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AN ELECTROPHYSIOLOGICAL ANALYSIS OF THE EFFECTS OF NORADRENALINE AND α-RECEPTOR ANTAGONISTS ON NEUROMUSCULAR TRANSMISSION IN MAMMALIAN MUSCULAR ARTERIES

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1 The effects of exogenously applied noradrenaline (NA) and α-adrenoceptor antagonists on the mechanical and intracellularly recorded responses to perivascular nerve stimulation were examined in the rabbit ear artery, rabbit saphenous artery and rat tail artery.

2 Excitatory junction potentials (e.j.ps) and action potentials recorded from these smooth muscles were not blocked or depressed by phentolamine, phenoxybenzamine, prazosin, or labetolol in concentrations as high as 10 μg/ml. Phentolamine (1 to 10 μg/ml) depressed neurally-evoked contractions of the ear and saphenous, but not the tail artery, and also depressed the contractions produced by direct muscle stimulation in the ear and saphenous arteries. Prazosin and labetolol (0.1 to 10 μg/ml) had no effect on the neurally evoked contractile response in any of the arteries examined.

3 The amplitude of the steady-state e.j.p. during repetitive stimulation at 0.45 to 2 Hz was increased by phentolamine or phenoxybenzamine but not by prazosin or labetolol. Phentolamine and phenoxybenzamine also increased the amplitude of the e.j.p. evoked by a single stimulus in the majority of the preparations.

4 Concentrations of NA $\geqslant$ 1 μg/ml depolarized the smooth muscle while concentrations $\geqslant$ 0.5 μg/ml depressed the amplitude of the e.j.ps recorded from these arteries. α-Antagonists did not suppress either the NA-induced membrane depolarization or depression of e.j.ps.

5 These observations call into question the physiological relevance of both pre- and postsynaptic α-receptors in regard to adrenergic neuromuscular transmission in muscular arteries.

Introduction

There is evidence that in many different preparations, *in vitro*, α-adrenoceptor antagonists block contractions evoked by exogenous application of noradrenaline (NA) more readily than they do neurally evoked contractions (see, for example, Nickerson, 1949). It may be possible to explain such findings on the basis of the observation that many α-antagonists also increase the amount of NA released during nerve stimulation, presumably by their action on presynaptic α-receptors which mediate a negative feedback regulation of NA release (see Starke, 1977; Westfall, 1977). Under these circumstances the increased transmitter released in the presence of α-blockers might be sufficient to overcome the blockade of postsynaptic α-receptors. Although this seems a reasonable hypothesis in relation to competitive α-antagonists it is rather more difficult to explain the action of irreversible antagonists in this way.

The inability of α-blockers to reduce postganglionic transmission in the vas deferens of some species has been the basis for the case against the assumption that NA is the transmitter liberated from some sympathetic motor nerves (Ambache & Zar, 1971; Ambache, Dunk, Verney, & Zar, 1972), a view recently supported by von Euler & Hedqvist (1975). On the other hand, Hotta (1969) has suggested that the dichotomy of the actions of α-antagonists on exogenous and nerve released NA in the vas deferens may be due to the existence of two types of postsynaptic receptors, 'extrajunctional' receptors that correspond to classical α-receptors and 'junctional' receptors that are activated by neurally released NA and are insensitive to these drugs. The existence of two such populations of excitatory receptors in arterial smooth muscle might also explain the findings that exogenously applied NA can cause contractions in the

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absence of any change in membrane potential of the smooth muscle (Droogmans, Raemaekers & Casteels, 1977; Casteels, Kitamura, Kuriyama & Suzuki, 1977; Holman & Surprenant, 1979) whereas contractions evoked by perivascular nerve stimulation are associated with large depolarizations and action potentials (Hirst, 1977; Holman & Surprenant, 1979; 1980; Surprenant, 1980).

The aim of the present study was to examine the effects of exogenously applied NA and α-antagonists on neuromuscular transmission in a number of mammalian muscular arteries, *in vitro*. Both α-antagonists known to have presynaptic receptor blocking activity and others considered to have predominantly or exclusively postsynaptic receptor blocking properties have been used in these experiments.

Methods

Saphenous and central ear arteries were obtained from young New Zealand white rabbits (800 to 1500 g) while tail arteries were obtained from hooded or Wistar rats (200 to 300 g). The saphenous artery was removed as previously described (Holman & Surprenant, 1979). A 1 to 1.5 cm segment of the central ear artery was removed from the base of the ear. Rats were stunned and bled. The tail was severed, skinned and a 1 to 2 cm length of the artery was dissected from the proximal end of the tail. In all arteries, intact segments were used which were free of any side branches which could be visualized under the dissecting microscope. The arteries were pinned out in an organ bath (volume 2 ml) through which a modified Krebs solution [composition (mM): NaCl 120, KCl 5, $CaCl_2$ 2.5, $MgSO_4$ 1, $NaHCO_3$ 25 and glucose 11, gassed with 95% O_2 and 5% CO_2] flowed continuously at a rate of 5 to 6 ml/min. The temperature was maintained at 35°C.

Intracellular recordings were made from the smooth muscle cells via the adventitial surface using high resistance (140 to 200 MΩ) microelectrodes filled with 2 M KCl. The criteria for a successful impalement were a final resting membrane potential of −60 mV or greater (in control solution) which remained stable for at least 30 min, less than 5 mV change in tip potential when the electrode was withdrawn from the cell and, in the case of the rat tail artery, the presence of spontaneous excitatory junction potentials $\geqslant 3$ mV in amplitude (see Surprenant, 1980).

A suction electrode around the proximal portion of the artery was used for stimulation of the perivascular nerve supply in the majority of experiments. In some experiments the electrical and mechanical activity produced by nerve stimulation were recorded simultaneously as described previously (Holman & Surprenant, 1980). In these experiments Pt ring electrodes, as described by Bevan & Su (1975) were used for perivascular nerve stimulation. Stimulus durations ranged from 0.01 to 0.1 ms. A BIOMAC 1000 Signal Averager was used in some experiments to obtain averaged responses.

In a separate series of experiments, spiral strips of the arteries were prepared as previously described (Holman & Surprenant, 1979), mounted under a resting tension of 0.5 g and longitudinal tension recorded with a Grass FT03 transducer and displayed on a Grass polygraph pen recorder. Stimulating electrodes as described by Birmingham & Wilson (1963) were used for nerve stimulation (0.25 ms duration, 100 V setting of a Grass S4 Stimulator) or direct muscle stimulation (a single pulse of 500 ms duration, 9 to 40 V setting). Guanethidine sulphate (10 μg/ml) abolished the contractile responses produced by the former but not the latter regime. The contractions evoked by 25 nerve stimuli at each frequency examined were used to obtain frequency-response curves in the three arteries.

The following drugs were used: phentolamine mesylate (Regitine, Ciba), phenoxybenzamine sulphate HCl (SKF), prazosin HCl (Pfizer), labetolol (Glaxo), guanethidine sulphate (Ciba-Geigy); and (−)-noradrenaline bitartrate (l-arterenol bitartrate; Sigma).

Differences of means were assessed using a paired Student's t test. Unless otherwise stated n = number of preparations, all means are given with ±s.e. mean.

Results

Effects of exogenously applied noradrenaline

The effects of the addition of NA to the perfusion solution on the resting membrane potential (r.m.p.) of the smooth muscle of the rabbit ear, saphenous and rat tail artery were examined for concentrations ranging from 0.01 to 50 μg/ml. Figure 1 shows the results obtained when multiple penetrations were made at varying concentrations of NA, the NA being washed out for 30 to 40 min before a new concentration was added to the bath. Concentrations of NA $\leqslant$ 1 μg/ml had no effect on the r.m.p. of these arterial smooth muscles but higher concentrations depolarized the membrane in a dose-dependent manner. In one cell from each of the three different arteries examined in these experiments an impalement was maintained throughout the addition, at 15 min intervals, of progressively higher concentrations of NA. Each of these impalements was maintained during washout of NA and the membrane potential returned to within 2 to 4 mV of the original value (Figure 2). A similar depolarization was seen in these three cells.

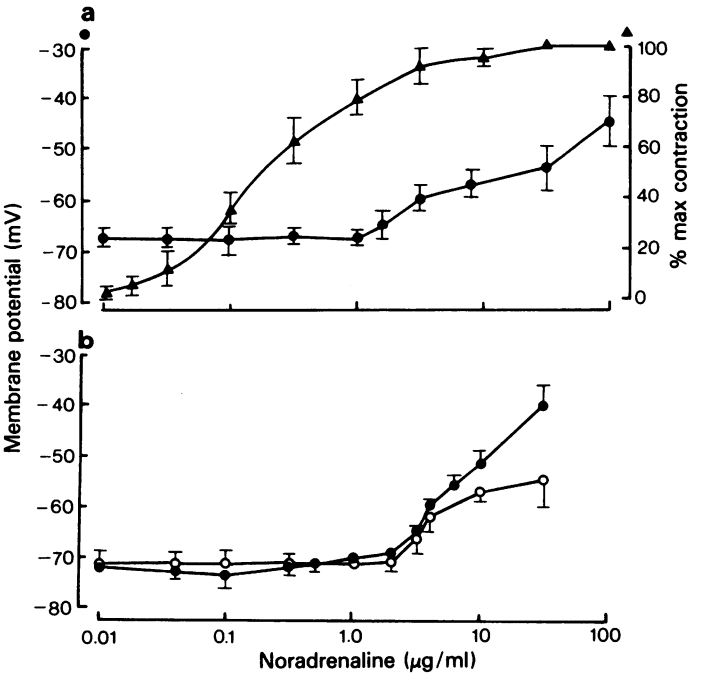

Figure 1 (a) Effect of different concentrations of noradrenaline (NA) on membrane potential (●) recorded from rabbit intact saphenous arteries and on tension development (▲) in spiral strip preparations of this artery. The tension is expressed as a percentage of the maximal contraction induced by NA (n = 15). (b) Membrane potential recorded in the rabbit ear artery (●) and rat tail artery (○) as a function of NA concentration. $n \geq 4$ for all points; total no. of cells ≥ 9 for all points.

The concentration range over which no change in membrane potential occurred produces 70 to 100% of the maximum contraction in response to exogenous NA in these arteries (rabbit saphenous artery, Bevan & Purdy, 1973; rat tail artery, Hermsmeyer, 1976; rabbit ear artery, Droogmans *et al.*, 1977; see also Figure 1a).

Spontaneous oscillations in the membrane potential, which could not be attributed to mechanical artifact, were never observed in any of the arteries examined during application of NA to the bathing solution. In contrast, in the rabbit common carotid artery, concentrations of adrenaline ($\geq$0.1 μg/ml) have been shown to depolarize the membrane by 4 to 7 mV and induce spontaneous oscillations of 5 mV amplitude (Mekata & Niu, 1972).

Excitatory junction potentials (e.j.ps) of similar time course can be recorded from all cells of the rabbit saphenous, ear and rat tail arteries in response to perivascular nerve stimulation (Surprenant, 1980; see also Figure 3). Concentrations of NA $\geq$ 0.5 μg/ml depressed the amplitude of the e.j.ps recorded during single and repetitive (0.45 to 2 Hz) stimulation but had no significant effect on their time course. Figure 3 shows averaged e.j.ps recorded from one cell of the rat tail artery and rabbit ear artery before and 15 min after the addition of 1 μg/ml NA. This concentration produced no membrane depolarization but depressed the amplitude of the e.j.ps by approximately 35% in these cells. Excitatory junction potentials recorded from the rabbit ear and saphenous artery were completely abolished by 50 μg/ml NA (which depolarized the membrane by approximately 25 mV). In contrast, this concentration of NA depolarized the smooth muscle of the rat tail artery by about 15 mV and depressed e.j.ps recorded from this tissue by only 68 to 90%.

Effects of α-antagonists on neuromuscular transmission

Single excitatory junction potentials Phentolamine, phenoxybenzamine, prazosin and labetolol, in concentrations as high as 10 μg/ml, failed to depress the

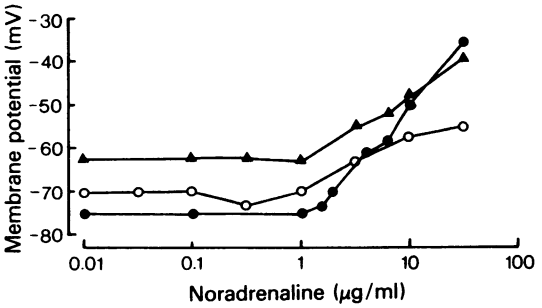

Figure 2 Membrane potential recorded from one cell in rabbit ear artery (●), rabbit saphenous artery (▲) and rat tail artery (○) during successive application of increasing concentrations of noradrenaline; 15 min after washout the membrane potential in each case had returned to within 2 to 4 mV of the original baseline.

e.j.ps evoked during single or repetitive nerve stimulation at frequencies between 0.3 and 2 Hz during exposure to these drugs for up to 3 h (Figure 4). These α-blockers had no effect on the r.m.p. or the time course of the e.j.ps recorded from these arteries.

In a large proportion of preparations (9 of 14 ear, 6 of 9 saphenous and 6 of 9 tail arteries) a single stimulus evoked an e.j.p. whose amplitude was increased above control values when either phentolamine or phenoxybenzamine was present (Figure 4). In these preparations the amplitude of the e.j.p. was increased from 10 to 200% above control values when the frequency at which nerve stimuli were delivered was as low as once every 2 min. This is in contrast to the mouse vas deferens where phentolamine and phenoxybenzamine markedly depress the amplitude of the e.j.p. evoked by a single stimulus (Bennett & Middleton, 1975). No increase in the amplitude of the e.j.p. evoked by a single stimulus was observed in any artery when prazosin or labetolol was added to the bathing fluid.

Excitatory junction potentials during repetitive stimulations During stimulation at frequencies between 0.45 and 1 Hz the amplitudes of successive e.j.ps recorded from the rabbit ear, saphenous and rat tail artery reach a steady-state level after approximately 3 to 7 impulses (see Surprenant, 1980). The steady-state amplitudes of the e.j.ps evoked during trains of stimuli at 0.5 and 1 Hz were increased by phentolamine and phenoxybenzamine in all preparations

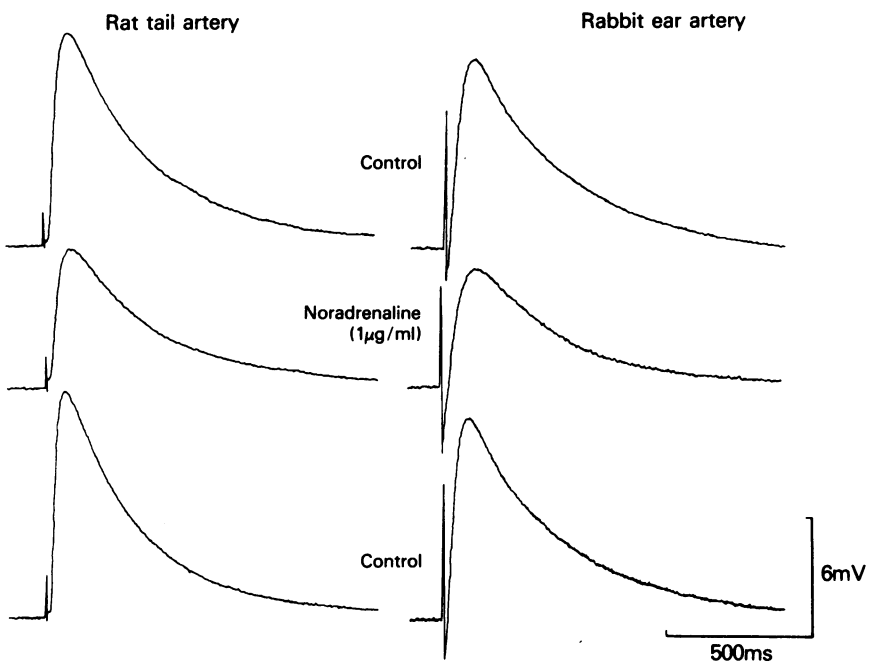

Figure 3 Depression of e.j.p. amplitude induced by applied noradrenaline (NA). Averaged response to 64 stimuli at 0.45 Hz recorded in the same cell in control solution, 15 min after the addition of 1 μg/ml NA to the bath and 15 min after washout of the NA. This concentration of NA failed to depolarize the membrane.

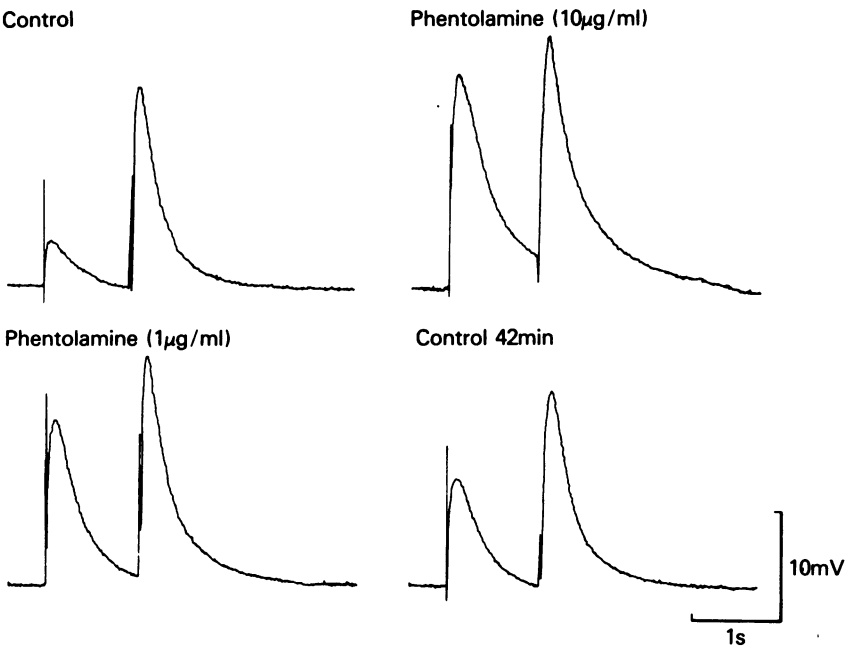

Figure 4 Increase in e.j.p. amplitude in the presence of phentolamine. Records obtained from one cell in the rabbit ear artery. Two stimuli, 1 s apart, delivered at 2 min intervals. Eight responses averaged in each case. Phentolamine increased the amplitude of the initial as well as the subsequent e.j.p. The effects of phentolamine were slowly reversible; 42 min after phentolamine (10 μg/ml) washout the e.j.ps were still increased over control values. Stimulus intensity constant throughout.

(Table 1). This effect was apparent at concentrations of 0.1 μg/ml; lower concentrations were not examined. The increase in the steady-state response during repetitive stimulation at 0.5 Hz varied widely among different preparations of the same artery. For example, in the rabbit ear artery phentolamine (1 μg/ml) increased the steady-state response by 2 to 210% in different preparations ($n = 14$) while in the rat tail artery the increase in the steady-state response produced by phenoxybenzamine (1 μg/ml) ranged

Table 1 Effects of α-receptor antagonists on steady state excitatory junction potential (e.j.p.) amplitude in muscular arteries

	% increase in steady state e.j.p. amplitude (as % of control)		
Drug (1 μg/ml)	*Rabbit ear artery*	*Rabbit saphenous artery*	*Rat tail artery*
Phentolamine	30 ± 15 (14)	28 ± 8 (7)	20 ± 6 (8)
Phenoxybenzamine	68 ± 14 (4)	14 ± 4 (2)	23 ± 13 (5)
Prazosin	0 ± 1.3 (8)	−1.2 ± 0.7 (2)	0.8 ± 1 (5)
Labetolol	−1.6 ± 0.4 (4)	0.9 ± 0.1 (3)	0 ± 1.1 (2)

Number of preparations is given in parentheses.

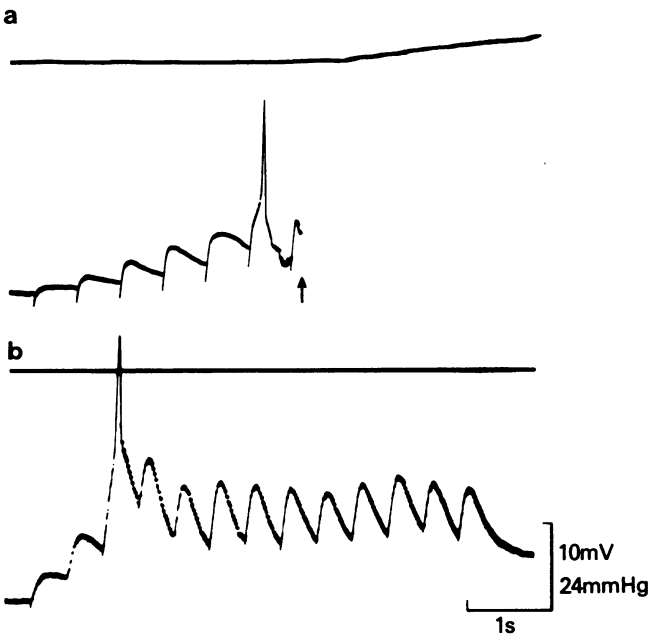

Figure 5 Pressure change (upper traces) and intracellularly recorded action potentials (lower traces) in the rabbit ear artery. (a) Action potential recorded in control solution in response to 2 Hz stimuli with associated increase in pressure. Arrow marks dislodgement of microelectrode due to contraction of the artery. (b) Action potential recorded 62 min after the addition of phentolamine (1 μg/ml). No change in the pressure trace or evidence of contraction was observed. Stimulus intensity constant throughout.

from 4 to 80% ($n = 5$). The effects of phentolamine were only slowly reversible, requiring 20 to 30 min to return to control values after exposure to a concentration of 1 μg/ml and up to 1.5 h when 10 μg/ml phentolamine was applied (Figure 4). The reversibility of the effects of phenoxybenzamine was not examined.

Prazosin and labetolol had no effect on the amplitude of the e.j.p. evoked by repetitive stimulation at frequencies between 0.45 and 2 Hz. Averaged responses of e.j:ps were obtained in the same cell during repetitive stimulation at 0.5 Hz in control solution and 15 min after the addition of phentolamine, phenoxybenzamine, prazosin or labetolol (1 μg/ml). The mean change in amplitude of the averaged e.j.p. expressed as a percentage of control value, for all experiments, is given in Table 1.

Active responses and action potentials Perivascular stimulation of the rabbit ear artery at frequencies greater than 1 Hz evokes e.j.ps which sum with each other and an all-or-nothing action potential of 42 to 50 mV amplitude is initiated from the peak of the e.j.p. (Surprenant, 1980). Such action potentials are invariably associated with a contraction of the artery. In contrast, neurally-evoked contractions in the rabbit saphenous artery and rat tail artery are associated with 'active responses'; that is, a change in membrane potential of the smooth muscle which is graded in amplitude (up to 55 mV) and time course, possibly related to the rate of depolarization during stimulation (Holman & Surprenant, 1979; Surprenant, 1980). The α-antagonists used in these experiments, in concentrations as high as 10 μg/ml, did not abolish or depress the ability of any of these arteries to generate active responses or action potentials, although phentolamine and phenoxybenzamine did depress the neurally evoked contractions in the saphenous and ear arteries (see below). Figure 5 shows intracellular recordings obtained in the rabbit ear artery before and 65 min after the addition of phentolamine (1 μg/ml). In control solution a short train of stimuli at 2 Hz evoked the characteristic action potential and associated contraction (Figure 5a). In phentolamine a longer train of stimuli evoked an action potential without any observable change in pressure or evidence of movement artifact (Figure 5b).

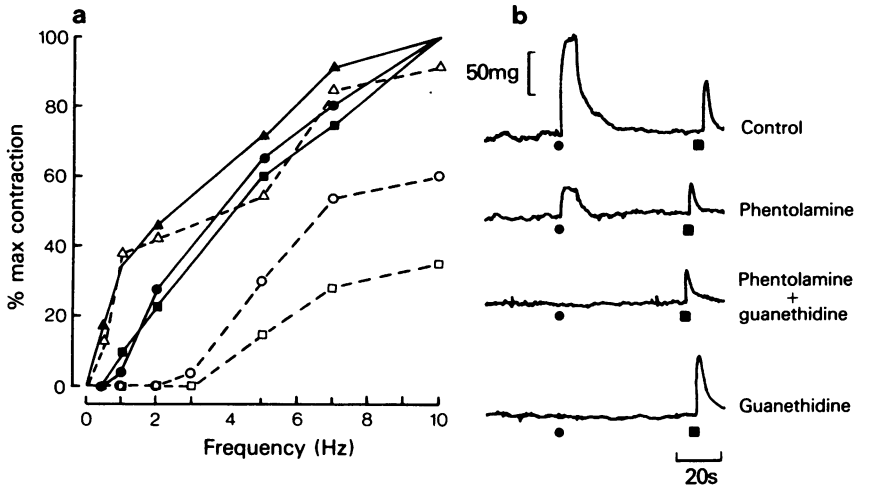

Figure 6 (a) Frequency-response curves obtained from spiral strip preparations of the rabbit ear artery (●○), rabbit saphenous artery (■□) and rat tail artery (▲△). Tension expressed as percentage of contraction produced by 10 Hz stimulation. Phentolamine depressed the neurally evoked contractions in ear and saphenous but not tail arteries. Filled symbols = control; open symbols = phentolamine (10 μg/ml). $n \geq 4$ for all arteries, s.e. mean $\leq 8\%$ for all points. (b) Tension records obtained from one preparation of the rabbit saphenous artery in response to nerve stimulation (●) and direct muscle stimulation (■); 20 min after the addition of phentolamine (10 μg/ml) both responses were depressed. The addition of guanethidine (10 μg/ml) completely abolished the nerve response but had no effect on the direct muscle response. The direct muscle response had returned to control levels 1.5 h after washout of phentolamine.

Effects of α-blockers on mechanical responses evoked by electrical stimulation

Frequency-response curves were obtained in control solution from spiral strip preparations of the three arteries examined for frequencies between 0.5 and 10 Hz (Figure 6a). Phentolamine (≥ 1 μg/ml) reduced the nerve-evoked contractions in both the rabbit ear and saphenous arteries but had no significant effect on the responses produced in the rat tail artery at concentrations ≤ 10 μg/ml. The saphenous artery was most sensitive to the phentolamine-induced depression of neurally evoked contractions (Figure 6a). Concentrations of phentolamine ≥ 1 μg/ml completely abolished contractile responses in the ear and saphenous arteries in response to low frequency (less than 3 Hz) stimulation.

Prazosin and labetolol in concentrations up to 10 μg/ml had no detectable effect on neurally evoked contractions (up to 10 Hz, 25 impulses at each frequency) in any of the arteries ($n = 5$ ear, 4 saphenous and 6 tail arteries).

Whereas phentolamine completely abolished the contractions produced by NA within seconds, its action in depressing the neurally evoked contractions developed gradually, requiring 15 to 20 min for the maximum effect to occur. No further depression in the neurally evoked contractions was observed when phentolamine was left in the bath for up to 2 h.

Contractions in response to application of NA (up to 10 μg/ml) were completely abolished when 0.1 to 1.0 μg/ml of any of the α-blockers used in these experiments was present in the organ bath.

In view of the finding that active responses or action potentials evoked during low frequency (less than 3 Hz) nerve stimulation were not blocked by phentolamine at a time when the contractions associated with these responses were abolished it was considered possible that the ability of this drug to depress neurally evoked contractile responses was due to a direct effect on the smooth muscle. Therefore the effects of phentolamine on the responses produced by direct muscle stimulation were examined on spiral strip preparations of the ear and saphenous arteries. Figure 6b shows records obtained from a preparation of the saphenous artery in response to nerve stimulation (0.25 ms pulses, 10 Hz for 10 s) and direct muscle stimulation (a single 500 ms pulse). Phentolamine (10 μg/ml) depressed both responses; 15 min after the addition of guanethidine (10 μg/ml) the nerve

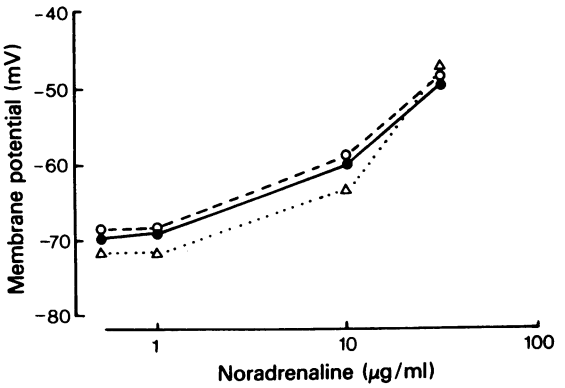

Figure 7 Effect of applied noradrenaline (NA) on membrane potential recorded from one preparation of the rabbit saphenous artery in control solution and in the presence of phentolamine. The NA-induced depolarization was not prevented by either concentration of phentolamine. Each point in control solution (●) represents the average membrane potential recorded from ⩾6 cells; s.e. mean within limits of symbols. Points obtained in the presence of phentolamine, 1 μg/ml (○) and 10 μg/ml (△) are from a single impalement in each case; 1.5 h was allowed for washout between each run.

response was completely abolished while no further depression of the direct muscle response was observed. The response produced by direct muscle stimulation returned to control values within 1.5 h after phentolamine was washed out. The extent of the phentolamine-induced depression of these responses (in the presence of guanethidine) ranged from 45 to 100% in the saphenous artery ($n = 7$) and 34 to 60% in the ear artery ($n = 5$).

Interaction between applied noradrenaline and α-blockers

Resting membrane potential The effects of applied NA on the r.m.p. were examined in the presence of phentolamine, phenoxybenzamine and prazosin. Whereas these α-antagonists completely abolished NA-induced contractions they did not prevent the depolarization of the membrane caused by high concentrations of NA in any of the three arteries examined.

In four preparations of the rabbit saphenous artery the relationship between membrane depolarization and NA concentration was examined in detail. Dose-membrane depolarization curves were obtained for applications of NA between 1 and 50 μg/ml in the presence of 1 μg/ml and 10 μg/ml phentolamine. Membrane potentials were recorded in control solution and 15 min after the addition of each successively higher concentration of NA. After the dose-response curve for NA was obtained, 1.5 h was allowed for washout and membrane potentials were again recorded in control solution and 15 min after the addition of NA plus phentolamine. Figure 7 shows the results obtained from one such experiment. No significant shift in the dose-membrane depolarization curves was apparent when either concentration of phentolamine was present.

Excitatory junction potentials The presence of α-blockers at concentrations up to 10 μg/ml failed to prevent the depression of the e.j.ps induced by NA. Results obtained in the rabbit ear artery and rat tail artery are shown in Figure 8. As described previously, phentolamine and phenoxybenzamine alone increased the steady-state amplitude of the e.j.p. Concentrations of NA ⩾ 1 μg/ml resulted in approximately the same level of depression as that observed with NA alone (Figure 8). However, the decrease in e.j.p. amplitude was more marked when prazosin was present in the bathing fluid and was observed at lower concentrations than in NA alone (Figure 8). Similar results were obtained in the rabbit saphenous artery.

Discussion

Concerning postsynaptic α-receptors

Concentrations of α-antagonists, that completely blocked NA-induced contractions, had little or no effect on neurally-evoked contractions in any of the arteries examined. When depression of the neurally-evoked contraction was observed (i.e. with phentolamine) it developed slowly and required a 10 to 100 fold higher concentration. In particular, prazosin, the effects of which are considered to be exclusively postsynaptic (Cambridge, Davey & Massingham, 1977), was completely ineffective in blocking the neurally-evoked contractions. Excitatory junction potentials were not depressed by high concentrations of any of these blockers even when these were present in the bathing solution for up to 3 h. All of these findings indicate that these α-receptor antagonists fail to block the postsynaptic receptors that are activated by neurally released transmitter when the frequency of stimulation is limited to the physiological range (less than 10 Hz).

This discrepancy between the action of exogenous NA and endogenous transmitter observed in the rabbit saphenous, ear and rat tail artery is remarkably similar to that often described in the vas deferens (Hotta, 1969; Ambache & Zar, 1971; Swedin, 1971; Ambache *et al.*, 1972). Ambache and his colleagues suggested that the most likely explanation for their

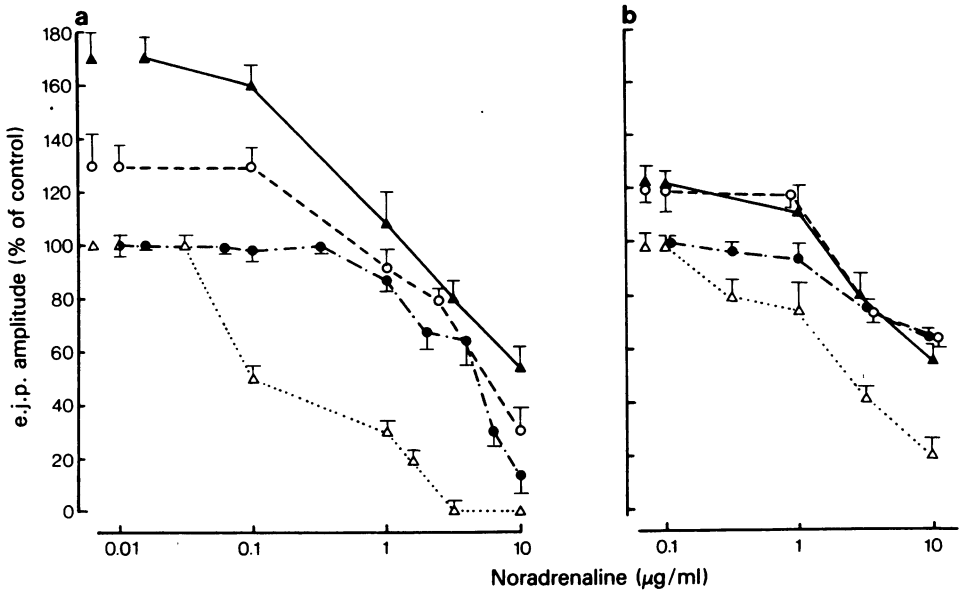

Figure 8 Effects of applied noradrenaline (NA) on depression of e.j.ps in control solution (●), and in the presence of phenoxybenzamine (▲), phentolamine (○), or prazosin (△) in the rabbit ear artery (a) and rat tail artery (b). Averaged responses to 64 stimuli at 0.45 or 0.5 Hz obtained in each case; $n \geq 4$ for all points; total no. of cells ≥ 7 for all points. Phentolamine and phenoxybenzamine alone increased the steady-state e.j.p. amplitude but did not prevent the depression of the e.j.ps induced by NA. Prazosin alone had no effect on the e.j.ps but caused a much greater depression of the e.j.ps than that induced by NA. Concentration of α-antagonists = 1 μg/ml.

results was that NA was not the excitatory transmitter in the vas deferens and our present experiments could be explained in terms of their hypothesis. An alternative idea, namely that NA was released from the sympathetic nerves in the vas deferens but that this acted on receptors with a different pharmacology from that of α-receptors, was first put forward by Hotta (1969). Hirst & Neild (1980) studied the changes in membrane potential and calibre of small segments of intestinal arterioles, *in vitro*, when NA was applied in a highly localized way by iontophoresis and came to the conclusion that postsynaptic α-receptors were not involved in neuromuscular transmission. This could also be true for muscular arteries studied in our experiments. This would be in accordance with our observation that α-blockers not only failed to depress the e.j.ps but also the depolarization caused by high concentrations of NA added to the bathing fluid.

How then, is it possible to account for reports that α-blockers depress mechanical responses to nerve stimulation? Phentolamine completely abolished contractions evoked by low frequency (less than 3 Hz) nerve stimuli in the rabbit saphenous and ear arteries but had no effect on the ability of these arterial smooth muscles to respond to nerve stimulation with action potentials or active responses. In addition, prolonged exposure to phentolamine depressed the contraction produced by direct muscle stimulation of these arteries. It seems likely that the effect of phentolamine in depressing the stimulation-induced contractile response is due to its direct effect on the smooth muscle, possibly by uncoupling some step in the excitation-contraction mechanism.

Concerning presynaptic α-receptors

Phentolamine and phenoxybenzamine, but not prazosin, increased the amplitude of the steady-state e.j.ps recorded from all arteries examined. These results are in accordance with data obtained from overflow studies in that both phentolamine and phenoxybenzamine enhance the release of NA from the nerve terminals while prazosin has no effect on stimulation-induced overflow of NA (See Starke, 1977; Westfall, 1977; Cambridge *et al.*, 1977). Data obtained from

overflow studies have given rise to the hypothesis that α-receptors exist on presynaptic terminals which act to depress subsequent release of NA (the negative-feedback hypothesis) (Farnebo & Hamberger, 1971; Kirpekar & Puig, 1971; Starke, 1971; see Starke 1977). The depression of e.j.ps by exogenous NA in concentrations that had no effect on the membrane potential of the smooth muscle is in accord with this suggestion. However, in our experiments this effect of NA was not prevented by α-blockers which is in direct contrast with the results of overflow studies (see Table 6 in Starke, 1977). Furthermore, prazosin, which itself had no effect on the amplitude of the e.j.ps, appeared to interact synergistically with exogenous NA to produce marked depression of the e.j.p.

Casteels *et al.* (1977) have suggested that NA may cause an increase in conductance in arterial smooth muscle even in the absence of a change in membrane potential. If this were the case in our experiments it could help to account for the depression in the e.j.ps. However, in the rabbit saphenous artery, no consistent changes in the amplitude or time course of electrotonic potentials have been recorded in the presence of concentrations of NA (1 μg/ml) which had no effect on the r.m.p. (Surprenant, unpublished observations).

We considered whether it would be possible to explain the depressant effect of exogenous NA on the amplitude of the e.j.ps on the basis of desensitization of postsynaptic receptors which are activated by neurally-released NA, as is the case for acetylcholine at the skeletal neuromuscular junction (Thesleff, 1955; Katz & Thesleff, 1957). Although desensitization of postsynaptic receptors may provide an adequate explanation for the depression of the e.j.ps, it is difficult to reconcile such an explanation with the repeatedly observed findings from overflow studies that NA actually depresses the amount of NA released by the nerve terminals. In addition, the finding that the NA-induced membrane depolarization was similar when cumulative doses of NA were added to the bath; i.e. under conditions where the effects of desensitization could be considered greatest, or when it was washed out prior to each new concentration being added, suggests that desensitization is unlikely to account significantly for the depression of the e.j.ps.

The potentiation of single e.j.ps by phentolamine and phenoxybenzamine in response to stimuli delivered at intervals as long as 2 min is also difficult to explain by the negative-feedback hypothesis. In order to do so it would be necessary to postulate that the NA released by nerve stimulation remained active and available to the presynaptic receptor for as long as 2 min after its release, or that the action of NA was very prolonged at the presynaptic receptor. We therefore agree with Kalsner (1979) who doubts the physiological relevance of the proposed α-receptor mediated feedback mechanism.

It is clear from the present study that further experiments concerning the pharmacology of action of NA in inhibiting its release by nerve stimulation, the actions of α-antagonists in enhancing transmitter release, as well as the interactions between NA and α-receptor blockers on transmitter release, are required before an adequate explanation for these results can be found.

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