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INHIBITORY EFFECTS OF CLONIDINE ON RESPONSES TO SYMPATHETIC NERVE STIMULATION IN THE PITHED RAT

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1 The spinal sympathetic outflow to the eyelid, heart, splanchnic blood vessels, vas deferens and anococcygeus muscle was stimulated in pithed rats.

2 Clonidine inhibited sympathetic outflow to all of the tissues studied. The inhibitory effects of clonidine on cardiac nerves and hypogastric nerves were antagonized by phentolamine.

3 Clonidine produced a postsynaptic α-adrenoceptor agonist action on the eyelid, splanchnic blood vessels and the anococcygeus muscle. These effects were also antagonized by phentolamine.

4 The effects of clonidine, naphazoline and oxymetazoline on pre- and postsynaptic α-adrenoceptors were determined.

5 The presynaptic α-adrenoceptors employed were situated in either the sympathetic cardiac or hypogastric nerve terminals. Increases in diastolic blood pressure were used to assess concurrent postsynaptic α-adrenoceptor agonist activity.

6 The presynaptic α-adrenoceptor agonist potencies of clonidine, naphazoline and oxymetazoline were very similar on cardiac nerve terminals whereas on the hypogastric nerve terminals oxymetazoline was about 6 times more potent than either naphazoline or clonidine.

7 The results support the view that presynaptic α-adrenoceptors regulate transmitter release in sympathetic nerves. There appear to be subtle differences between the presynaptic α-adrenoceptors of different sympathetic nerve endings.

Introduction

Current concepts on neurotransmission in sympathetic nerves suggest that sympathetic nerve endings possess α-adrenoceptors that control the release of noradrenaline (Langer, 1974). The studies of Pacha, Salzmann & Scholtysik (1975) suggested that these presynaptic α-adrenoceptors are not distributed uniformly throughout the peripheral sympathetic nervous system. These workers found that in the spinal cat, cardiac sympathetic nerves appeared to be more sensitive to α-adrenoceptor-mediated inhibition of impulse transmission than sympathetic nerves supplying the nictitating membrane. Armstrong & Boura, (1970) also suggested that cardiac adrenergic nerves might be more susceptible to depression by blocking agents than other peripheral sympathetic nerves.

The spinal sympathetic outflow to the eyelid, heart, splanchnic blood vessels, vas deferens and anococcygeus muscle was stimulated in the pithed rat. The inhibitory effects of clonidine on sympathetic stimulation were studied and where possible antagonism by phentolamine was investigated. In other experiments the activity of oxymetazoline, naphazoline and clonidine at presynaptic α-adrenoceptors was studied in preparations where the innervated organ did not respond directly to these α-adrenoceptor agonists. Thus presynaptic α-adrenoceptor agonist activity was assessed by estimation of the inhibition of sympathetic transmission in either cardiac or hypogastric nerves. Postsynaptic α-adrenoceptor agonist activity was assessed by measurement of increases in diastolic blood pressure produced by the compounds.

During the final stages of the experimental work similar methods for estimating α-adrenoceptor activity using selective cardiac nerve stimulation and pressor responses were reported by Drew (1976).

Methods

Pithed rats

Male rats in the weight range 250–300 g were bilaterally adrenalectomized and then pithed during a brief period of halothane anaesthesia. The animals were subsequently artificially respired (100 strokes/min, 1 ml/100 g) with room air. Blood pressure was recorded from the left common carotid artery with a Hewlett Packard 1280 c pressure transducer linked to a Hewlett Packard 7700 recorder. Heart rate was

recorded using the pressure wave to trigger a ratemeter. The left femoral vein was cannulated to facilitate intravenous administration of drugs. Where contractions of the eyelid, anococcygeus muscle and vas deferens were recorded, an isometric transducer was used (Statham Gold Cell, UC3). In all experiments tubocurarine 1 mg/kg was injected intravenously before stimulation began.

The pithing rod was used to stimulate either the entire sympathetic outflow (Gillespie & Muir, 1967) or discrete segments of the spinal cord (Gillespie, Maclaren & Pollock, 1970). The tissues to which the sympathetic outflow was stimulated and the stimulus parameters used are shown in Table 1.

The effects of clonidine on electrically induced sympathetic outflow to various organs were studied. Clonidine was injected into the femoral vein, the series of nerve stimulations being applied before and 10 min after each dose of clonidine. Saline (0.9% w/v NaCl solution) was injected into further animals for control studies.

Quantitative assessment of pre- and postsynaptic α-adrenoceptor agonist activity

Presynaptic α-adrenoceptor agonist activity was assessed by determining the inhibition of either stimulation-induced cardiac acceleration or stimulation-induced contractions of the vas deferens. The stimulation parameters for cardiac acceleration were 1 Hz, 10 V, 0.5 ms for 10 s stimulation being applied every 2 minutes. The hypogastric nerve was stimulated at 2 Hz, 20 V, 200 μs for 3 s every 30 seconds. Postsynaptic activity was assessed by measurement of the pressor responses to the compounds studied.

The agonists studied were injected intravenously in increasing doses; sufficient time was allowed between doses of agonists for blood pressure to return to pre-injection levels. The increase in diastolic blood pressure was plotted against the logarithm of the individual dose of agonist. Percentage inhibition of either stimulation-induced cardiac acceleration or vas deferens contractions was plotted against logarithm of either dose or, cumulative dose of agonist. This procedure was adopted since at high doses the inhibition of cardiac and hypogastric nerves was of considerably longer duration than the pressor response produced by the agonists.

Table 1 Tissues used and stimulation parameters

Tissue	*Stimulation parameters*
Eyelid	20V; 500 μs; 0.5–6Hz; 10 s
Blood pressure	20 V; 500 μs; 0.5–6Hz; 10 s
Heart rate	10V; 500 μs; 0.5–6Hz; 30 s
Vas deferens	20V; 50 μs; 3–12Hz; 3 s
Anococcygeus	20V; 500 μs; 1–6Hz; 30 s

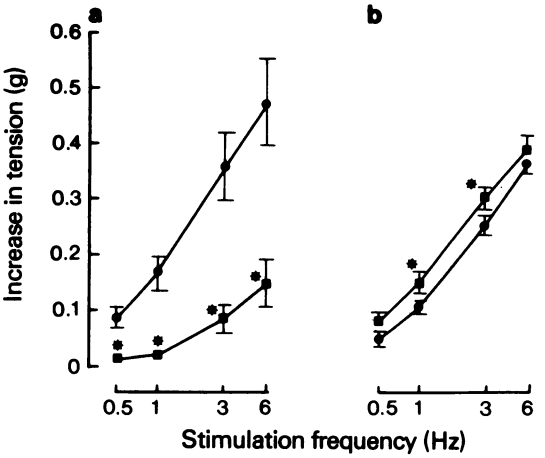

Figure 1 Responses of the eyelid to stimulation of the spinal sympathetic outflow in pithed rats before (●) and after (■) either (a) clonidine 30 μg/kg, intravenously or (b) saline 1.0 ml/kg intravenously. The results are the mean of 5 experiments. Vertical lines show s.e. mean. Significant difference from control: $^*P < 0.05$.

Student's *t* test for paired data was used throughout the study.

The following drugs were used: clonidine hydrochloride (Boehringer Ingelheim), naphazoline nitrate (CIBA), oxymetazoline hydrochloride (Allen and Hanbury), phentolamine mesylate (CIBA) and tubocurarine chloride (Burroughs Wellcome). Unless otherwise stated all doses in the text refer to the respective salt.

Results

Contractions of the eyelid of pithed rats

Clonidine administered intravenously in a dose of 30 μg/kg increased the resting tension of the eyelid by 0.45 ± 0.07 g (mean ± s.e.mean of 5 experiments). Clonidine inhibited stimulation-induced contractions of the tissue (Figure 1a). The inhibition of stimulation appeared to be a true effect and not the result of the increase in tone produced by clonidine since the increase in tone was of short duration and the tension had returned to near control levels before stimulation was started. The increase in resting eyelid tone produced by clonidine was antagonized by phentolamine (1.0 mg/kg i.v.). In a separate control study the effects of sympathetic stimulation were increased slightly after an intravenous injection of saline, (Figure 1b).

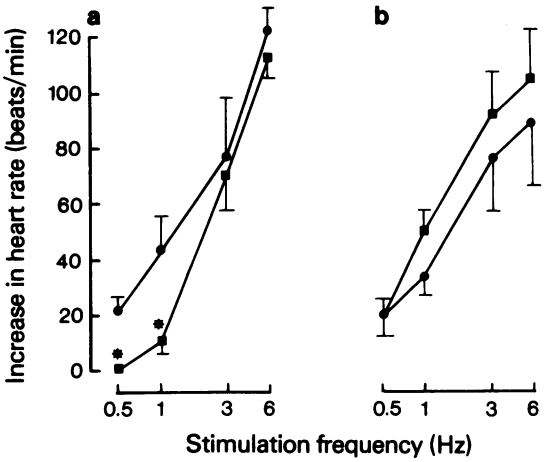

Figure 2 Responses of heart rate to stimulation of the cardiac sympathetic outflow in pithed rats before (●) and after (■) either (a) clonidine 30 μg/kg, intravenously or (b) saline 1.0 ml/kg, intravenously. The results are the mean of 5 experiments. Vertical lines show s.e. mean. Significant difference from control: $*P < 0.05$.

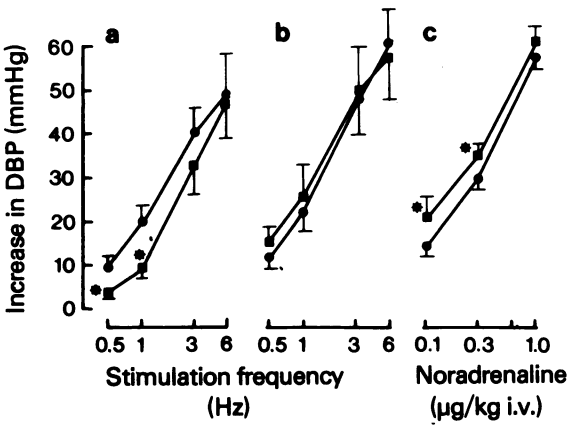

Figure 3 Responses of blood pressure to stimulation of the spinal sympathetic outflow in pithed rats before (●) and after (■) either (a) clonidine 30 μg/kg, intravenously or (b) saline 1.0 ml/kg, intravenously. (c) The pressor responses to noradrenaline before (●) and after (■) clonidine 30 μg/kg, intravenously. The results are the mean of 5 experiments. Vertical lines show s.e. mean. Significant difference from control: $*P < 0.05$.

Cardiac acceleration in pithed rats

The resting heart rate of pithed rats was unaffected by clonidine (30 μg/kg, i.v.). The tachycardia induced by low frequency stimulation of the cardiac nerves was inhibited by this dose of clonidine (Figure 2a), whereas the effects of high frequency stimulation were unaffected. In a control study saline had no effect on the stimulation-induced tachycardia (Figure 2b).

Pressor responses in pithed rats

Clonidine (30 μg/kg, i.v.) caused an increase in the diastolic blood pressure (74.7 ± 2.5 mmHg, $n = 5$) of pithed rats and inhibited the increases in blood pressure produced by low frequency stimulation of the sympathetic outflow (Figure 3a). Pressor responses evoked by high frequency stimulation were unaffected by this dose of clonidine. No changes in stimulation-induced pressor responses were seen in control experiments (Figure 3b). In a separate series of experiments noradrenaline pressor responses were potentiated by clonidine despite the large pressor response which clonidine itself produced (Figure 3c). The clonidine pressor response was antagonized by phentolamine (1 mg/kg, i.v.).

Contractions of the vas deferens of pithed rats

The resting tension of the vas deferens was unaffected by clonidine (30 μg/kg, i.v.) but electrically induced contractions of the tissue were inhibited (Figure 4a). Both low and high frequency stimulation induced contractions were inhibited by clonidine. Injections of saline in control experiments (Figure 4b) produced no effect on electrically induced contractions of the vas deferens.

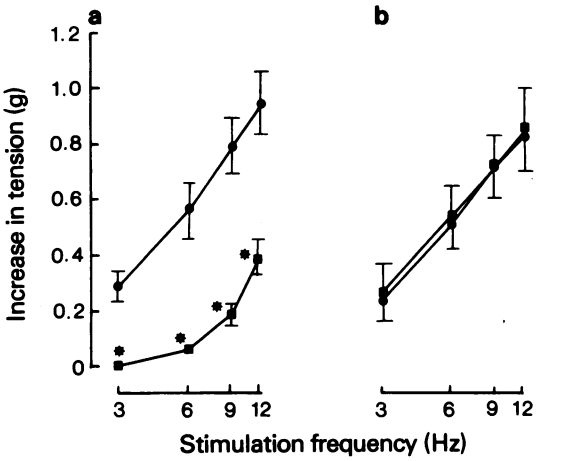

Figure 4 Responses of vas deferens to stimulation of the spinal sympathetic outflow in pithed rats before (●) and after (■) either (a) clonidine 30 μg/kg, intravenously or (b) saline 1.0 ml/kg, intravenously. The results are the mean of 5 experiments. Vertical line show s.e. mean. Significant difference from control: $*P < 0.05$.

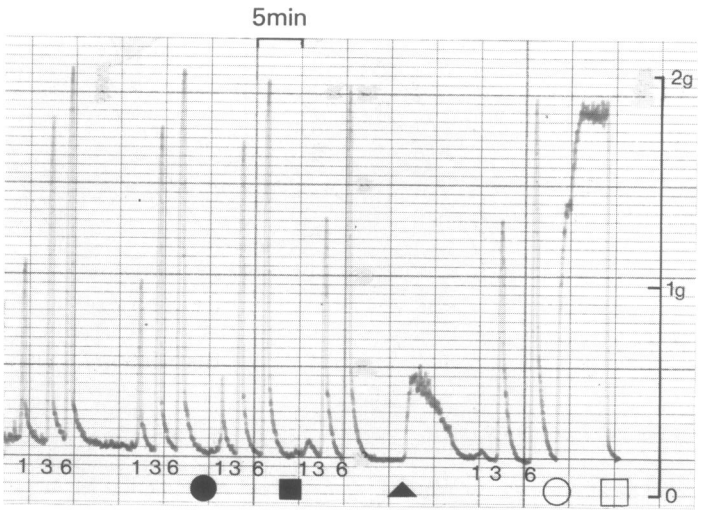

Figure 5 Responses of the anococcygeus muscle to stimulation of the spinal sympathetic outflow at frequencies of 1, 3 or 6Hz in pithed rats. Clonidine was injected in intravenous doses of 1 μg/kg (●), 3 μg/kg (■), 10 μg/kg (▲), and 30 μg/kg (○). Phentolamine, 1 mg/kg, intravenously was also injected (□).

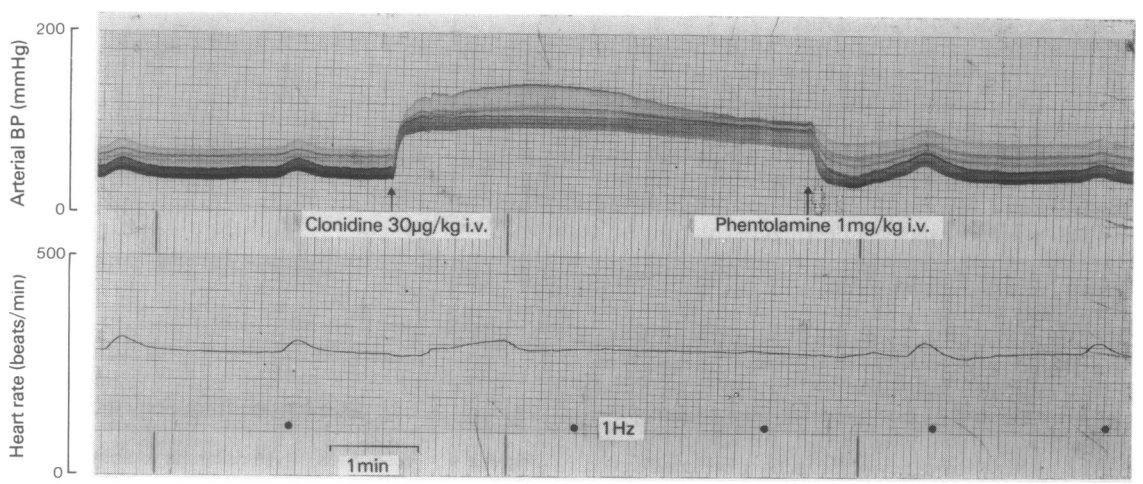

Figure 6 The effects of clonidine (30 μg/kg, i.v.) on blood pressure and cardiac acceleration and its reversal by phentolamine (1 mg/kg, i.v.) in pithed rats. Cardiac nerves were stimulated at 1Hz, 0.5 ms and 10V for 10 s every 2 minutes.

Contractions of the anococcygeus muscle of pithed rats

Clonidine (30 μg/kg, i.v.) caused a marked and long lasting (> 15 min) increase in the resting tension of the anococcygeus muscle (Figure 5). Because of the large increase in tension and the subsequent instability of the muscle the effects of this dose of clonidine on stimulation-induced contractions of the anococcygeus muscle could not be studied. With lower doses of clonidine (1-10 μg/kg) the resting tone was less affected and inhibition of stimulation-induced contrac-

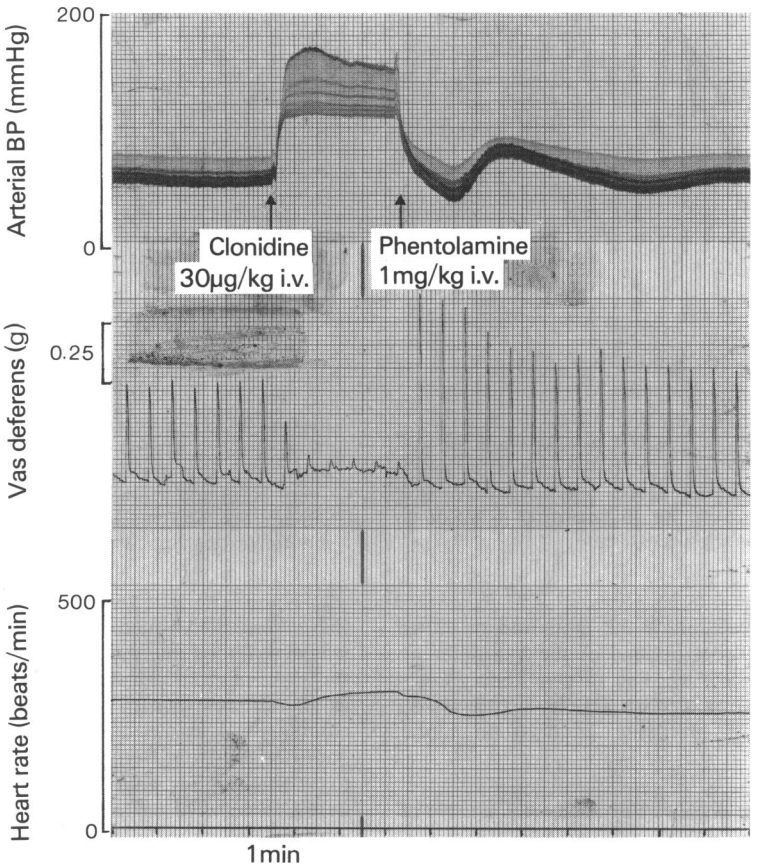

Figure 7 The effects of clonidine (30 μg/kg, i.v.) on blood pressure and contractions of the vas deferens and its reversal by phentolamine (1 mg/kg, i.v.) in pithed rats. The hypogastric nerves were stimulated at 6 Hz, 50 μs and 20V for 3 s every 30 seconds.

tions was seen. Again the contractions induced by low frequency stimulation were more susceptible to inhibition by clonidine than those produced by higher frequencies (Figure 5). The contractile response produced by clonidine was antagonized by phentolamine (1 mg/kg, i.v.).

The effect of an α-adrenoceptor antagonist on clonidine-mediated inhibition of cardiac acceleration and vas deferens contractions

Two of the tissues examined above did not produce a response to clonidine itself i.e. the heart and the vas deferens. The effects of an α-adrenoceptor antagonist on the inhibition produced by clonidine in these tissues were studied.

Stimulation of the sympathetic cardiac nerves produced an increase in heart rate and blood pressure (Figure 6). Clonidine caused a rise in blood pressure and inhibited the effects of cardiac stimulation. Phentolamine (1 mg/kg, i.v.) antagonized the pressor effect of clonidine and restored the effects of cardiac nerve stimulation. The fact that cardiac nerve stimulation increased blood pressure in the presence of phentolamine indicated that this slight pressor response was due to increased cardiac performance and not stimulation of vasoconstrictor nerves.

The inhibitory effects of clonidine on electrically induced contractions of the vas deferens were antagonized by phentolamine (1 mg/kg, i.v., Figure 7).

Potency of agonists at pre- and postsynaptic α-adrenoceptors

The presynaptic α-adrenoceptor agonist activity of clonidine, naphazoline and oxymetazoline was

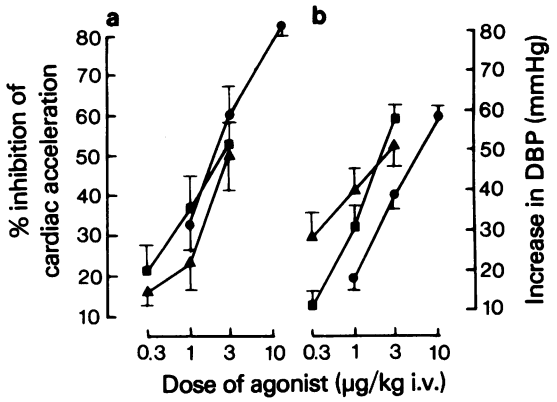

Figure 8 The effects of clonidine (●), naphazoline (■) and oxymetazoline (▲) on (a) stimulation-induced cardiac acceleration (1Hz, 10V, 0.5 ms for 10 s every 2 min) and (b) diastolic blood pressure (DBP) in pithed rats. The results are the mean of 5 experiments. Vertical lines show s.e. mean.

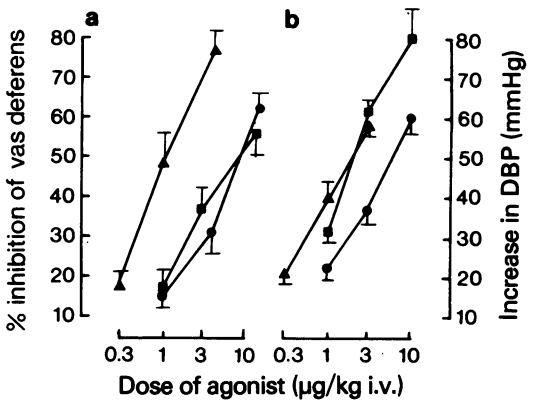

Figure 9 The effects of clonidine (●), naphazoline (■) and oxymetazoline (▲) on (a) stimulation-induced vas deferens contractions (2 Hz, 200 μs, 20V for 3 s every 30 s) and (b) diastolic blood pressure (DBP) in pithed rats. The results are the mean of 5 experiments. Vertical lines show s.e. mean.

assessed by measurement of the inhibition of either stimulation-induced contractions of the vas deferens or stimulation-induced cardiac acceleration. Postsynaptic α-adrenoceptor agonist activity was assessed by measurement of increases in diastolic blood pressure.

Inhibition of cardiac acceleration as an assessment of presynaptic α-adrenoceptor agonist activity. Clonidine, naphazoline and oxymetazoline produced a dose-related inhibition of stimulation-induced cardiac acceleration and a dose-related increase in diastolic blood pressure (Figure 8). By use of data derived from Figure 8 the ratio of presynaptic α-adrenoceptor agonist activity was assessed by referring to the activity of clonidine. The intravenous dose of clonidine (base) which inhibited cardiac acceleration by 50% was determined and the pressor response produced by this dose of clonidine was calculated (Table 2) i.e. the ratio of presynaptic to postsynaptic activity for clonidine was assumed to be unity. Similarly by use of data derived from Figure 8, the ratios of presynaptic to postsynaptic activity for oxymetazoline and naphazoline were calculated (Table 2). It can be seen in Table 2 that the selectivity of naphazoline for presynaptic α-adrenoceptors was less than that of clonidine but more than that of oxymetazoline.

Inhibition of vas deferens contractions as an assessment of presynaptic α-adrenoceptor agonist activity. Clonidine, naphazoline and oxymetazoline (Figure 9) produced a dose-related inhibition of stimulation-

Table 2 Relative potencies of agonists at presynaptic and postsynaptic α-adrenoceptors in the pithed rat

Agonist	*Presynaptic Dose (μg/kg) giving 50% inhibition of cardiac acceleration*	*Postsynaptic Dose (μg/kg) giving 32.5 mmHg increase in diastolic blood pressure*	*Ratio of presynaptic to post-synaptic potency*
Clonidine	1.7	1.7	1.0
Naphazoline	1.8	0.8	0.44
Oxymetazoline	2.4	0.4	0.17

The results are derived from Figure 8.

Table 3 Relative potencies of agonists at presynaptic and postsynaptic α-adrenoceptors in the pithed rat

Agonist	*Presynaptic Dose (μg/kg) causing 50% inhibition of vas deferens contraction*	*Postsynaptic Dose (μg/kg) giving 57 mmHg rise in diastolic blood pressure*	*Ratio of presynaptic to post-synaptic potency*
Clonidine	7.3	7.3	1.0
Naphazoline	6.2	2.0	0.32
Oxymetazoline	1.0	2.4	2.4

The results are derived from Figure 9.

induced contractions of the vas deferens. An identical procedure to that used in the previous section was adopted for determining pre- and postsynaptic activity (Table 3). It can be seen in Table 3 that the use of the stimulated vas deferens as a model for presynaptic activity resulted in different results being found from those obtained by use of cardiac acceleration. On the vas deferens oxymetazoline was more selective for presynaptic α-adrenoceptors than clonidine, whilst naphazoline was less selective than clonidine for presynaptic α-adrenoceptors.

Discussion

The effects of clonidine on spinal sympathetic outflow from different segments of the spinal cord indicated that in the rat all sympathetic nerves were inhibited by clonidine. The inhibitory effects of clonidine on sympathetic outflow to the heart, blood vessels and anococcygeus muscle occurred preferentially at low stimulation frequencies. The same effect of clonidine on cardiac acceleration has been described previously by Kobinger (1967), Armstrong & Boura (1973), Scriabine & Stavorski (1973) and Pacha *et al.*, (1975) and can be explained by its agonist action at presynaptic α-adrenoceptors (Langer, 1974). The proposed presynaptic action of clonidine was supported by the finding that phentolamine antagonized the inhibition of sympathetic cardioacceleration (Pacha *et al.*, 1975, Drew, 1976).

The inhibitory effects of clonidine on sympathetic nerves supplying the splanchnic blood vessels appeared to be presynaptic in origin since the effects of injected noradrenaline on blood pressure were potentiated by clonidine. Confirmation that clonidine stimulated presynaptic α-adrenoceptors was not possible since phentolamine itself blocked the effects of sympathetic stimulation postsynaptically. In addition to its presynaptic inhibitory action on splanchnic nerves, clonidine produced a pressor response in pithed rats which was antagonized by phentolamine.

Clonidine also had a dual action on the anococcygeus muscle. Low frequency stimulation was antagonized; this action appeared to be pre-synaptic in origin since noradrenaline evoked contractions of the tissue were not inhibited by clonidine (Idowu & Zar, 1976). Higher doses of clonidine caused a contraction of the anococcygeus muscle, this effect being antagonized by phentolamine.

Contractions of the eyelid and vas deferens induced by sympathetic stimulation were inhibited by clonidine but there was no evidence of preferential inhibition between the frequencies studied. The inhibitory action of clonidine on sympathetic nerves supplying the eyelid was presynaptic in origin since clonidine had no effect on the sensitivity of the eyelid to close arterial injections of noradrenaline (J.C. Doxey unpublished observations). The contractile effect of clonidine on the eyelid was anatagonized by phentolamine.

The inhibitory effects of clonidine on sympathetic nerves supplying the vas deferens and its antagonism by phentolamine have been demonstrated previously by Vizi, Somogyi, Hadhazy & Knoll, (1973) using isolated tissues. Similar results were seen in the present studies with pithed rats. The fact that clonidine did not cause a contraction of the vas deferens and that the presynaptic inhibition was antagonized by phentolamine despite the presence of a postsynaptic α-adrenoceptor (Gillespie & McGrath, 1975) reflected the unusual nature of the motor response in the vas deferens. The role of noradrenaline in the transmission process of the vas deferens has been questioned by Ambache & Zar (1971) and Ambache, Dunk, Verney & Zar, (1972). If however, as suggested by Gillespie & McGrath (1975), noradrenaline is the transmitter a possible explanation could be that hypogastric nerves have a very high population of presynaptic α-adrenoceptors in comparison with other sympathetic nerves or that the postsynaptic α-adrenoceptor is less accessible to exogenously administered agonists and antagonists.

The properties seen with clonidine can be explained in terms of its pre- and postsynaptic agonist activity. Stimulation of presynaptic α-adrenoceptors caused inhibition of sympathetic outflow and all tissues containing postsynaptic α-adrenoceptors were contracted by clonidine, the exceptions being the heart where the postsynaptic adrenoceptors were β in nature and the vas deferens which appears to have an unusual motor

Table 4 Summary of presynaptic and postsynaptic α-adrenoceptor agonist activity

	Presynaptic activity			*Postsynaptic activity*		
Compound (base)	**Rabbit pulmonary artery (nM)*	*Cardiac pithed rat (μg/kg, i.v.)*	*Vas deferens pithed rat (μg/kg, i.v.)*	**Rabbit pulmonary artery (nM)*	*Small pressor responses (μg/kg, i.v.)*	*Large pressor responses (μg/kg, i.v.)*
Clonidine	12 (4)	1.7 (0.7)	7.3 (7)	74 (4)	1.7 (4)	7.3 (3)
Oxymetazoline	3 (1)	2.4 (1)	1.0 (1)	18 (1)	0.4 (1)	2.4 (1)
Naphazoline	15 (5)	1.8 (0.8)	6.2 (6)	38 (2)	0.8 (2)	2.0 (0.8)

*Starke *et al.*, 1975a.
The figures in parentheses are equiactive doses/concentrations taking oxymetazoline activity as unity.

response. Unlike the studies of Pacha *et al.*, (1975) which showed that cardiac sympathetic nerves appeared to be more sensitive to inhibition by clonidine than nerves supplying the nictitating membrane, these studies showed no such selectivity in rats.

In experiments in which the presynaptic and postsynaptic agonist potencies of clonidine, naphazoline and oxymetazoline were studied, presynaptic potency depended on the system used. Where presynaptic activity was assessed using inhibition of cardiac acceleration there appeared to be little difference between the potency of naphazoline, oxymetazoline and clonidine. However, when inhibition of the vas deferens was used oxymetazoline was more potent than both naphazoline and clonidine. The results obtained on the vas deferens were very similar to those obtained by Starke, Montel & Endo, (1975b) (Table 4).

In postsynaptic studies the relative potencies of naphazoline, oxymetazoline and clonidine in producing pressor responses depended on which part of the dose-response curve was used. For small increases in blood pressure i.e. 32.5 mmHg, oxymetazoline was the most potent compound followed by naphazoline and clonidine. These results were identical to those found by Starke *et al.*, (1975b) (Table 4). For larger increases in blood pressure i.e. 57 mmHg, oxymetazoline had a potency intermediate between that of naphazoline and clonidine.

Because of the arbitrary nature of the choice of criteria for assessing pre- and postsynaptic activity it was not possible to say whether any of the compounds studied was more active at presynaptic than postsynaptic α-adrenoceptors. However, it was possible to relate the activity of naphazoline and oxymetazoline to that of clonidine. When cardiac nerve stimulation was used as a measure of presynaptic activity, clonidine was more selective for presynaptic α-adrenoceptors than either naphazoline or oxymetazoline. With the vas deferens, oxymetazoline was more selective and naphazoline less selective for presynaptic α-adrenoceptors than clonidine.

In conclusion, the work reported here endorses the view that pre- and postsynaptic α-adrenoceptors are different (Starke, Borowski & Endo, 1975a; Drew, 1976). The finding that the order of presynaptic agonist activities of the three imidazolines on cardiac and hypogastric nerves was different indicated that there are differences between presynaptic α-adrenoceptors of different peripheral sympathetic nerves.

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