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COMPARISON OF MIANSERIN WITH DESIPRAMINE, MAPROTILINE AND PHENTOLAMINE ON CARDIAC PRESYNAPTIC AND VASCULAR POSTSYNAPTIC α-ADRENOCEPTORS AND NORADRENALINE REUPTAKE IN PITHED NORMOTENSIVE RATS

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1 The cardiovascular effects of intravenous desipramine (0.03 and 0.1 mg/kg), maprotiline (0.5 mg/kg), mianserin (1.0 and 3.0 mg/kg) and phentolamine (0.25 mg/kg) were examined and compared in pithed rats. Several experimental procedures were used in order to distinguish between the effects of the compounds on cardiac presynaptic α-adrenoceptors and on neuronal noradrenaline reuptake, as inhibition of either mechanism produces an increase of neurotransmitter concentration within the sympathetic synapse and therefore results in a greater end organ response.

2 Pressor responses elicited by noradrenaline were potentiated by desipramine and maprotiline, reduced by phentolamine and not significantly modified by mianserin. However, all four compounds inhibited the pressor action of tyramine. Furthermore, mianserin reduced the pressor response to adrenaline.

3 Desipramine, maprotiline and mianserin, but not phentolamine enhanced the positive chronotropic effects of noradrenaline, without affecting those of isoprenaline.

4 All four compounds abolished the clonidine-induced inhibition of heart rate responses to short term electrical stimulation of the spinal cord. Moreover, in rats with a persistent tachycardia (induced by continuous stimulation of the thoracic spinal cord) desipramine, maprotiline and mianserin further increased heart rate. This effect was also observed in animals pretreated with phentolamine, administered in order to inhibit cardiac presynaptic α-adrenoceptors.

5 In rats with a sustained tachycardia (100 beats/min produced by electrical stimulation of the spinal cord) both mianserin and phentolamine, in contrast to desipramine, shifted the clonidine heart rate dose-response curve to the right. Phentolamine was about 34 times more potent than mianserin in this respect.

6 In pithed, reserpine-treated rats, the pressor responses to clonidine were not significantly modified by desipramine. The dose-response curves were shifted to the right by phentolamine (0.25 mg/kg) and mianserin (3.0 mg/kg).

7 These results indicate that mianserin is an antagonist of both cardiac presynaptic and vascular postsynaptic α-adrenoceptors and also inhibits the neuronal reuptake of noradrenaline.

Introduction

Mianserin, a tetracyclic piperazino-azepine, has been shown to be effective in the treatment of depressive states (Brogden, Heel, Speight & Avery, 1978). This compound is classified as an atypical antidepressant agent since, in contrast to classical tricyclic drugs, it does not antagonize reserpine-induced hypothermia (Van Riezen, 1972) and it increases the turnover of noradrenaline in the rat brain (Kafoe & Leonard, 1973).

It has been suggested that mianserin blocks presynaptic α-adrenoceptors in the rat cortex (Baumann & Maître, 1975; 1977). More recently, Robson, Antonaccio, Saelens & Liebman (1978) advanced the hypothesis that mianserin might selectively antagonize cardiac presynaptic α-adrenoceptors in pithed spontaneously hypertensive rats since it reversed the clonidine-induced inhibition of tachycardia to short

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term electrical stimulation of the spinal cord, without affecting the pressor responses to endogenous or exogenous noradrenaline. However, these authors did not describe the effects of mianserin on the chronotropic responses to exogenous noradrenaline or spinal cord stimulation. The latter experiment is necessary to verify whether an action of mianserin on noradrenaline reuptake could possibly account for some of their findings, since either the latter mechanism or inhibition of presynaptic α-adrenoceptors increase the concentration of the neurotransmitter within the sympathetic synaptic gap.

Mianserin has been reported to inhibit noradrenaline reuptake in rat brain (Baumann & Maître, 1975; Raiteri, Angelini & Bertollini, 1976) and rabbit heart (Goodlet, Mireylees & Sugrue, 1977) as well as in the rat cardiovascular system (Goodlet & Sugrue, 1974; Doxey, Everitt & Metcalf, 1978). Mianserin also possesses vascular postsynaptic α-adrenoceptor blocking properties in the rat (Van Zwieten, 1975; Doxey *et al.*, 1978) and dog (Vargaftig, Coignet, De Vos, Grijsen & Bonta, 1971).

The purpose of the present study was to compare in the pithed normotensive rat the cardiovascular effects of mianserin with those of the α-adrenoceptor antagonist, phentolamine, and of two noradrenaline neuronal reuptake inhibitors, maprotiline and desipramine. Maprotiline is a tetracyclic compound possessing a pharmacological profile similar to that of conventional tricyclic antidepressants (Brunner, Hedwall, Meier & Bein, 1971; Maître, Staehelin & Bein, 1971; Pinder, Brogden, Speight & Avery, 1977). In particular, experiments were designed in order to distinguish heart rate effects due to an action on presynaptic α-adrenoceptors and those resulting from an inhibition of neuronal reuptake of noradrenaline. Part of this work was presented to the British Pharmacological Society (Cavero, Gomeni, Lefèvre-Borg & Roach, 1979).

Methods

General

Male normotensive rats (Sprague Dawley, C. River) weighing 230 to 260 g were anaesthetized with sodium pentobarbitone (55 mg/kg, i.p.), pithed and artificially respired (Harvard respirator, model 680) with room air (1 ml/100 g at a rate of 40 to 50 strokes/min).

The carotid artery and the femoral vein were cannulated for blood pressure measurement and intravenous drug administration. The blood pressure was measured with a Statham transducer (model P23Dd) connected to a preamplifier (Grass, model 7P1). The pulse pressure signals were used to trigger a cardiotachometer (Grass, model 7P44). Heart rate and blood pressure were recorded on a polygraph (Grass, model 7D).

Before initiating the experimental procedure (30 to 45 min after pithing) the cardiovascular reactivity of each animal was tested 2 to 4 times with noradrenaline (0.5 μg/kg, i.v.) until heart rate and blood pressure responses to two successive doses of this amine were stable.

Electrical stimulation of the thoracic spinal cord

The pithing metal rod was insulated with varnish so as to allow electrical stimulation of the thoracic spinal (C7-T2) cord with continuous square wave trains of 0.06 to 0.8 Hz, 0.5 ms duration and 40 to 50 V intensity (Grass S88 Stimulator or SRI Dual Stimulator). The frequency of stimulation was adjusted to give between 45 to 60 and 90 to 110 beats/min increases in heart rate. Moreover, heart rate frequency-response curves to short term (20 s) electrical stimulation (0.25, 0.5 and 1.0 Hz, 1.0 ms, 60 V) were constructed.

About 15 min before starting electrical stimulation of the spinal cord, atropine (1.0 mg/kg) plus (+)-tubocurarine (3.0 mg/kg) were administered intravenously to block muscarinic receptors and reduce skeletal muscle movements, respectively.

Experimental design

In several groups of pithed rats heart rate was increased (approx. 50 beats/min) by electrical stimulation of the thoracic spinal cord. Once this effect became stable either 0.9% w/v NaCl solution (saline, 0.075 ml/min) or phentolamine (25 μg kg^{-1} min^{-1}) were infused over a 10 min period. Approximately 2 min later another intravenous infusion (over 10 min) of either desipramine (3 μg kg^{-1} min^{-1}), maprotiline (50 μg kg^{-1} min^{-1}) or mianserin (100 μg kg^{-1} min^{-1}) was performed; then clonidine (5 μg/kg, i.v.) was injected and its effects on heart rate were followed for 5 min. Clonidine was also studied in animals in which no drug was given and heart rate was further elevated by 50 beats/min by increasing the frequency of stimulation of the spinal cord. The doses of desipramine, maprotiline and mianserin were chosen on the basis that they produced equivalent increases in heart rate lasting over 15 min in this preparation (see result section).

In another series of experiments heart rate was elevated by approx. 100 beats/min by stimulation of spinal cord and heart rate decrease dose-response curves to clonidine (0.3 to 300 μg/kg, i.v.: 5 to 10 min interval between two successive doses) were constructed in rats pretreated 10 min earlier with either intravenous (infused over 5 min) desipramine (0.1 mg/kg), mianserin (3.0 mg/kg), phentolamine (0.25 mg/kg) or saline (0.075 ml/min) to determine the

effects of each treatment on cardiac presynaptic α-adrenoceptors. The effects of these four treatments on the blood pressure dose-response curves to clonidine were also studied in pithed reserpine-treated rats to evaluate their effects on vascular postsynaptic α-adrenoceptors. Reserpine (5.0 mg/kg) was given subcutaneously 24 h before the experimental procedure.

In different groups of rats the effects of mianserin (3.0 mg/kg, i.v.) were evaluated on the heart rate and blood pressure responses to intravenous adrenaline (0.25 to 1 μg/kg), noradrenaline (0.125 to 0.5 μg/kg), isoprenaline (0.125 to 0.5 μg/kg) and tyramine (100 μg/kg) as well as 20 s electrical stimulation (0.25, 0.5 and 1.0 Hz; 1.0 ms; 60 V) of the spinal cord. Experiments with noradrenaline, isoprenaline and tyramine were also performed with intravenous desipramine (0.03 mg/kg), maprotiline (0.5 mg/kg) and phentolamine (0.25 mg/kg).

Frequency-response curves to short term spinal cord electrical stimulation were constructed before and 5 min after clonidine (30 μg/kg, i.v.) and then again 15 min after either intravenous desipramine (0.03 mg/kg), maprotiline (0.5 mg/kg), mianserin (3.0 mg/kg) or phentolamine (0.25 mg/kg). Before terminating the experiment, a 4th frequency-response curve was obtained after the administration of phentolamine (0.25 mg/kg, i.v.). Suitable control experiments were performed to check the reproducibility of 4 frequency-response curves over 1 h and to assess the duration of the inhibiting effect of clonidine over a 30 min period (see Figure 5).

Analysis of data

Data are given as means ± s.e. mean.

Dose-response (expressed as % of the maximum) curves to clonidine were fitted with a general logistic model. The doses producing 50% of maximal response and its standard deviation (ED_{50} ± s.d.) as well as the slope of the curve were calculated (Gomeni & Gomeni, 1978). The data used for the fitting were the doses of clonidine and the corresponding group mean responses weighted by the inverse of the variance of the mean.

To facilitate the analysis of the 3 point dose- or frequency-response profiles, the areas under these curves were calculated using the trapezoidal rule. Two-way analysis of variance was then used to assess significant differences within sequential treatments and between different compounds. Although in a few figures the control values from several separate groups of the same experiment were pooled for graphical reasons, the statistical analysis was always performed on individual group results.

Two-way analysis of variance was used to compare steady state heart rate responses to infusions of saline or phentolamine followed by mianserin, desipramine and then clonidine (Figures 6 and 7).

Drugs

Atropine sulphate (E. Merck), clonidine hydrochloride (Boerhinger Ingelheim), desipramine hydrochloride (Ciba-Geigy), adrenaline bitartrate (Sigma), isoprenaline hydrochloride (Labaz), maprotiline hydrochloride (Ciba-Geigy), mianserin hydrochloride (Organon), noradrenaline bitartrate (Sigma), sodium pentobarbitone (Abbott), pancuronium bromide (Organon), phentolamine mesylate (Ciba-Geigy), reserpine (Ciba-Geigy), (+)-tubocurarine chloride (Abbott) and tyramine hydrochloride (Sigma) were used. All doses in the text refer to the bases of the compounds.

Results

The basal mean carotid blood pressure and heart rate levels after the initial stabilization period were 59 ± 1 mmHg, and 241 ± 5 beats/min in a group of 60 pithed rats randomly selected from the present study.

The changes in heart rate (ΔHR: beats/min) and blood pressure (ΔBP: mmHg) produced at the end of a 10 min infusion of desipramine (3 μg kg^{-1} min^{-1}: ΔBP: 4 ± 2; ΔHR 1 ± 3, $n = 9$), maprotiline (50 μg kg^{-1} min^{-1}: ΔBP: 4 ± 1; ΔHR: −4 ± 2, $n = 7$), mianserin (100 μg kg^{-1} min^{-1}: ΔBP: −7 ± 1; ΔHR: −6 ± 2, $n = 5$; 300 μg kg^{-1} min^{-1}: ΔBP: 15 ± 1; ΔHR: 11 ± 5; $n = 12$) and phentolamine (25 μg kg^{-1} min^{-1}: ΔBP −3 ± 3: ΔHR −1 ± 4: $n = 10$) were minor and generally disappeared within 10 to 15 min before the studies described below were started.

Effects of desipramine, maprotiline, mianserin and phentolamine on cardiovascular responses to several adrenoceptor agonists

Intravenous desipramine (30 μg/kg), maprotiline (0.5 mg/kg) and mianserin (3.0 mg/kg) did not modify the increase in heart rate elicited by isoprenaline but enhanced that of noradrenaline (Figure 1). Phentolamine (0.25 mg/kg, i.v.) did not influence the chronotropic effect of either agonist. However, whereas pressor responses to noradrenaline were not changed by mianserin, they were reduced by phentolamine and potentiated by desipramine and maprotiline (Figure 1).

In the pithed rat adrenaline increased mean carotid blood pressure in a dose-dependent manner. After mianserin these response were significantly reduced and were followed by small decreases in blood pressure (Figure 2). In control animals, propranolol pretreatment shifted the adrenaline blood pressure dose-response curve to the left (Figure 2).

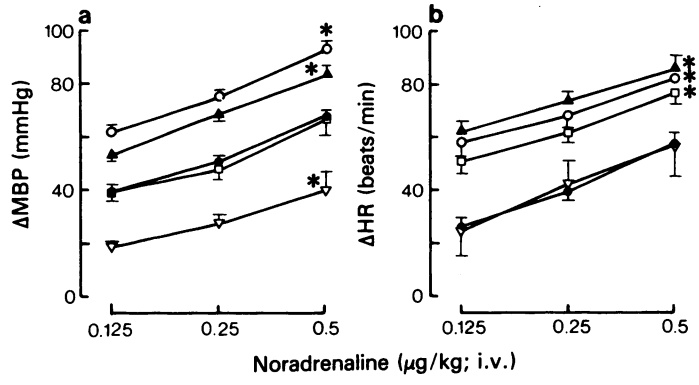

Figure 1 Peak mean carotid blood pressure (MBP) and heart rate (HR) responses to intravenous noradrenaline in pithed rats (n = 4–5/group) before (●) and after intravenous administration of desipramine (0.1 mg/kg: ○), maprotiline (0.5 mg/kg: ▲), mianserin (3 mg/kg: □) and phentolamine (0.25 mg/kg: ▽). An asterisk indicates a significant difference of all the points from control ($P < 0.05$).

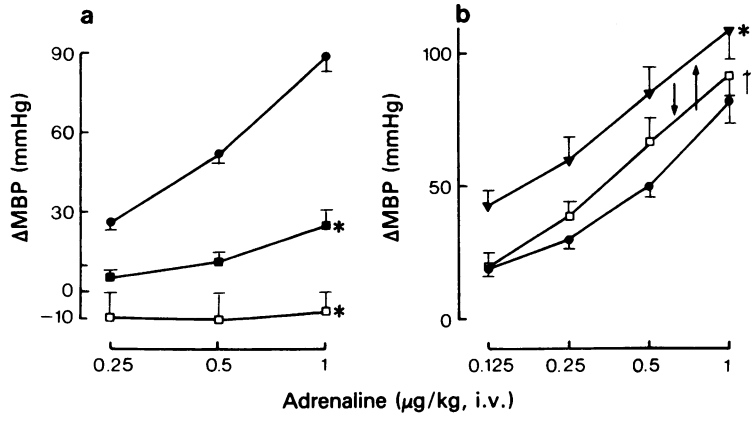

Figure 2 Peak mean carotid blood pressure (MBP) responses to adrenaline in pithed rats (n = 4) before (control: ●) and after mianserin (3.0 mg/kg, i.v.). The responses after mianserin (a) were biphasic, a rise (■) followed by a fall (□). In (b), the responses to adrenaline were studied in rats (n = 4) given propranolol (0.75 mg/kg, i.v.: ▼) followed by i.v. mianserin (3.0 mg/kg: □). An asterisk indicates a significant difference from control, a cross indicates that mianserin significantly inhibited the adrenaline responses after propranolol ($P < 0.05$).

Under these experimental conditions, the pressor effects of adrenaline were significantly inhibited by mianserin but remained monophasic (Figure 2).

Mianserin significantly ($P < 0.05$) enhanced heart rate responses to adrenaline (e.g.: 0.5 μg/kg: $\Delta HR = 44 \pm 5$ beats/min in control; $\Delta HR = 69 \pm 7$, after mianserin, $n = 4$).

Desipramine, maprotiline, mianserin and phentolamine inhibited blood pressure responses to tyramine (Figure 3). However, the heart rate effects of this amine were not significantly changed by the four compounds (Figure 3). The latter result suggests that desipramine, maprotiline and mianserin reduced the quantity of noradrenaline displaced by tyramine from sympathetic nerve endings, otherwise, a potentiation of heart rate responses would have occurred, as observed after these compounds with exogenous (Figure 1) and endogenous noradrenaline (next section).

Effects of desipramine, maprotiline, mianserin and phentolamine on the tachycardia to electrical stimulation of spinal cord and on its inhibition by clonidine

Desipramine (0.03 mg/kg), maprotiline (0.5 mg/kg), mianserin (1.0 mg/kg) and phentolamine (0.25 mg/kg) enhanced the heart rate responses to short term electrical stimulation by 28%, 29%, 66% and 22% respect-

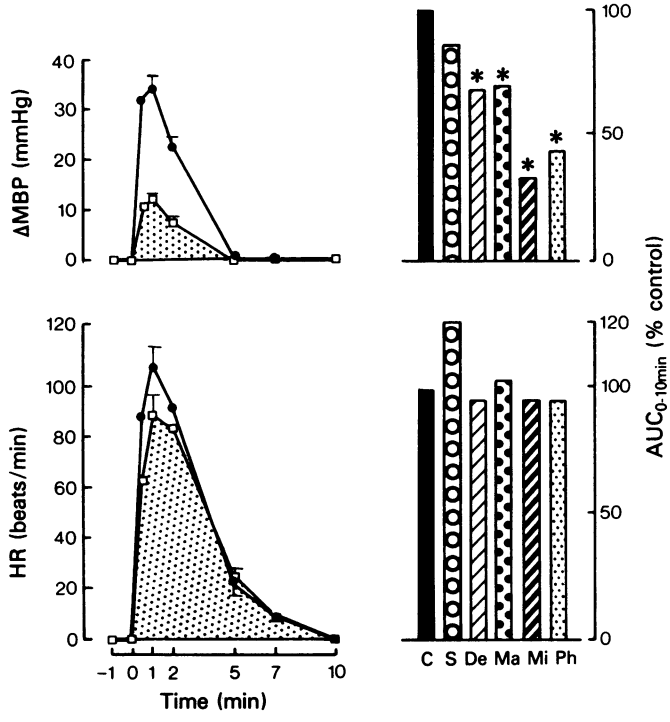

Figure 3 Time-course mean carotid blood pressure (MBP) and heart rate (HR) responses to i.v. tyramine (100 μg/kg) in pithed rats (n = 4–5/group) given i.v. saline (control: ●), and mianserin (3.0 mg/kg: □) (panels at right). The area (shaded surface) under the effect-time (10 min) profile (AUC) as percentage of control (C) response (= 100%) is shown for saline (S), desipramine (De, 30 μg/kg, i.v.) mianserin (Mi, 3.0 mg/kg, i.v.), maprotiline (Ma, 0.5 mg/kg i.v.) and phentolamine (Ph, 0.25 mg/kg, i.v.). All treatments except saline significantly reduced pressor responses with respect to control ($P < 0.05$: two-way analysis of variance on the area under the response-time profile).

ively (calculation performed on the area under the heart rate frequency (0.25 to 1.0 Hz)-response curve: as shown in Figure 4). All four compounds potentiated the response to the lowest frequency of stimulation more than the highest one (e.g.: 0.25 and 1.0 Hz produced ΔHR: 38 ± 4 and 80 ± 6 beats/min, n = 6, under control conditions respectively, and 68 ± 7 and 92 ± 7 beats/min after desipramine). However, with the highest frequency the heart rate response after these compounds was close to maximum. In a matched control group given saline there was only a 1% change between the first and second heart rate frequency-response curve (Figure 5).

In pithed rats short-term electrical stimulation of the thoracic spinal cord induced positive cardiac chronotropic effects which were frequency-dependent (Figure 4). These frequency-response curves were very reproducible over the period of the experimental procedure as indicated by the areas (Figure 4) under each of the 4 successive frequency-peak heart rate response curves obtained at 15 min intervals (Figure 5). The heart rate frequency-response curve was depressed by clonidine (Figures 4 and 5) and this inhibition was still present 30 min later (Figure 5). Desipramine, maprotiline, mianserin and phentolamine antagonized this action of clonidine. Mianserin was the most effective of the four compounds since the heart rate responses after this drug were greater than those obtained in the control period or after the three other compounds. The administration of phentolamine after desipramine and maprotiline enhanced heart rate responses to a level similar to that obtained with mianserin (Figure 5).

Experiments in rats with a heart rate elevated experimentally by sustained electrical stimulation of spinal cord

In pithed rats continuous stimulation (0.06 to 0.3 Hz) of the thoracic spinal cord resulted in a stable tachycardia (50 beats/min) lasting over 30 min (Figure 6). Under these experimental conditions, administration of clonidine produced a negative chronotropic effect (62 ± 8%; n = 7). A similar decrease (57 ± 5%, n = 5)

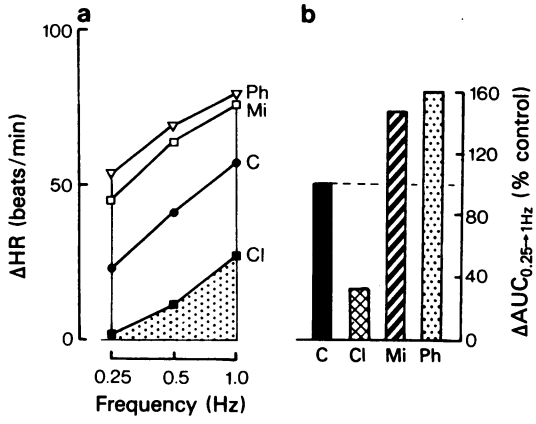

Figure 4 In (a) peak heart rate (HR)-frequency response curves to 20 s electrical stimulation of the thoracic spinal cord in pithed rats (n = 5) is shown before (C = control: ●) and after clonidine (Cl, 30 μg/kg, i.v.: ■) followed by mianserin (Mi, 3 mg/kg, i.v.: □) and then phentolamine (Ph, 0.25 mg/kg, i.v.: ▽). In (b) the areas under the HR-frequency (0.25–1.0 Hz: represented (a) as the surface within vertical lines) response profile (AUC: as % of control) is given to facilitate the comparison and the representation of the effects due to several successive treatments. For statistical analysis, see Figure 5.

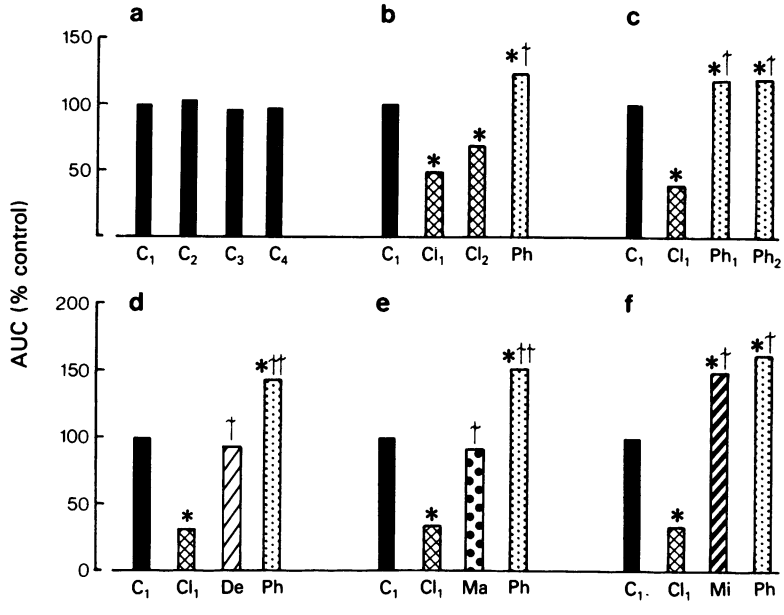

Figure 5 Effects of various treatments on the heart rate frequency-response curve to 20 s electrical stimulation of the thoracic spinal cord in pithed rats (n = 4–5/group). The data are given as areas (see Figure 4) under the frequency (0.25–1.0 Hz)-response curves (AUC). The control (C_1) AUC is considered as 100%. In (a) the reproducibility of responses over the experimental period is shown (C_1–C_4 control). In (b) AUC obtained 15 (Cl_1) and 30 (Cl_2) min after clonidine (30 μg/kg, i.v.) followed by phentolamine (Ph) are shown. In panels (c), (d), (e) and (f) the curve 30 min after clonidine was obtained 15 min after intravenous administration of desipramine (De, 0.1 mg/kg), phentolamine (Ph, 0.25 mg/kg), maprotiline (Ma, 0.5 mg/kg) and mianserin (Mi, 3.0 mg/kg), respectively. Phentolamine (Ph, 0.25 mg/kg, i.v.) was then administered after each of the latter treatments. Ph_1 and Ph_2 indicates that in this group phentolamine was given twice with a 15 min interval between each administration.

An asterisk indicates a significant difference from control, a dagger indicates a significant difference from clonidine response and a double dagger indicates a significant difference from the treatment preceding phentolamine (Ph) ($P < 0.05$).

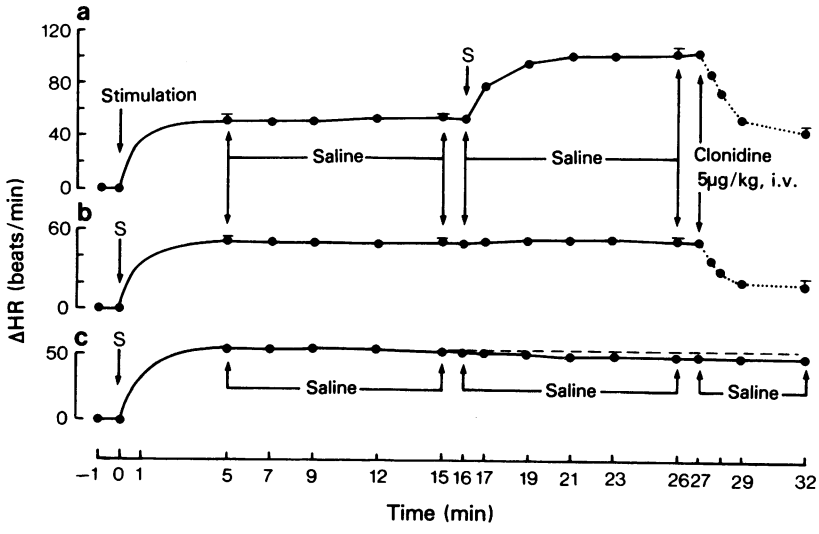

Figure 6 Effects of continuous stimulation (S) of the spinal cord for approx. 30 min (c) during which time saline was infused (0.1 ml/min) repeatedly; (a) and (b) show the effects of clonidine in rats with approx. 50 and 100 beats/min tachycardia, respectively.

was produced by clonidine when administered to animals in which heart rate was further elevated by approximately 50 beats/min (i.e. a final tachycardia of 100 beats/min) by raising the frequency from 0.1 to 0.2 Hz (Figure 6).

Phentolamine (25 μg kg^{-1} min^{-1} infused over 10 min) always induced a significant increase in heart rate (26 ± 3 beats/min, $n = 17$, Figure 7) in rats with a pre-existing cardiac acceleration elicited by electrical stimulation of spinal cord. Under the same experimental conditions a 10 min infusion of desipramine (3 μg kg^{-1} min^{-1}), maprotiline (50 μg kg^{-1} min^{-1}) or mianserin (100 μg kg^{-1} min^{-1}) enhanced heart rate by a similar amount in rats pretreated with either saline or phentolamine (Figure 7). The respective values for this effect were 55 ± 6, 49 ± 4 and 49 ± 3 after saline pretreatment and 52 ± 7, 58 ± 7 and 41 ± 5 beats/min after phentolamine. About 2 min after the termination of these infusions, clonidine was administered and significantly decreased heart rate in saline pretreated preparations. The extent of this effect was about 55% of the tachycardia present before injection of clonidine for the groups of animals treated with desipramine or maprotiline and only 25% for the animals given mianserin (Figure 7). Thus, clonidine produced comparable effects in animals in which the pre-existing sympathetic tachycardia was enhanced by electrical stimulation of the spinal cord (Figure 6) or by desipramine or maprotiline (Figure 7).

In phentolamine (0.25 mg/kg, i.v.) pretreated rats, in which heart rate was increased by 50 beats/min, mianserin infused over 10 min (10, 30 or 100 μg kg^{-1} min^{-1}) produced maximal heart rate increases of 15 ± 2, 28 ± 6 and 49 ± 4 beats/min ($n = 2$ to 5), respectively. With the two higher doses, this effect persisted for over 15 min after termination of the infusion.

In pithed rats in which heart rate was elevated by 100 beats/min, administration of clonidine in cumulative doses reduced heart rate in a dose-dependent manner (Figure 8). The highest dose of clonidine reduced the tachycardia by 90 to 100%. Phentolamine produced a parallel shift to the right of the control dose-responses (Figure 8). The doses of clonidine producing a 50 beats/min fall in heart rate (ED_{50} ± s.d.: μg/kg) were 3.1 ± 0.3 and 29.0 ± 3.5 in saline (control) and phentolamine pretreated animals, respectively. Mianserin (3.0 mg/kg) moderately inhibited this clonidine response (ED_{50} = 10.3 ± 0.8) whereas desipramine (0.10 mg/kg), like saline, did not significantly alter clonidine effects (ED_{50} = 3.7 ± 0.1). Thus, mianserin was approx. 34 times less potent (on a dose basis) than phentolamine against the cardiac presynaptic α-adrenoceptor stimulant properties of clonidine.

Effects of desipramine, mianserin and phentolamine on blood pressure responses to clonidine

In reserpine-treated pithed rats, intravenous administration of clonidine produced dose-related pressor responses. The dose producing 50% of the maximal

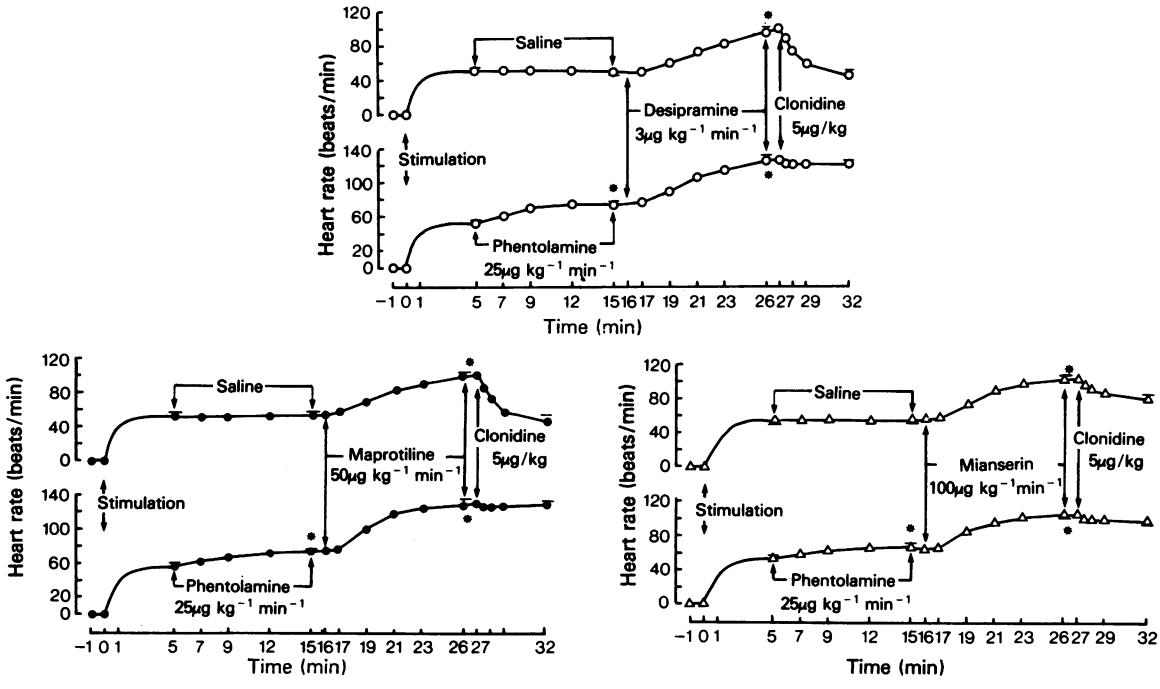

Figure 7 Pithed rat (n = 4–5/group) preparation in which baseline heart rate was increased by 50 beats/min by continuous electrical stimulation of the spinal cord. Desipramine, maprotiline and mianserin were infused in animals pretreated with either phentolamine or saline. The effects of clonidine after the three compounds are shown. The steady state responses after phentolamine are significantly different (*$P < 0.05$) from those obtained during infusion of saline. Similarly the response to desipramine, maprotiline and mianserin are significantly different from those observed with the immediately preceding treatment. In the saline-treated animals the heart rate response to clonidine was significantly different from that in phentolamine pretreated animals.

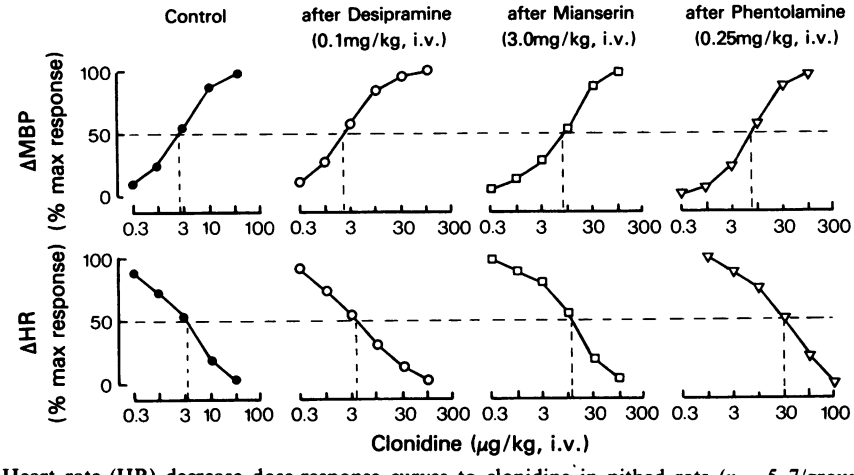

Figure 8 Heart rate (HR) decrease dose-response curves to clonidine in pithed rats (n = 5–7/group) in which baseline heart rate was increased by approx. 100 beats/min. The carotid mean blood pressure (MBP) dose-response curves to clonidine were obtained in reserpinized rats (n = 5/group). The effects of i.v. desipramine (0.1 mg/kg), mianserin (3.0 mg/kg) and phentolamine (0.25 mg/kg) on the clonidine mean carotid blood pressure and heart rate are shown. The slopes of the sigmoid curves were similar after each treatment. The values of the doses producing 50% of the maximal response and their standard deviation are given in the text.

blood pressure increase ($ED_{50} \pm$ s.d.: μg/kg) was 2.6 ± 0.3 (Figure 8). Desipramine (0.1 mg/kg) failed to affect significantly the dose-response curve to clonidine (ED_{50}: 2.9 ± 0.3). In contrast, mianserin (3.0 mg/kg) and phentolamine (0.25 mg/kg) moved the clonidine dose-response curve to the right (ED_{50} values 9.5 ± 1.0 and 8.5 ± 0.6 respectively). Thus, phentolamine was about 11 times more potent on a dose basis than mianserin as an inhibitor of vascular postsynaptic α-adrenoceptors.

The maximal mean blood pressure increases obtained with clonidine were 106 ± 4, 101 ± 3, 96 ± 3, 90 ± 4 mmHg ($n = 5$ to 7) for the groups used to study the effects of saline, desipramine, mianserin and phentolamine, respectively.

Relative activity of clonidine, desipramine, mianserin and phentolamine on cardiac pre- and vascular postsynaptic α-adrenoceptors

When the ratio ED_{50} blood pressure increases (vascular postsynaptic α-adrenoceptors)/ED_{50} heart rate decrease (cardiac presynaptic α-adrenoceptors) to clonidine was calculated for each treatment, it appeared that in animals pretreated with saline, clonidine had a similar potency at vascular postsynaptic and cardiac presynaptic α-adrenoceptors. Desipramine, in the dose used, did not possess a significant inhibitory action on either type of receptor, whereas mianserin similarly inhibited pre- and postsynaptic receptors. Phentolamine was about 3 times more effective in antagonizing the cardiac pre- than the vascular postsynaptic α-adrenoceptors.

Discussion

The complex cardiovascular effects of mianserin on the peripheral cardiovascular system of the rat are the result of concurrent actions on both sympathetic nerve endings and effector tissues. Mianserin enhanced heart rate responses due to adrenaline, noradrenaline and electrical stimulation of the spinal cord, but did not affect those to isoprenaline. This pharmacological behaviour resembles that of desipramine and maprotiline and is entirely compatible with an impairment of normal noradrenaline reuptake into neurones innervating the cardiac pacemaker. Such a mechanism enhances the availability at β-adrenoceptor sites of endogenously released or exogenously injected noradrenaline. This action of mianserin has been demonstrated by studing blood pressure responses to tyramine in the pithed rat (Goodlet & Sugrue, 1974; Doxey *et al.*, 1978) and by biochemically assessing noradrenaline reuptake into the rat (Baumann & Maître, 1975; 1977) and rabbit (Harper & Hughes, 1977) heart and into synaptosomes and slices from rat brain (Goodlet & Sugrue, 1974; Raiteri *et al.*, 1976; Goodlet *et al.*, 1977; Baumann & Maître, 1975; 1977).

In contrast to desipramine, mianserin failed to enhance pressor responses to intravenous noradrenaline or electrical stimulation of the spinal cord. This finding implies that mianserin exerted other pharmacological effects at the level of the vascular effector system. In pithed rats pretreated with mianserin, the control pressor responses to adrenaline became smaller and were followed by a blood pressure decrease. The latter effect was abolished by propranolol, suggesting that it resulted from vascular β_2- adrenoceptor stimulating properties of adrenaline generally unmasked by α-adrenoceptor antagonists (adrenaline reversal). Furthermore, mianserin inhibited the pressor responses of clonidine, an α-adrenoceptor agonist not undergoing neuronal reuptake (Autret, Schmitt, Fénard & Petillot, 1971). This observation favours the conclusion that mianserin possessed vascular α-adrenoceptor blocking properties which were responsible for the lack of potentiation of the noradrenaline pressor responses. The latter effect is generally observed with compounds which inhibit neuronal reuptake (desipramine and maprotiline) without affecting, at least in the doses used in this investigation, vascular postsynaptic α-adrenoceptors. The effectiveness of mianserin as an antagonist of postsynaptic α-adrenoceptors has been shown in intact dogs (Vargaftig *et al.*, 1971) and pithed rats (Van Zwieten, 1975; Doxey *et al.*, 1978). However, Robson *et al.* (1978) discounted this effect since mianserin neither antagonized nor enhanced (as observed in our experiments) blood pressure responses to noradrenaline and spinal cord stimulation in pithed, spontaneously hypertensive rats. However, examination of pressor responses to exogenous and endogenous noradrenaline is insufficient to draw a meaningful conclusion concerning the vascular α-adrenoceptor blocking properties of a compound which also inhibits reuptake of noradrenaline.

Substantial evidence favours the presence of α-adrenoceptors on symapthetic nerve endings. Activation of these presynaptic receptors produces a decrease in the quantity of noradrenaline liberated per single nerve impulse which in turn is followed by a smaller end organ response. The inverse occurs when the responsiveness of presynaptic α-adrenoceptors is impaired with suitable blocking agents (Langer, 1977; Starke, 1977; Westfall, 1977). According to these concepts, the clonidine-induced reduction of positive chronotropic responses to electrical stimulation of the spinal cord in the pithed rat resulted from activation of cardiac presynaptic α-adrenoceptors and its antagonism by phentolamine was due to blockade of these receptors. However, in a series of experiments whereby the heart rate response to short term electrical

stimulation of the spinal cord was performed (see Figure 5) the cardiac presynaptic agonist action of clonidine was abolished not only by phentolamine, but also desipramine and maprotiline. These findings may indicate that the latter two compounds, in the doses used, either possessed significant cardiac presynaptic α-adrenoceptor blocking activity or their antagonism against clonidine was due to their well known noradrenaline neuronal reuptake blocking properties. The latter suggestion is supported by our experimental results. Firstly, desipramine, but not phentolamine, failed to modify significantly the decrease in heart rate dose-response curve to clonidine (Figure 8). Secondly, in rats in which desipramine and maprotiline had already abolished the effects of clonidine on the positive chronotropic effects to short period electrical stimulation of the spinal cord, phentolamine further enhanced heart rate response (Figure 5). In contrast, the heart rate effect of phentolamine was not further potentiated by a second dose of phentolamine, suggesting that the first dose of this compound was sufficient to inhibit cardiac presynaptic α-adrenoceptors. Finally, clonidine reduced the sympathetic tachycardia enhanced by maprotiline and desipramine, but it failed to do so when the latter two compounds were given in animals pretreated with phentolamine (Figure 7). Thus, if a compound abolishes the clonidine-induced inhibition of heart rate responses to short term spinal cord stimulation, then it does not necessarily follow that this effect results from blockade of cardiac presynaptic α-adrenoceptors since drugs inhibiting noradrenaline reuptake also effectively antagonize this effect of clonidine. This observation casts doubt on the conclusion of Robson *et al.* (1978), that in the spontaneously hypertensive rat, mianserin is a selective antagonist at presynaptic α-adrenoceptors. That mianserin possessed this property is clearly demonstrated by its ability to shift the clonidine heart rate dose-response curve in rats with sustained tachycardia. However, mianserin in this respect was found to be about 34 times less potent than phentolamine. Furthermore, the dose of mianserin, which effectively blocked cardiac presynaptic α-adrenoceptors, did not differ from that blocking vascular postsynaptic α-adrenoceptors as assessed from the blood pressure dose-response curve to clonidine. Therefore, mianserin in our experiments, as well as those of Doxey *et al.* (1978), appears not to be a selective antagonist at cardiac presynaptic α-adrenoceptors.

Doxey *et al.* (1978) reported that desipramine did not affect the clonidine-induced inhibition of short term stimulation of spinal cord in pithed normotensive rats. This is only an apparent discrepancy with our results since Doxey *et al.* (1978) administered desipramine immediately after the injection of clonidine (and not 15 min later as did Robson *et al.* (1978) or ourselves) when presumably the latter compound had completely inhibited the release of endogenous noradrenaline (as indicated by the absence of heart rate response). The finding that mianserin under the same experimental conditions reversed the effect of clonidine was probably due to both properties of this compound, namely, inhibition of presynaptic α-adrenoceptors and noradrenaline reuptake. This conclusion is supported by the fact that the heart rate response after mianserin appeared to be larger than that observed before giving clonidine; this not being the case after the presynaptic α-adrenoceptor blocker, yohimbine.

This investigation warns against the indiscriminate use of heart rate (or any end organ) responses to assess the effectiveness of compounds as agonists or antagonists of cardiac presynaptic α-adrenoceptors unless suitable precautions are taken. For instance, the antagonism of the heart rate responses to short term sympathetic nerve stimulation by clonidine may be affected by mechanisms unrelated to presynaptic α-adrenoceptors (eg. drugs blocking noradrenaline reuptake). However, the adoption of either the sequence of treatments shown in Figures 5 and 6 or the procedure whereby the compound is intravenously infused during continuous stimulation of the spinal cord (Figure 7) are two simple ways to verify whether the response to a drug under study is solely due to an action on presynaptic α-adrenoceptors. Furthermore, these qualitative tests should preferably be completed by determining the potency of drugs as cardiac presynaptic α-adrenoceptor antagonists by constructing heart rat decrease dose-response curves to clonidine (or other suitable agonists) as shown in Figure 8.

In the past few years, the simple demonstration that a compound exerted presynaptic α-adrenoceptor agonist or antagonist effects in a tissue was considered sufficient to attribute this property to similar receptors in other tissues (as, Robson *et al.* (1978) did for mianserin). This extrapolation is premature and should be avoided since evidence is now accumulating favouring the view that presynaptic α-adrenoceptors in different tissues of the same species (Doxey & Everitt, 1977; Arbilla & Langer, 1978; Dubocovich, 1979) or for the same tissue in different species (Roach, Lefèvre & Cavero, 1978; Roach, Lefèvre-Borg & Cavero, 1978) are far from being a homogenous population.

In conclusion, mianserin possesses a complex cardiovascular profile, especially if one adds to the above described properties an ability to inhibit pressor responses to 5-hydroxytryptamine in the pithed rat (personal observation; Robson *et al.*, 1978) and histamine-induced vasodepression in intact rats (personal observation). It is possible that these neuronal and receptor effects occur concomitantly within the cen-

tral nervous system. In fact, mianserin blocks presynaptic α-adrenoceptors and neuronal amine reuptake in the rat brain (Baumann & Maître, 1977). Furthermore, drugs inhibiting histaminergic and 5-hydroxytryptaminergic receptors exert central nervous system effects. Therefore, the atypical behaviour of mianserin (Brogden *et al.*, 1978) in classical pharmacological models of depression is conceivably the result of multiple actions which may mask, prevent or modify behavioural and biochemical effects generally associated with the antidepressant activity of the classical noradrenaline reuptake inhibitors.

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