Neurally evoked responses of human isolated resistance arteries are mediated by both α_1 - and α_2 -adrenoceptors

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1 Human subcutaneous resistance arteries (internal diameter 113–626 μm) were mounted in an isometric myograph. Electrical field stimulation was applied either continuously in the form of a frequency-response curve or intermittently at 16 Hz. The magnitude of the maximum contraction induced by continuous stimulation expressed as a percentage of the response to a supramaximal concentration of noradrenaline (10 μM) was highly variable but unrelated to vessel calibre. Contractile responses to both continuous and intermittent stimulation were abolished by 1 μM tetrodotoxin.

2 Prazosin (100 nM and 1 μM , α_1 -adrenoceptor antagonist) inhibited responses to continuous stimulation over a range of frequencies (2–8 Hz). The response to continuous stimulation at 8 Hz was inhibited by $78 \pm 6\%$ by 1 μM prazosin. Rauwolscline (100 nM, α_2 -adrenoceptor antagonist) had a smaller effect on contractions induced by continuous stimulation. Rauwolscline inhibited the response at 8 Hz by $36 \pm 6\%$. Rauwolscline at a higher concentration (1 μM) caused further inhibition of the response to continuous stimulation. Prazosin and rauwolscline in combination almost completely inhibited the response to continuous stimulation at concentrations of 1 μM .

3 Prazosin and rauwolscline inhibited responses to intermittent stimulation in a concentration-dependent manner. The IC_{50} for this action of prazosin was 3.7 ± 1.6 nM and the maximum inhibition induced by 100 nM prazosin was $78 \pm 6\%$. The IC_{50} of rauwolscline was 12.0 ± 1.3 nM and 100 nM rauwolscline caused a $86 \pm 7\%$ reduction in the response to intermittent stimulation. Prazosin and rauwolscline in combination (each at 100 nM) caused marked inhibition of the response to intermittent stimulation leaving only $7.0 \pm 2.6\%$ of the response.

4 These data suggest that neurally released noradrenaline evokes contractions of human resistance arteries by activation of both α_1 - and α_2 -adrenoceptors postjunctionally.

Keywords: Prazosin-pharmacology; peripheral-nerves-drug-effects; peripheral-vascular; rauwolscline; α -adrenoceptors; resistance-vessel

Introduction

Studies using exogenously applied agonists and antagonists have demonstrated functional postjunctional α_1 - and α_2 -adrenoceptors in human arteries. Moulds & Jauernig (1977) were the first to describe *in vitro* a 'prazosin-resistant' component of the response to noradrenaline in human isolated digital arteries, and subsequently, responses mediated by arterial postjunctional α_2 -adrenoceptors have been demonstrated both *in vivo* (Elliott & Reid, 1983; Jie *et al.*, 1984) and *in vitro* (Stevens & Moulds, 1981; Nielsen *et al.*, 1989).

The contribution of postjunctional α_2 -adrenoceptors to neurally evoked responses is not yet clarified. It was originally proposed that the α_2 -adrenoceptors would only be activated by circulating catecholamines and not by neuronally released noradrenaline; i.e. an extra-synaptic location of the α_2 -adrenoceptor (Timmermans & Van Zwieten, 1982; Jie *et al.*, 1987). Studies using terminal arterioles of the rat cremaster muscle, however, have suggested that the α -adrenoceptor being stimulated postjunctionally by neurally-released noradrenaline is mainly of the α_2 -subtype (Ohyanagi *et al.*, 1991) and results from investigations on human isolated digital arteries indicate that both α_1 - and α_2 -adrenoceptors are activated postjunctionally by neurally released noradrenaline (Stevens & Moulds, 1985).

Responses to exogenously applied noradrenaline in human subcutaneous resistance arteries are mediated by both α_1 - and α_2 -adrenoceptors (Nielsen *et al.*, 1990). The objective of this study was to assess the possible contributions of the post-

junctional α_1 - and α_2 -adrenoceptors to neurally-evoked contractions of these vessels. The normal pattern of nerve firing is highly irregular under *in vivo* conditions (Adrian *et al.*, 1982; Nilsson *et al.*, 1985) with impulses occurring in bursts separated by periods of quiescence. We therefore chose to activate perivascular nerves both continuously in a frequency-dependent manner and intermittently at high frequency (16 Hz) in bursts of 5 s duration. Some of the data have been presented previously as an abstract (Parkinson *et al.*, 1991).

Methods

Subjects and preparations

Biopsies of subcutaneous fat (approximately $2 \times 1 \times 1$ cm) were obtained from patients undergoing surgery. This was approved by local ethics committees. Under the microscope, two small arteries (113–626 μm internal diameter) were dissected out from each biopsy and mounted as ring segments (approximately 2 mm long) in a microvascular isometric myograph by threading the vessels onto two 40 μm stainless steel wires and securing the wires across two mounting supports (Mulvany & Halpern, 1977). After a 1 h equilibration period at 37°C, the vessels were set to a normalised internal circumference, estimated to be 0.9 times L_{100} , where L_{100} is the circumference they would maintain when relaxed and exposed to a transmural pressure of 100 mmHg (Mulvany & Halpern, 1977). At 0.9 L_{100} , the contraction produced is close to maximal (Mulvany & Warshaw, 1979), and internal diameters of the arteries were calculated as $l_{100} = L_{100}/\pi$. After

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normalisation, the arteries were initially exposed three times to $10\ \mu\text{M}$ noradrenaline for 2 min at 5 min intervals to determine vessel viability and maximal noradrenergic response. Any vessel which was not able to produce an active pressure equivalent to at least 100 mmHg (approximately 10% of all vessels mounted) was discarded.

Electrical field stimulation

The mounting supports contained two $200\ \mu\text{m}$ thick platinum electrodes. Each electrode had dimensions of approximately $1 \times 2\ \text{mm}$ and was fixed in a plane perpendicular to the plane containing the wires and about $800\ \mu\text{m}$ from the nearest wire (Angus *et al.*, 1988). Square wave field pulses were passed between the electrodes in a monophasic manner, either continuously or intermittently.

Continuous field stimulation studies were carried out at the Institute of Pharmacology, University of Aarhus, Denmark. The current was controlled with a battery driven, low-output-resistance stimulator (Biophysics Institute, University of Aarhus, Denmark) and the pulses were monitored with a two-channel oscilloscope. Two frequency-response curves (FRCs) were generated at 12 V and a pulse width of 0.3 ms, starting at 0.5 Hz, and increasing the frequency until a plateau was reached. In preliminary studies it was found that the response to 8 Hz was near to maximal. Stimulation at frequencies in excess of 8 Hz decreased responses of subsequent frequency-response curves and so was not used. In the 1 h interval between the two FRCs, the vessels were challenged three times, once every 15 min with $10\ \mu\text{M}$ noradrenaline for 2 min. The first FRC was always generated in the absence of drugs, whereas the second FRC was performed either (a) in the presence of prazosin alone (α_1 -adrenoceptor antagonist), (b) in the presence of rauwolscine alone (α_2 -adrenoceptor antagonist), (c) in the presence of the two antagonists in combination, (d) in the presence of tetrodotoxin ($1\ \mu\text{M}$), or (e) in the absence of any drugs (i.e. time-control curves). When antagonists were used, the arteries were preincubated for 15 min with the drugs which remained in the solution during generation of the second FRC; for TTX, however, preincubation lasted for 1 h. Vessels failing in the first FRC to produce more than 10% of the response to exogenously applied noradrenaline were excluded from the study (12% of the arteries exposed to continuous field stimulation).

Intermittent field stimulation studies were carried out at the Department of Clinical Pharmacology, St. Mary's Hospital, London. The stimulation parameters were controlled with a Grass S48 stimulator and the pulses monitored on an oscilloscope (Solartron). Complete sensitivity of field stimulation-evoked responses to TTX was ensured in each vessel prior to stimulation: after 10 min preincubation with $1\ \mu\text{M}$ TTX, the voltage was adjusted for each artery to the highest value (5–9 V) where contractile responses due to direct stimulation were absent. TTX added at the start of each experiment had no effect on the ability of the vessels to contract to exogenously applied noradrenaline. TTX was then washed out over 1 h whilst exposing the vessels three times to noradrenaline every 15 min to determine viability and maximal adrenergic activity. Following washout, field stimulation (pulse width 0.3 ms) was applied intermittently at 16 Hz for 5 s every 2 min for 50 min. Stable responses were obtained within 10 min and, unless time-control experiments were performed, three cumulative concentrations of either an α_2 -adrenoceptor agonist (rauwolscine or yohimbine) or an α_1 -adrenoceptor antagonist (prazosin or doxazosin) were then introduced into the bath 20, 30 and 40 min, respectively, after starting intermittent stimulation. In five arteries stimulation was continued for an extra 10 min to enable prazosin and rauwolscine to be introduced into the bath in combination.

Statistics

Values are expressed as mean $\pm$ s.e.mean. Based on results from the intermittent stimulation studies, IC_{50} values, i.e. the antagonist concentration needed to inhibit 50% of the responses, were calculated by linear interpolation. Statistical differences between two means were determined by a two-tailed, paired Student's *t* test and, when multiple comparisons were made, the Bonferroni procedure. The nominal level of significance was set at $P = 0.05$.

Drugs and solutions

The composition of the physiological saline solution (PSS) was (mM): NaCl 119, KCl 4.7, $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ 2.5, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 1.17, NaHCO_3 25, KH_2PO_4 1.18, Na_2EDTA 0.026 and glucose 5.5, bubbled with 95% O_2 and 5% CO_2 . Noradrenaline bitartrate, prazosin HCl, and tetrodotoxin were obtained from Sigma Chemical Co. (U.S.A.). Doxazosin-HCl was a gift from Pfizer, U.K. Rauwolscine HCl was obtained from Research Biochemical Inc. (U.S.A.).

Results

Continuous stimulation

A sample trace is shown in Figure 1a. In the absence of any drugs, stable tone in response to stimulation was observed to occur more rapidly at higher than at lower frequencies. The maximum response obtained with the first control FRC (usually at 8 Hz) was equivalent to $47 \pm 5\%$ (78 vessels) of the response to $10\ \mu\text{M}$ noradrenaline applied exogenously. The size of the maximum response to continuous stimulation was quite variable, however, and no relationship with artery calibre was found (Figure 1b).

In the absence of any drugs, the maximum response of the

Figure 1 The upper panel (a) shows the response to exogenously applied noradrenaline (NA, $10\ \mu\text{M}$) and to continuous nerve stimulation at increasing frequencies (0.5–8 Hz). The lower panel (b) shows the individual points and regression line of the maximum response to continuous nerve stimulation as a percentage of the response to exogenously applied noradrenaline ($10\ \mu\text{M}$) plotted against normalised internal diameter (I_{100} , see Methods).

second FRC was $108 \pm 12\%$ ($n = 8$) of the maximum response of the first FRC (Figure 2a). This response was almost totally inhibited by $1 \mu\text{M}$ TTX (Figure 2b).

Preincubation of vessels with prazosin (100 nM) reduced responses over the frequency range 4–8 Hz and significantly inhibited the maximum response to continuous stimulation by $63 \pm 7\%$ (Figure 3a). At a higher concentration, prazosin markedly reduced responses over the frequency range 2–8 Hz and significantly inhibited the response to continuous stimulation at 8 Hz by $74 \pm 7\%$ (Figure 3b).

The effect of rauwolfscine (100 nM) on the frequency-response relationship was less marked; rauwolfscine caused a small reduction in the response to continuous stimulation over the range 4–8 Hz (Figure 3d). Only the inhibition of response to stimulation at 8 Hz ($36 \pm 6\%$) differed significantly from control. A higher concentration of rauwolfscine ($1 \mu\text{M}$) produced more pronounced inhibition of responses across the range of frequencies used (Figure 3e), reducing the response to stimulation at 8 Hz by $68 \pm 5\%$ ($P < 0.05$).

Prazosin (100 nM) and rauwolfscine (100 nM) in combination significantly, but not completely, inhibited the neurogenic response (Figure 3c), leaving $48 \pm 8\%$ of the first FRC at 8 Hz. At higher concentrations, rauwolfscine ($1 \mu\text{M}$) and prazosin ($1 \mu\text{M}$) in combination caused near complete inhibition of the neurogenic response (Figure 3f).

Intermittent stimulation

Figure 4 shows a sample trace of contractile responses to intermittent stimulation and the effect of adding increasing concentrations of rauwolfscine to the bath. Both prazosin alone and rauwolfscine alone caused significant inhibition of the contractile responses to intermittent stimulation in a concentration-dependent manner (Figure 5). The IC_{50} values

Figure 2 (a) Two consecutive frequency response curves (FRCs) in response to continuous field stimulation and in the absence of drugs (12 vessels), (○) first FRC; (□) second FRC. Error bars have been omitted for clarity in this panel, but did not exceed 9% of the maximum response. In (b), the effect of tetrodotoxin (TTX $1 \mu\text{M}$) on the second FRC (■, 8 vessels) is shown. All responses are expressed relative to the response at 8 Hz of the first FRC as arithmetic means ($\pm$ s.e.mean, vertical bars); *response of second FRC significantly different from response of first FRC.

Figure 3 The effects of prazosin (Prz) and rauwolfscine (Rwl) alone and in combination on responses to continuous nerve stimulation. The panel shows contractile responses of the first frequency response curve (FRC) (×) and the effects on the second FRC of: (a) 100 nM prazosin alone (□, 6 vessels), (b) 1 μM prazosin alone (■, 10 vessels), (c) 100 nM prazosin and 100 nM rauwolfscine in combination (◇, 10 vessels), (d) 100 nM rauwolfscine alone (○, 8 vessels), (e) 1 μM rauwolfscine alone (●, 12 vessels), and (f) 1 μM prazosin and 1 μM rauwolfscine in combination (◆, 14 vessels). All responses are expressed relative to the maximum response at 8 Hz of the first FRC as arithmetic mean ($\pm$ s.e.mean, vertical bars); *response of second FRC significantly different from response of first FRC.

Figure 4 Trace showing contractile responses of human isolated resistance arteries to intermittent field stimulation and the effect of cumulatively adding increasing concentrations of rauwolfscine (Rwl).

for prazosin (5 vessels) and rauwolfscine (5 vessels) were $3.7 \pm 1.5 \text{ nM}$ and $12 \pm 1.3 \text{ nM}$, respectively. Prazosin (100 nM) inhibited the response to intermittent stimulation by $78 \pm 6\%$ and rauwolfscine (100 nM) inhibited the response to intermittent stimulation by $86 \pm 7\%$. Similar results were also obtained in other studies using yohimbine, ($\text{IC}_{50} = 12 \pm 1 \text{ nM}$, 11 vessels) and doxazosin ($\text{IC}_{50} = 5 \pm 2 \text{ nM}$, 9 vessels) as selective α_2 - and α_1 -adrenoceptor antagonists respectively (Parkinson *et al.*, 1991). Prazosin and rauwolfscine in combination (each at 100 nM) caused marked inhibition of the response to intermittent stimulation leaving only $7.0 \pm 2.6\%$ of the response.

Figure 5 The inhibition of the responses to intermittent field stimulation produced by prazosin (Prz) and rauwolscine (Rwl). The panels show the effects of antagonists at concentrations of (a) 1 nM, (b) 10 nM, and (c) 100 nM. All responses are expressed relative to the responses obtained in the 10 min stimulation period preceding the addition of drugs as arithmetic mean ($\pm$ s.e.mean, vertical bars). Time-control responses are shown by open columns. *Significant reductions in relative response.

Discussion

These studies demonstrate that electrical field stimulation causes contraction of isolated subcutaneous resistance arteries. The sensitivity to TTX and the near total inhibition of the responses to field stimulation by prazosin and rauwolscine in combination imply that the responses to field stimulation were due to activation of perivascular sympathetic nerves.

Previous studies *in situ* of resistance arteries from rat skeletal muscle (Boegehold & Johnson, 1988; Ohyanagi *et al.*, 1991) have shown inverse relationships between resistance artery diameter and sensitivity to sympathetic nerve stimulation, and the large variability in the maximum response to continuous nerve stimulation (Figure 1b) could be due to differences in innervation density of the vessels. This variation was apparently not dependent on vessel calibre (Figure 1b) and alternatively, the variability may be due to different degrees of degeneration of nerve terminals during transportation, dissection and mounting, since responses to exogenously applied noradrenaline did not show noticeable variation.

The sensitivity of the neurally evoked contractile responses of human subcutaneous resistance arteries to prazosin and rauwolscine suggests that the responses are mediated by both α_1 - and α_2 -adrenoceptors. This conclusion depends, however, on the selectivities of prazosin and rauwolscine for α -adrenoceptor subtypes in this tissue. Prazosin has a much higher affinity for α_1 - than for α_2 -adrenoceptors (Cubeddu, 1988) and earlier work in the same arteries from the same source as used in this study has shown that responses to the α_1 -adrenoceptor agonist, phenylephrine, are antagonized competitively by prazosin (Nielsen *et al.*, 1990) and that responses to the selective α_2 -adrenoceptor agonist B-HT 933 (Timmermans & Van Zwieten, 1980) are unaffected by 1 μ M

prazosin. These findings indicate that prazosin at concentrations up to 1 μ M is selective for α_1 -adrenoceptors in human subcutaneous resistance arteries. Rauwolscine is generally accepted to be a selective antagonist for α_2 -adrenoceptors (Weitzell *et al.*, 1979) and rauwolscine competitively antagonizes responses to B-HT 933 in human subcutaneous resistance arteries with a potency similar to that described in other preparations (pA₂ 7.6, Nielsen unpublished data). We have no data regarding the α_1 -antagonist properties of rauwolscine in human resistance arteries, but in other species these are generally seen at concentrations above or around 1 μ M (Wilson *et al.*, 1991). At the lower concentrations used in this study, prazosin (100 nM) and rauwolscine (100 nM) can probably be considered selective for α_1 - and α_2 -adrenoceptors, respectively. However, although the higher concentration of prazosin used in these studies can also probably be regarded as selectively antagonizing α_1 -adrenoceptor-mediated responses, 1 μ M rauwolscine is likely to show significant α_1 -adrenoceptor antagonist properties and the effects of the drug at this higher concentration should not be attributed solely to α_2 -adrenoceptor antagonism. Bearing this in mind it is noticeable that the effects of prazosin and rauwolscine appear to differ when continuous and intermittent stimulations are compared. Prazosin (100 nM and 1 μ M) produced marked inhibition of contraction induced by continuous stimulation across the range of frequencies used in this study. In contrast, 100 nM rauwolscine had a much less marked effect on tone induced by continuous stimulation and only significantly reduced the response at the highest frequency. Although 100 nM rauwolscine may not be sufficient to abolish α_2 -adrenoceptor-mediated responses completely, we would suggest that the more profound effect of 1 μ M rauwolscine may represent α_1 -adrenoceptor antagonism by this drug. This seems to be borne out when these results are compared with the findings using intermittent stimulation. In this case both prazosin and rauwolscine (10 and 100 nM) caused marked inhibition of the response to field stimulation at concentrations that are probably selective for α_1 - and α_2 -adrenoceptors, respectively. These findings appear to indicate that the response to intermittent stimulation involves postjunctional α_2 -adrenoceptors to a greater extent than does the response to continuous stimulation. Others, on the basis of studies in various species, including man (Timmermans & Van Zwieten, 1982; Jie *et al.*, 1984) have suggested that α_2 -adrenoceptors may be largely located extrajunctionally. We do not know whether the stimulation modes used in our studies activate extra- or intrajunctional α -adrenoceptors predominantly, not to mention possible preferential activation of prejunctional vs. postjunctional α_2 -adrenoceptors. Clearly further work is needed in this context, possibly using uptake-1 blockers, such as cocaine to increase extrajunctional 'spillover', antagonists selective for pre- vs. postjunctional adrenoceptors, or future highly selective α_2 -adrenoceptor antagonists.

It is not possible to estimate the relative contributions of these receptors to neurally evoked responses accurately, since concurrent blockade of presynaptic α_2 -adrenoceptors by rauwolscine may enhance neuronal release of noradrenaline. This action will, if anything, lead to an underestimation of the contribution by postjunctional α_2 -adrenoceptors. Even if the impact of prazosin and rauwolscine were attributed solely to postjunctional effects, estimation of the relative contribution of each receptor to the response is difficult as we have no information on the receptor-occupancy relationships for the interaction of noradrenaline with either receptor subtype, or the affinity of noradrenaline for either receptor subtype in this tissue. Although the relative importance of postjunctional α_1 - and α_2 -adrenoceptors to neurally evoked responses in human subcutaneous resistance arteries cannot be deduced from our data, the substantial inhibition by both α -adrenoceptor antagonists individually suggests an important contribution of both subtypes to neurally evoked responses in these arteries.

The innervation of vascular postjunctional α_1 - and α_2 -adrenoceptors varies, as indicated in the Introduction, from one arterial preparation to another. The postjunctional α -adrenoceptor stimulated by neurally released noradrenaline is of the α_1 -subtype in canine aorta (Bobik & Anderson, 1983), in large arterioles of cremaster skeletal muscle (Ohyanagi *et al.*, 1991) and in femoral skeletal muscle arteries of the rat (Medgett & Ruffolo, 1988). In contrast, the predominant contractile effect of nerve stimulation is mediated by postjunctional α_2 -adrenoceptors in terminal arterioles in rat cremaster skeletal muscle (Ohyanagi *et al.*, 1991) and in the cutaneous microcirculation of cats (Koss *et al.*, 1991) and rats (Wilette *et al.*, 1991). These findings suggest that the importance of postjunctional α_2 -adrenoceptors as mediators of nerve stimulation may increase as arteries get smaller. Although the suggestion is still speculative, these considerations emphasise the problems associated with uncritical extrapolation of the results from this study to other vascular beds, in particular to arteries with calibres different from those examined in the present study. If is of note, however, that postjunctional α_2 -adrenoceptors mediate in part responses to exogenously applied noradrenaline not only in human subcutaneous resistance arteries (Nielsen *et al.*, 1990) but also in human resistance arteries isolated from a variety of other vascular beds (Nielsen *et al.*, 1991).

Purinergic transmission has been demonstrated in a variety of animal blood vessels *in vitro* (Sneddon & Burnstock, 1984) and *in vivo* (Bullock & McGrath, 1988). In man it has been suggested that ATP may account for a non-adrenergic component of sympathetic transmission in the forearm (Taddei *et al.*, 1989; 1990). Lower body negative pressure, the stimulus to sympathetic activation used in these studies, produces an increase in sympathetic nerve firing similar to the pattern of

intermittent perivascular nerve stimulation (Sundlof & Wallin, 1978). Although our study has addressed noradrenergic neurotransmission, the near total abolition of the responses to perivascular field stimulation by α -adrenoceptor antagonists does not support a substantial role for purines in mediating contractile responses of subcutaneous resistance arteries. On the other hand, our data on the effect of prazosin and rauwolscine in combination do not totally exclude a modest non- α -adrenoceptor-mediated contractile response of human resistance arteries to nerve stimulation.

In conclusion, the data from this study strongly suggest that neurally evoked responses of human isolated subcutaneous resistance arteries are mediated postjunctionally not only by α_1 - but also by α_2 -adrenoceptors. Arteries of the size examined in this study contribute substantially to precapillary resistance (Mulvany & Aalkjaer, 1990). The postjunctional α_2 -adrenoceptor may consequently be an important target for sympatholytic therapy in the management of essential hypertension, particularly in view of recent observations suggesting that postjunctional α_2 -adrenoceptors can be differentiated pharmacologically from those located prejunctionally (Ruffolo *et al.*, 1987; Akers *et al.*, 1991).

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