at 1 to 10 Hz (Figure 1a). At 10^{-6} M, mianserin reduced the magnitude of the maximal response to field stimulation in all preparations tested ($n = 8$, see Table 2) but potentiated the responses at some lower frequencies, i.e., 2 and 5 Hz (Figure 2b). The responses to field stimulation at 1 and 2 Hz and at 5 to 40 Hz were abolished and greatly reduced, respectively, by 10^{-5} M mianserin (Figure 1c).

Mianserin (10^{-9} to 10^{-5} M) had no effect on the magnitude of the maximal responses to (−)-noradrenaline (Table 2). The responses to (−)-noradrenaline were unaffected by 10^{-9} M mianserin (Table 3). At 10^{-7} and 10^{-6} M, mianserin potentiated responses to (−)-noradrenaline to a similar extent, i.e. ×11, whereas 10^{-5} M mianserin inhibited the responses (Table 3).

Mianserin 10^{-9} M had no effect on the maximum responses to tyramine (Table 2) and inhibited the responses at lower concentrations (Figure 2a) with a

Table 1 The effect of mianserin and nortriptyline on (−)-[^{3}H]-noradrenaline accumulation in the rat anococcygeus muscle

		Tissue/medium ratios (Mean ± s.e. mean)				
(A)	Control (Mianserin)	7.40 ± 0.76 (6)	(B)	Control (Nortriptyline)	6.56 ± 0.78 (6)	NS
	10^{-9} M	5.22 ± 0.65 (8)*		10^{-9} M	4.38 ± 0.47 (5)*	NS
	10^{-8} M	5.78 ± 0.20 (8)		10^{-8} M	3.66 ± 0.28 (4)	**
	10^{-7} M	4.70 ± 0.56 (6)		10^{-7} M	2.82 ± 0.42 (6)	**
	10^{-6} M	2.67 ± 0.19 (4)		10^{-6} M	2.25 ± 0.54 (6)	NS
	10^{-5} M	0.88 ± 0.08 (4)		10^{-5} M	0.98 ± 0.15 (6)	NS

* $P < 0.05$, compared to control by unpaired t test.
The tissue/medium ratios in the presence of the same concentration of mianserin or nortriptyline were compared by unpaired t test; NS = not significantly different, ** $P < 0.0125$.
(n) = number of observations.

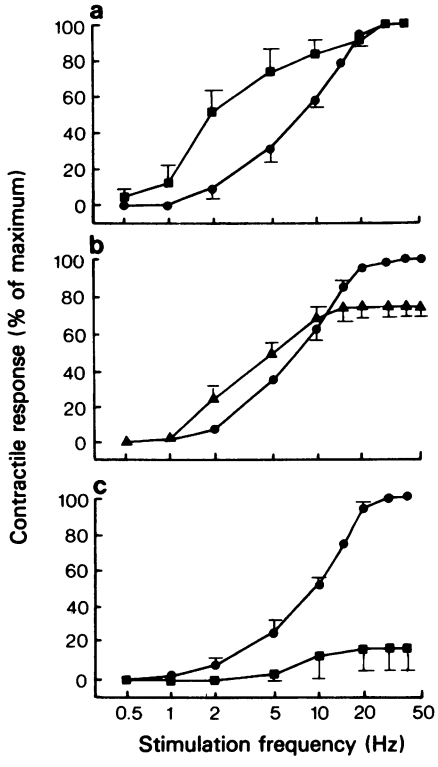

Figure 1. Effect of mianserin on responses to field stimulation in the rat anococcygeus muscle. Responses to field stimulation in Krebs solution (●), and in the presence of (a) 10^{-7} M mianserin (■), (b) 10^{-6} M mianserin (▲) and (c) 10^{-5} M mianserin (■). Responses in (a) are expressed as a percentage of the maximum response of the individual dose-response curves and in (b) and (c) as a percentage of the maximum response of the control dose-response curve. Each value is the mean from 4 to 6 preparations; vertical lines show s.e. mean.

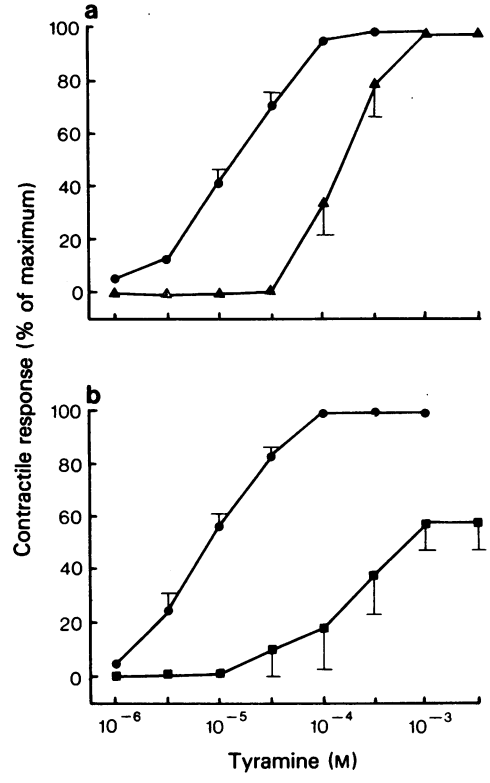

Figure 2 Effect of mianserin on responses to tyramine in the rat anococcygeus muscle. Responses to tyramine in Krebs solution (●), and in the presence of (a) 10^{-9} M mianserin (▲) and (b) 10^{-8} M mianserin (■). Responses in (a) are expressed as a percentage of the maximum response of the individual dose-response curves and in (b) as a percentage of the maximum response of the control dose-response curve. Each value is the mean from 4 preparations; vertical lines show s.e. mean.

Table 2 The effect of mianserin alone in Krebs solution on maximal responses to field stimulation, (−)-noradrenaline and tyramine in rat anococcygeus muscle

		Maximum response (Mean ± s.e. mean)		
		Field stimulation	*(−)-Noradrenaline*	*Tyramine*
(a) (i)	Control	4.01 ± 0.70 (4)	6.54 ± 1.21 (4)	4.70 ± 0.67 (4)
(ii)	Mianserin 10^{-9} M	4.40 ± 1.16 (4)	7.26 ± 0.65 (4)	3.31 ± 1.50 (4)
(b) (i)	Control	6.04 ± 0.42 (4)	6.56 ± 0.64 (4)	6.41 ± 0.78 (4)
(ii)	Mianserin 10^{-8} M	5.66 ± 0.71 (4)	7.01 ± 1.08 (4)	3.51 ± 0.26 (4)*
(c) (i)	Control	6.33 ± 0.38 (4)	6.90 ± 0.45 (5)	6.45 ± 0.37 (4)
(ii)	Mianserin 10^{-7} M	4.63 ± 1.04 (4)	6.26 ± 0.93 (5)	3.64 ± 0.89 (4)*
(d) (i)	Control	7.71 ± 0.56 (8)	7.63 ± 0.59 (10)	4.71 ± 0.99 (4)
(ii)	Mianserin 10^{-6} M	5.49 ± 0.41 (8)**	7.28 ± 0.27 (10)	0.19 ± 0.13 (4)**
(e) (i)	Control	5.40 ± 0.79 (5)	7.63 ± 0.76 (4)	—
(ii)	Mianserin 10^{-5} M	0.77 ± 0.68 (5)**	6.71 ± 0.32 (4)	—

* $P < 0.025$, paired t test; ** $P < 0.0025$, paired t test.
(n) = number of observations.

dose-ratio of 9; this inhibition was competitive in nature as the slopes of the dose-response curves, in the presence and absence of 10^{-9} M mianserin, were not significantly different. With higher concentrations of mianserin (10^{-8}, 10^{-7} and 10^{-6} M; for results with 10^{-8} M mianserin see Figure 2b), the inhibitory effect on responses to tyramine included a reduction in the magnitude of the maximum response and was, therefore, non-competitive.

In the presence of nortriptyline (10^{-6} M) 10^{-8}, 10^{-7} and 10^{-6} M mianserin had no effect on the magnitude of the maximum responses to field stimulation (Table 4). The responses to lower frequencies of stimulation were potentiated by mianserin (10^{-8} to 10^{-6} M); 10^{-8} M was the most potent and potentiated responses at 5 to 20 Hz (Figure 3a), 10^{-7} M mianserin potentiated responses at 5 to 15 Hz, and 10^{-6} M mianserin was the least potent in this aspect and only potentiated responses at 5 and 10 Hz (Figure 3b). In the presence of 10^{-6} M nortriptyline, 10^{-5} M mianserin abolished all responses to field stimulation (Table 4).

In the presence of nortriptyline (10^{-6} M), the magnitude of the maximum response to (−)-noradrenaline was unaltered by mianserin, 10^{-8}, 10^{-7} and 10^{-5} M (Table 5). Mianserin inhibited the responses to (−)-noradrenaline (Table 5) and this inhibition was not dose-related; thus the responses were inhibited $\times 7.6 \pm 3.3(5)$ [mean dose-ratio (inhibition) ±s.e. mean $n = 5$], $\times 6.7 \pm 2.6(6)$, $\times 6.6 \pm 0.7(4)$, $\times 2.5 \pm 0.5(5)$ by 10^{-8}, 10^{-7}, 10^{-6} and 10^{-5} M mianserin, respectively. As a consequence of this, the apparent pA_2 values for mianserin decreased with increasing concentrations of mianserin (Table 5). Furthermore, the pA_2 values obtained with different concentrations of mianserin were significantly differ-

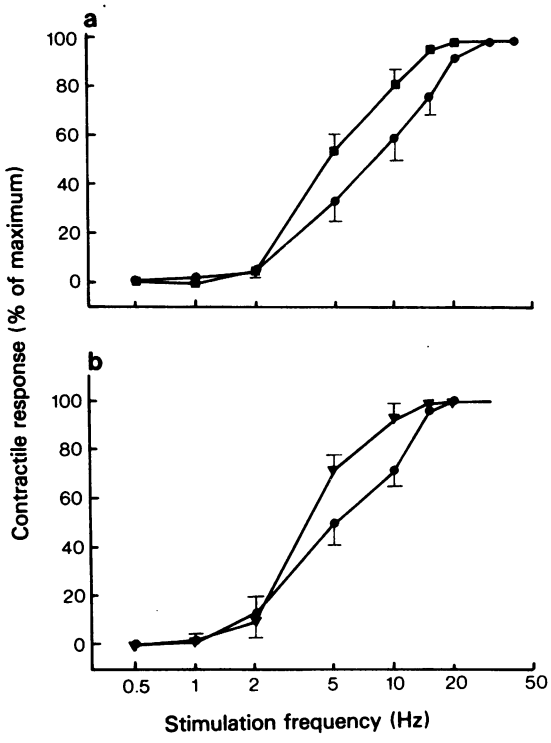

Figure 3 In the presence of nortriptyline, the effect of mianserin on responses to field stimulation in the rat anococcygeus muscle. All responses were obtained in the presence of 10^{-6} M nortriptyline; control responses (●) and responses in the presence of (a) 10^{-8} M mianserin (■) and (b) 10^{-6} M mianserin (▼) are shown. All responses are expressed as a percentage of the maximum response of the individual dose-response curve. Each value is the mean from 4 to 5 preparations; vertical lines show s.e. mean.

Table 3 The effect of mianserin alone in Krebs solution on responses to (−)-noradrenaline

		(−)-Noradrenaline pD_2	(−)-Noradrenaline Dose-ratio
(a)	(i) Control	5.58 ± 0.12 (4)	
	(ii) Mianserin 10^{-9} M	5.82 ± 0.13 (4)	
(b)	(i) Control	5.81 ± 0.18 (4)	
	(ii) Mianserin 10^{-8} M	6.02 ± 0.09 (4)	
(c)	(i) Control	5.47 ± 0.19 (4)	11.01 ± 5.84 (4)
	(ii) Mianserin 10^{-7} M	6.29 ± 0.19 (4)*	(Potentiation)
(d)	(i) Control	5.43 ± 0.10 (6)	10.45 ± 6.10 (6)
	(ii) Mianserin 10^{-6} M	6.12 ± 0.25 (6)*	(Potentiation)
(e)	(i) Control	5.48 ± 0.07 (4)	3.24 ± 0.68 (4)
	(ii) Mianserin 10^{-5} M	5.00 ± 0.09 (4)*	(Inhibition)

Each value is the mean ± s.e. mean (n) where n = number of observations.
* $P < 0.01$, paired t test.

ent; thus the pA_2 values obtained in the presence of 10^{-6} M mianserin were significantly larger than the pA_2 values obtained in the presence of 10^{-5} M mianserin, and those values obtained in the presence of 10^{-8} M mianserin were greater than the pA_2 values obtained with 10^{-7} M mianserin (comparison of individual values by unpaired t test).

After 6-hydroxydopamine (10^{-3} M) *incubation* for 3 h, the responses to (−)-noradrenaline are potentiated and the responses to tyramine and other indirectly acting amines are abolished in the rat anococcygeus muscle (Doggrell & Woodruff, 1978). After such treatment, mianserin (10^{-7}, 10^{-6} and 10^{-5} M), nortriptyline (10^{-7} and 10^{-6} M) and phentolamine (5×10^{-8}

Table 4 In the presence of 10^{-6} M nortriptyline, the effect of mianserin on maximum responses to field stimulation

	In the presence of Nortriptyline 10^{-6} M	*Field stimulation maximum response* (*g*) (Mean ± s.e. mean)
(a)	(i) Control	3.59 ± 0.38 (4)
	(ii) Mianserin 10^{-8} M	4.25 ± 0.64 (4)
(b)	(i) Control	4.05 ± 0.77 (4)
	(ii) Mianserin 10^{-7} M	3.55 ± 0.56 (4)
(c)	(i) Control	3.05 ± 0.50 (4)
	(ii) Mianserin 10^{-6} M	3.39 ± 0.51 (4)
(d)	(i) Control	3.53 ± 0.47 (6)
	(ii) Mianserin 10^{-5} M	0.00 (6)*

* $P < 0.0005$, paired t test.
(n) = number of observations.

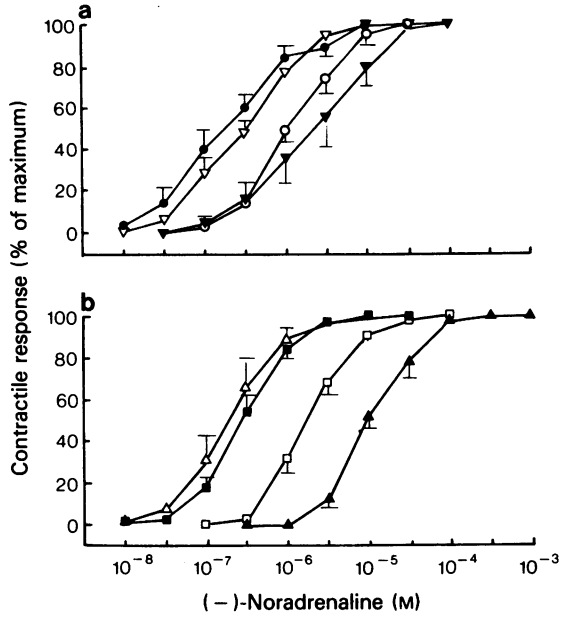

Figure 4 Following 6-hydroxydopamine incubation, the effect of mianserin and phentolamine on responses to (−)-noradrenaline in the rat anococcygeus muscle. All responses were obtained following 6-hydroxydopamine incubation (10^{-3} M for 3 h): (a) (i) control (●) and in the presence of 10^{-7} M mianserin (○), and (ii) control (▽) and in the presence of 10^{-6} M mianserin (▼); (b) (iii) control (■) and in the presence of 5×10^{-8} M phentolamine (□) and (iv) control (△) and in the presence of 5×10^{-7} M phentolamine (▲). All responses are expressed as a percentage of the maximum response of the individual dose-response curve. Each value is the mean from 4 to 6 preparations; vertical lines show s.e. mean.

Table 5 In the presence of 10^{-6} M nortriptyline: the effect of mianserin on responses to (−)-noradrenaline in the rat anococcygeus muscle

	In the presence of 10^{-6} M *nortriptyline*	(−)-*Noradrenaline* *Maximum response* (*g*)	*pD₂ value*	*pA₂ value*
(a)	(i) Control	5.59 ± 0.82 (5)	6.35 ± 0.19 (5)	8.56 ± 0.26 (5)
	(ii) Mianserin 10^{-8} M	6.28 ± 0.52 (5)	5.63 ± 0.05 (5)*	
(b)	(i) Control	5.07 ± 0.71 (6)	6.37 ± 0.14 (6)	7.36 ± 0.33 (6)
	(ii) Mianserin 10^{-7} M	5.04 ± 0.70 (6)	5.74 ± 0.13 (6)*	
(c)	(i) Control	5.23 ± 0.30 (4)	6.71 ± 0.15 (4)	6.72 ± 0.06 (4)
	(ii) Mianserin 10^{-6} M	5.09 ± 0.56 (4)	5.89 ± 0.11 (4)**	
(d)	(i) Control	5.57 ± 0.61 (5)	6.10 ± 0.12 (5)	5.10 ± 0.14 (5)
	(ii) Mianserin 10^{-5} M	5.08 ± 0.49 (5)	5.73 ± 0.11 (5)**	

Each value is the mean ± s.e. mean (n), where n = number of observations.
* $P < 0.0025$, paired t test.
** $P < 0.0005$, paired t test.

and 5×10^{-7} M) had no effect on the magnitude of the maximum responses to (−)-noradrenaline and inhibited the submaximal responses (Table 6). Under these conditions, the inhibitory effect of mianserin on responses to (−)-noradrenaline was not dose-related; 10^{-7}, 10^{-6} and 10^{-5} M inhibited the responses $\times 11.4 \pm 4.7(6)$, $\times 10.7 \pm 4.6(6)$ and $\times 18.8 \pm 5.6(4)$, respectively. This lack of dose-related effect with mianserin resulted in apparent pA_2 values for mianserin ranging from 6.20 to 7.65 (Table 6). Furthermore, the pA_2 values obtained in the presence of 10^{-7} M mianserin were significantly greater than those obtained in the presence of 10^{-5} M mianserin.

Under similar conditions, the inhibitory effects of both nortriptyline and phentolamine were dose-related. Thus the responses to (−)-noradrenaline were inhibited $\times 4.6 \pm 1.4(5)$ and $\times 19.3 \pm 9.2(6)$ by 10^{-7} and 10^{-6} M nortriptyline respectively, and by $\times 8.7 \pm 3.3(4)$ and $\times 70.4 \pm 34.2(4)$ by 5×10^{-8} and 5×10^{-7} M phentolamine, respectively. The pA_2 values for nortriptyline and phentolamine were independent of concentration used and approximated 7.20 and 8.00, respectively (Table 6). The differing effects of mianserin and phentolamine on responses to (−)-noradrenaline are illustrated in Figure 4.

Discussion

Mianserin is a potent inhibitor of the accumulation of (−)-[^{3}H]-noradrenaline in the rat anococcygeus muscle. In the presence of low concentrations of noradrenaline, accumulation represents neuronal uptake minus any efflux. Noradrenaline releasing agents, e.g. guanethidine, labetalol, cause contractions of the rat anococcygeus muscle (Doggrell & Paton, 1978a, b). As mianserin had no effect on the tone of this preparation it seems unlikely that mianserin releases noradrenaline to any great extent. This suggests that mianserin is a potent inhibitor of neuronal uptake in this tissue.

Of a series of ten tricyclic antidepressants tested for their ability to inhibit noradrenaline uptake in the rat anococcygeus muscle, Doggrell & Woodruff (1977) demonstrated that nortriptyline was the most potent. In the present study at a concentration of 10^{-9} M, mianserin was equipotent with nortriptyline in producing a 30% inhibition of the neuronal uptake of noradrenaline. The effect of nortriptyline, but not of mianserin, on noradrenaline uptake was closely dose-related. Thus 10^{-9}, 10^{-8} and 10^{-7} M mianserin had similar inhibitory effects on neuronal uptake; the reason for this is not immediately apparent. Under these circumstances the calculation and comparison of an IC_{50} value (concentration causing 50% inhibition of uptake) for nortriptyline and mianserin is not valid. However, a valid comparison may be made at each concentration level. Thus the inhibition produced by 10^{-8} and 10^{-7} M mianserin was less than that observed with 10^{-8} and 10^{-7} M nortriptyline, respectively. At higher concentrations (10^{-6} and 10^{-5} M), mianserin and nortriptyline were equipotent.

Table 6 Following 6-hydroxydopamine incubation (10^{-3} M for 3 h): the effect of mianserin, nortriptyline and phentolamine on responses to (−)-noradrenaline in the rat anococcygeus muscle

Following 6-hydroxydopamine incubation (10^{-3} M *for 3 h*)		(−)-*Noradrenaline* *Maximum response*	*pD_2 value*	*pA_2 value*
(a)	(i) Control	5.91 ± 1.16 (6)	6.78 ± 0.17 (6)	7.65 ± 0.29 (6)
	(ii) Mianserin 10^{-7} M	4.80 ± 0.99 (6)	5.96 ± 0.10 (6)*	
(b)	(i) Control	4.49 ± 0.58 (6)	6.58 ± 0.11 (6)	6.68 ± 0.26 (6)
	(ii) Mianserin 10^{-6} M	5.10 ± 1.09 (6)	5.78 ± 0.26 (6)*	
(c)	(i) Control	5.46 ± 0.73 (4)	6.38 ± 0.08 (4)	6.20 ± 0.14 (4)
	(ii) Mianserin 10^{-5} M	5.08 ± 0.65 (4)	5.14 ± 0.09 (4)**	
(d)	(i) Control	5.46 ± 1.07 (5)	6.77 ± 0.07 (5)	7.40 ± 0.19 (5)
	(ii) Nortriptyline 10^{-7} M	7.26 ± 0.87 (5)	6.18 ± 0.09 (5)*	
(e)	(i) Control	4.89 ± 0.62 (6)	6.72 ± 0.14 (6)	7.08 ± 0.16 (6)
	(ii) Nortriptyline 10^{-6} M	6.89 ± 1.09 (6)	5.59 ± 0.18 (6)**	
(f)	(i) Control	5.40 ± 0.23 (4)	6.56 ± 0.12 (4)	7.99 ± 0.27 (4)
	(ii) Phentolamine 5×10^{-8} M	4.96 ± 0.20 (4)	5.76 ± 0.10 (4)*	
(g)	(i) Control	5.00 ± 1.15 (4)	6.71 ± 0.18 (4)	7.98 ± 0.22 (4)
	(ii) Phentolamine 5×10^{-7} M	4.40 ± 0.42 (4)	4.98 ± 0.10 (4)**	

Each value is the mean ± s.e. mean (n), where n = number of observations.
* $P < 0.0025$, paired t test.
** $P < 0.0005$, paired t test.

Although previous workers have suggested that mianserin is less potent than the tricyclic antidepressants in inhibiting the neuronal sites for noradrenaline uptake (rat brain, Raiteri *et al.*, 1976; Baumann & Maitre, 1977; de Paulis *et al.*, 1978; rat heart, Baumann & Maitre, 1977; rabbit brain, Goodlet *et al.*, 1977; rabbit heart, mouse atria and vas deferens, Harper & Hughes, 1977) significant inhibition has been observed with low concentrations of mianserin i.e. 10^{-7} M. Furthermore, the possibility of a significant inhibition with a very low concentration of mianserin (e.g., 10^{-9} M) has not been discounted in many of these studies. Thus, although mianserin is less potent than tricyclic antidepressants in inhibiting neuronal uptake into some tissues, it seems likely that this inhibitory effect may be an important determinant of the overall effect observed with mianserin.

In the presence of an inhibitor of neuronal uptake the contractile responses to noradrenaline are potentiated and the responses to tyramine are inhibited (Trendelenburg, 1972). In the present study, mianserin alone in Krebs solution at 10^{-7} and 10^{-6} M potentiated the contractile responses to field stimulation at low frequencies and to noradrenaline and inhibited the responses to tyramine. It seems likely that inhibition of neuronal uptake observed with these concentrations of mianserin is primarily responsible for these effects.

There was not a good correlation between the ability of mianserin to inhibit neuronal uptake and to potentiate contractile responses to noradrenaline. The postsynaptic α-adrenoceptor blocking activity of mianserin (Borowski *et al.*, 1976; Doxey *et al.*, 1978) may be responsible for this effect. Low concentrations of mianserin (10^{-9} and 10^{-8} M) had no effect on responses to noradrenaline. With these low concentrations, the effect of mianserin on neuronal uptake and, possibly, at α-adrenoceptors may be too small to produce an effect on contractile responses to noradrenaline. However, this seems unlikely as these concentrations of mianserin did inhibit responses to tyramine. This suggests that, in regard to responses to noradrenaline, with low concentrations of mianserin the inhibitory effect mianserin has on neuronal uptake is countered by its postsynaptic α-adrenoceptor blocking activity and, hence, no effect overall is observed on responses to noradrenaline. In the presence of 10^{-7} and 10^{-6} M mianserin, the inhibitory effect of mianserin on neuronal uptake appears to be the more prominent effect and, thus, the responses to noradrenaline are potentiated. With a high concentration of mianserin (10^{-5} M) the inhibitory effect on postsynaptic α-adrenoceptors becomes the pronounced effect and the responses to noradrenaline are inhibited.

It seems likely that these two inhibitory actions of mianserin, i.e. on neuronal uptake, and at postsynaptic α-adrenoceptors, are involved in its effects on contractile responses to tyramine. Firstly, by inhibiting the neuronal uptake process, mianserin will inhibit the uptake of tyramine. Consequently, less noradrenaline than usual will be released by tyramine. Secondly, the responses elicited by the released noradrenaline will be antagonized at the postsynaptic α-adrenoceptors by mianserin. The additive nature of these two effects with regard to inhibition of responses to tyramine explains the high potency mianserin has in this respect, inhibition of responses to tyramine being observed with 10^{-9} M mianserin.

To eliminate, as far as possible, any effects of mianserin due to the inhibition of neuronal uptake, further contractility studies were carried out in the presence of nortriptyline. In the presence of 10^{-6} M nortriptyline, mianserin (10^{-8}, 10^{-7} and 10^{-6} M) potentiated responses to field stimulation and inhibited responses to exogenously applied noradrenaline. There are two possible mechanisms which may account for this potentiating effect of mianserin on responses to field stimulation. Firstly, mianserin may be acting at presynaptic α-adrenoceptors. It is generally accepted that when an α-adrenoceptor antagonist potentiates the contractile responses to field stimulation but not to exogenously applied noradrenaline, the potentiating effect may be due to antagonism at presynaptic α-adrenoceptors. Presynaptic α-adrenoceptors have been demonstrated in all noradrenergically innervated tissues so far studied (see review by Starke, 1977). Thus in the rat anococcygeus muscle these receptors have been demonstrated both by direct methods, i.e. measuring ^{3}H overflow following preloading with [^{3}H]-noradrenaline (McGrath & Olverman, 1977; Idowu & Zar, 1978) and indirectly from contractility studies (Idowu & Zar, 1976). Such an antagonistic effect of mianserin at presynaptic α-adrenoceptors has previously been demonstrated in the rabbit pulmonary artery (Borowski *et al.*, 1976) in the rat brain (Baumann & Maitre, 1977) and in the pithed rat and vas deferens of the rat (Doxey *et al.*, 1978). Secondly, mianserin may reduce the effect of stimulation of the inhibitory nerves. Field stimulation of the rat anococcygeus muscle activates a noradrenergic motor innervation and an inhibitory innervation (Gillespie, 1972); thus any drug that reduces the inhibitory effect may potentiate the overall response to field stimulation.

The antagonism of responses to exogenously applied noradrenaline observed with mianserin was of an unusual nature. Thus, in the presence of nortriptyline, the degree of antagonism observed with mianserin was independent of concentration, i.e. the inhibitory effect of mianserin on responses to noradrenaline was similar whether 10^{-8} or 10^{-5} M mianserin was used. Such an effect with mianserin has not been reported by other workers (rabbit pulmonary artery, Borowski *et al.*, 1976; rat anococcygeus muscle,

Doxey *et al.*, 1978). In the present study, a similar non-dose-related antagonism was observed with mianserin after 6-hydroxydopamine incubation.

Following incubation of the rat anococcygeus muscle in the presence of 6-hydroxydopamine (10^{-3} M for 3 h) the contractile responses to tyramine were abolished, responses to noradrenaline were potentiated and [^{3}H]-noradrenaline accumulation was reduced (Doggrell & Woodruff, 1978). This suggests that the incuation with 6-hydroxydopamine causes a depletion of noradrenaline stores and a partial destruction of noradrenergic neurones. Under these conditions, any effects of mianserin on responses to exogenously applied noradrenaline must occur, predominantly, postsynaptically. Following this treatment, the antagonism of responses to noradrenaline observed with phentolamine and nortriptyline, but not with mianserin, was dose-related. As nortriptyline has a similar ability to mianserin to inhibit neuronal uptake in this tissue, this suggests the effect observed with mianserin is not related to any residual ability to inhibit neuronal uptake. Also it seems unlikely that the effect of mianserin postsynaptically is identical to that observed with phentolamine, a classical α-adrenoceptor antagonist.

One possible explanation of the lack of concentration-dependence of the inhibitory effect of mianserin on responses to noradrenaline is as follows. Firstly, there is an increasing antagonism at postsynaptic adrenoceptors with increasing concentrations of mianserin. Secondly, increasing concentrations of mianserin, in some way, increase the postsynaptic sensitivity to noradrenaline. These two effects would oppose each other and, thus, the degree of inhibition may not change with varying concentrations of mianserin.

In the rat anococcygeus muscle, a tissue with no cholinergic innervation (Gillespie, 1972; Burnstock, Cocks & Crowe, 1978), 10^{-7} M mianserin had no effect and 10^{-6} and 10^{-5} M mianserin potentiated responses, including maximal responses, to acetylcholine (Doggrell, 1979). This effect is in no way related to noradrenergic transmission as a similar potentiating effect of mianserin (10^{-6} and 10^{-5} M) on responses to acetylcholine is observed following 6-hydroxydopamine incubation (Doggrell, 1979). This suggests that mianserin increases the sensitivity to acetylcholine by an action at the level of, or distal to, the cholinoceptor. Mianserin does not have an identical sensitizing action on responses to noradrenaline as, under similar conditions, mianserin did not potentiate the maximal responses to noradrenaline. However, this does not exclude the possibility that mianserin may, by a somewhat similar and yet unknown mechanism, increase the sensitivity to noradrenaline at the level of, or distal to, the postsynaptic α-adrenoceptor. With regard to the responses to noradrenaline, it would be difficult to demonstrate any postsynaptic sensitizing effect of mianserin in the presence of its known antagonistic effect at postsynaptic α-adrenoceptors.

This study was supported by the Auckland Medical Research Foundation.

References

BAUMANN, P.A. & MAITRE, L. (1977). Blockade of presynaptic α-receptors and of amine uptake in the rat brain by the antidepressant mianserine. *Naunyn-Schmiedebergs Arch. Pharmac.*, **300**, 31–37.

BIEL, J.H. & BOPP, B. (1974). Antidepressant drugs. In *Medical Chemistry. A Series of Monographs.* Vol. 4. III Psychopharmacological Agents. ed. Gordon, M. pp. 283–341. New York; Academic Press.

BOROWSKI, E., EHRL, H. & STARKE, K. (1976). Relative pre- and postsynaptic potencies of α-adrenolytic drugs *Naunyn-Schmiedebergs Arch. Pharmac.*, **293**, Suppl R2.

BROGDEN, R.N., HEEL, R.C., SPEIGHT, T.M. & AVERY, G.S. (1978). Mianserin: A review of its pharmacological properties and therapeutic efficacy in depressive illness. *Drugs*, **16**, 273–301.

BURNSTOCK, G., COCKS, T. & CROWE, R. (1978). Evidence for purinergic innervation of the anococcygeus muscle. *Br. J. Pharmac.*, **64**, 13–20.

DOGGRELL, S.A. (1979). Mianserin potentiates responses to acetylcholine in the rat anococcygeus muscle. *Eur. J. Pharmac.*, **55**, 311–314.

DOGGRELL, S.A. & PATON, D.M. (1978a). Effect of labetalol and guanethidine on contractile responses to acetylcholine in the rat anococcygeus muscle. *J. Pharm. Pharmac.*, **30**, 453–454.

DOGGRELL, S.A. & PATON, D.M. (1978b). Release of noradrenaline by labetalol in the rat anococcygeus muscle. *Naunyn-Schmiedebergs Arch. Pharmac.*, **305**, 103–108.

DOGGRELL, S.A. & WOODRUFF, G.N. (1977). Effects of antidepressant drugs on noradrenaline accumulation and contractile responses in the rat anococcygeus muscle. *Br. J. Pharmac.*, **59**, 403–409.

DOGGRELL, S.A. & WOODRUFF, G.N. (1978). Effect of 6-hydroxydopamine incubation on noradrenaline accumulation and on contractile responses in the rat anococcygeus muscle. *Res. Comm. Chem. Path. Pharmac.*, **20**, 253–264.

DOXEY, J.C., EVERITT, J. & METCALF, G. (1978). Mianserin—An analysis of its peripheral autonomic actions. *Eur. J. Pharmac.*, **51**, 1–10.

GILLESPIE, J.S. (1972). The rat anococcygeus muscle and its response to nerve stimulation and to some drugs. *Br. J. Pharmac.*, **45**, 404–416.

GOODLET, I., MIREYLEES, S.E. & SUGRUE, M.F. (1977).

Effects of mianserin, a new antidepressant, on the *in vitro* and *in vivo* uptake of monoamines. *Br. J. Pharmac.*, **61**, 307–313.

HARPER, B. & HUGHES, I.E. (1977). A comparison in rabbit isolated hearts of the dysrhythmogenic potential of amitriptyline, maprotiline and mianserin in relation to their ability to block noradrenaline uptake. *Br. J. Pharmac.*, **59**, 651–660.

IDOWU, O.A. & ZAR, M.A. (1976). Inhibitory effect of clonidine on a peripheral adrenergic synapse. *Br. J. Pharmac.*, **58**, 278P.

IDOWU, O.A. & ZAR, M.A. (1978). Effects of clonidine and noradrenaline on the release of [^{3}H]-noradrenaline from the rat anococcygeus. *Br. J. Pharmac.*, **63**, 342–343P.

LEONARD, B.E. (1978). Some effects of mianserin on monoamine metabolism in the rat brain. *Br. J. clin. Pharmac.*, **5**, 11–12S.

MCGRATH, J.C. & OLVERMAN, H.J. (1977). Release of [^{3}H]-noradrenaline by field stimulation and by drugs from the anococcygeus muscle. *Br. J. Pharmac.*, **60**, 305–306P.

DE PAULIS, T., KELDER, D., ROSS, S.B. & STJERNSTRÖM, N.E. (1978). On the topology of the norepinephrine transport carrier in rat hypothalamus. The site of action of tricyclic uptake inhibitors. *Mol. Pharmac.*, **14**, 596–606.

PEET, M. & BEHAGEL, H. (1978). Mianserin: A decade of scientific development. *Br. J. clin. Pharmac.*, **5**, 5–9S.

RAITERI, M., ANGELINI, F. & BERTOLLINI, A. (1976). Comparative study of the effects of mianserin, a tetracyclic antidepressant, and of imipramine on uptake and release of neutrotransmitters in synaptosomes. *J. Pharm. Pharmac.*, **28**, 483–488.

STARKE, K. (1977). Regulation of noradrenaline release by presynaptic receptor systems. *Rev. Physiol. Biochem. Pharmac.*, **77**, 1–124.

STURMAN, G. (1971). Modification by a tricyclic series of compounds of the noradrenaline effect on the cat nictitating membrane. *J. Pharm. Pharmac.*, **23**, 142–143.

TRENDELENBURG, U. (1971). Classification of sympathomimetic amines. In *Catecholamines* ed. Blaschko, H. & Muscholl, E. pp. 336–362. Berlin: Springer.

(Received February 6, 1979.
Revised April 23, 1979.)