β -Adrenoceptor agonist mediated relaxation of rat isolated resistance arteries: a role for the endothelium and nitric oxide

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- 1 Isoprenaline (10^{-9} – 10^{-5} M) relaxed rat isolated mesenteric resistance arteries pre-contracted with K^+ (30–60 mM) (pEC_{50} (M) 8.03 ± 0.40 ; maximum relaxation $66.79 \pm 2.43\%$, $n = 7$). This relaxation was partially attenuated by the nitric oxide (NO) synthase inhibitor N^G -nitro-L-arginine methyl ester (L-NAME, 10^{-4} M).
- 2 The β_2 -adrenoceptor agonist, salbutamol (10^{-9} – 10^{-5} M), produced a modest maximum relaxation ($35.93 \pm 2.93\%$), which was not sensitive to L-NAME.
- 3 The β_1 -adrenoceptor agonist, dobutamine (10^{-9} – 10^{-5} M), relaxed arteries precontracted with K^+ . This relaxation was abolished by L-NAME (10^{-4} M) and also by propranolol (10^{-6} M), but not affected by D-NAME (10^{-4} M). The inhibition by L-NAME was partially reversed by L-arginine (10^{-3} M). Removal of the endothelium severely attenuated relaxation to dobutamine.
- 4 Contractile responses to depolarizing K^+ solutions were enhanced by the addition of L-NAME, and also by removal of the endothelium.
- 5 The above findings demonstrate that β_1 -adrenoceptor activation causes relaxation via NO release from the endothelium of rat mesenteric resistance arteries. In addition, contraction to K^+ is modified by release of NO from the endothelium, possibly in response to tension development.

Keywords: β -Adrenoceptors; vascular smooth muscle; endothelium; nitric oxide; rat resistance artery

Introduction

Relaxation of vascular smooth muscle by β -adrenoceptor agonists is conventionally held to occur via activation of adenylate cyclase and an increase in intracellular adenosine 3':5'-cyclic monophosphate (cyclic AMP) in the smooth muscle cell (Kukovetz *et al.*, 1981; Heeson & De Mey, 1990). Some reports, however, have suggested that the endothelium may play a facilitatory role in the relaxation to β -adrenoceptor agonists. These include the demonstration that removal of the endothelium from rat aorta (Kamata *et al.*, 1989) and canine coronary arteries (Rubanyi & Vanhoutte, 1985) attenuates isoprenaline-induced relaxation but does not affect the relaxation to forskolin (Kamata *et al.*, 1989). Not all are in agreement, however, as Moncada *et al.* (1991) found that isoprenaline-induced relaxation in rat aorta was neither endothelium-dependent, nor affected by inhibitors of nitric oxide (NO) synthase. Similarly, the absence of endothelium did not attenuate relaxation to isoprenaline in aorta and femoral artery of normotensive rats (Konishi & Su, 1983), although there was a slight reduction in the same arteries of spontaneously hypertensive rats. A study in the rat saphenous vein has also suggested that the endothelium is not involved in β -adrenoceptor agonist-induced relaxation in the venous circulation (De Mey & Vanhoutte, 1982).

Two studies have suggested more specifically that β -adrenoceptor-mediated relaxation may involve NO release from the endothelium of some preparations. In a preliminary report, Gray & Marshall (1991) found isoprenaline-induced relaxation to be endothelium-dependent in the rat isolated aorta, and the relaxation was attenuated by the nitric oxide (NO) synthase inhibitor N^G -nitro-L-arginine methyl ester (L-NAME). L-NAME also partially inhibited the vasodilatation caused by the β_2 -agonist, salbutamol, in the rat hindlimb *in vivo* (Gardiner *et al.*, 1991).

Other than the study by Gardiner *et al.* (1991) in the whole animal, all previous work has been carried out in isolated conduit arteries and no study has extensively investigated the

role of the endothelium in β -adrenoceptor-agonist-mediated relaxation in resistance arteries. Since the β -adrenoceptors are largely involved in control of peripheral resistance by affecting tone in small arteries, this investigation has been carried out to determine the role of NO in β -adrenoceptor-mediated relaxation in resistance arteries. In a previous report (Heeson & De Mey, 1990), the non-selective β -agonist isoprenaline, and forskolin (a direct activator of adenylate cyclase), were found to relax rat mesenteric resistance arteries. In this study, using the same preparation, we have investigated the role of NO in the relaxation induced by isoprenaline, salbutamol (β_2 -agonist), dobutamine (β_1 -agonist), and forskolin, all generally held to relax vascular smooth muscle via cyclic AMP-dependent mechanisms. Endothelium-dependence of relaxation to dobutamine was also investigated by mechanical removal of the endothelium.

A preliminary account of part of this work has been presented to the British Pharmacological Society (Graves & Poston, 1992).

Methods

Mulvany-Halpern myograph

Resistance arteries were isolated from male Wistar-Kyoto rats (225–250 g, Bantin & Kingman, Hull, UK). After killing by cervical dislocation, mesenteric resistance arteries (fourth order branches) were dissected and mounted as a ring preparation on a myograph capable of measuring isometric tension (Mulvany & Halpern, 1977). The organ bath was filled with physiological salt solution (PSS) of the following composition (mM): NaCl 119, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.17, $NaHCO_3$ 25, KH_2PO_4 1.18, EDTA 0.026 and glucose 5.5, pH 7.4, 37°C, bubbled with 5% CO_2 in O_2 . The passive tension-internal circumference characteristics of each vessel

was determined and the internal diameter estimated (mean diameter $322 \pm \text{s.e. mean } 3.1 \mu\text{m}$, $n = 115$). The arteries were then set to an internal circumference equivalent to 90% of that which they would have when relaxed *in situ* under a transmural pressure of 100 mmHg. The maximum active tension for the minimum resting tension is developed at approximately this circumference (Mulvany & Halpern, 1977).

To assess their contractile response, the arteries were then maximally contracted for 2 min every 10 min on five occasions. The first, second and fifth contractions were to $5 \mu\text{M}$ noradrenaline (NA) in 125 mM potassium solution (KPSS, equimolar substitution of KCl by NaCl in PSS); (NAK). The third was to $5 \mu\text{M}$ NA in PSS, and the fourth to KPSS. Any artery failing to produce a maximum active tension equivalent to a pressure of 100 mmHg on the final contraction was rejected. After the third ($5 \mu\text{M}$ NA) contraction, arteries were challenged with acetylcholine (ACh $5 \times 10^{-6} \text{ M}$) to check endothelial function; all arteries relaxed by $>85\%$ of the initial contraction following this procedure. For the experiments involving endothelial removal this protocol was modified as described below.

Response to β -agonists and the effect of NO synthase inhibitors

Isoprenaline

A control contraction (13 min) to KPSS (K^+) was carried out with a concentration of potassium (30–60 mM) which led to approximately 50% of the tension developed with NAK. Any artery which did not maintain tension over this period was omitted from the study. The K^+ was then washed out and after 10 min the arteries again contracted with the same concentration of K^+ . After 3 min of precontraction a cumulative concentration-response curve to isoprenaline (10^{-9} M – 10^{-5} M , 2 min intervals) was then carried out. Following washout for 5 min, arteries were then incubated with L-NAME (10^{-4} M , 10 min) and a further concentration-response curve to isoprenaline constructed in the continued presence of L-NAME. In other experiments, the reproducibility of the isoprenaline relaxation was investigated by using the same protocol, but omitting L-NAME before and during the third contraction. The ability of the arteries to maintain reproducible contractions to K^+ over the length of the protocol was also studied by contracting arteries 3 times with K^+ (for 13 min) and preincubating with L-NAME (10^{-4} M) for the third contraction. The effect of isoprenaline on arteries precontracted by a receptor agonist rather than K^+ was investigated using the same protocol, but with substitution of K^+ with $3 \mu\text{M}$ NA. This dose of NA gave approximately 90% of the maximal contraction and produced a stable and sustained contraction. To confirm the involvement of β -adrenoceptors in isoprenaline-induced relaxation, the effect of propranolol, a non-selective β -antagonist, was investigated. Arteries were precontracted with K^+ as described above and two concentration-response curves to isoprenaline carried out, the second in the presence of propranolol (10^{-6} M).

Salbutamol

The effect of the β_2 -agonist salbutamol (10^{-9} M – 10^{-5} M) on K^+ -induced contraction, and the effect of L-NAME upon salbutamol-induced relaxation were studied in the same arteries, following the protocol described above for isoprenaline.

Dobutamine

The effect of L-NAME upon relaxations elicited by dobutamine (β_1 -agonist) were similarly examined. In control experiments, sequential relaxations to dobutamine were not reproducible, the second of two concentration-response curves demonstrating considerable tachyphylaxis. Concen-

tration-response curves to dobutamine were therefore obtained and compared with those to dobutamine + L-NAME in another set of arteries. Arteries were submaximally precontracted with K^+ as described above and a concentration-response curve (10^{-9} M – 10^{-5} M) to dobutamine performed with or without L-NAME (10^{-4} M). The ability of L-arginine to reverse blockade by L-NAME was investigated by incubating with L-arginine (10^{-3} M) for 20 min before addition of L-NAME (10^{-4} M) and the subsequent concentration-response curve to dobutamine was performed in the presence of L-NAME and L-arginine. The effect of D-NAME (10^{-4} M), the inactive enantiomer of L-NAME, was also investigated using an identical protocol to that described for L-NAME. The response to dobutamine in the presence of propranolol (10^{-6} M) was also studied.

Removal of the endothelium

To examine the effect of endothelium removal on relaxation to dobutamine the luminal surface of the artery was gently rubbed with a human forearm hair after the first contraction of the run-up procedure. The second, third and fourth contractions were to $5 \mu\text{M}$ NA and the arteries were challenged with ACh ($5 \times 10^{-6} \text{ M}$) after each contraction. Failure to relax more than 15% in 2 min was adopted as the criterion for efficient endothelium removal. If the arteries relaxed more than 15% further gentle rubbing was performed. Control experiments were performed which confirmed that repeated exposure to 10^{-5} M ACh did not impair the arteries ability to relax to ACh. Dobutamine-induced relaxation was investigated after removal of the endothelium, using the protocol described above.

Effect of L-NAME on forskolin-induced relaxation

Arteries were contracted with K^+ (30–60 mM) for 17 min. After washout (10 min), the arteries were again contracted to K^+ and a concentration-response curve to forskolin (10^{-8} M – 10^{-5} M , 2 min intervals) carried out. Forskolin was first dissolved in ethanol and final concentrations of ethanol in the organ bath were less than 0.1%. In order to avoid the effects of tachyphylaxis, the above protocol was performed in a separate series of arteries, but with the addition of L-NAME (10^{-4} M) both for 10 min preceding and throughout the forskolin concentration-response curve.

Modification of K^+ contraction by NO

When studying agonist-induced relaxation it is important to produce similar tension in the test contracture to that in the control. In initial experiments using K^+ to precontract the arteries, considerable potentiation of the precontraction to K^+ occurred after incubation with L-NAME. This necessitated lowering the concentration of K^+ used in the presence of L-NAME to achieve similar tension. The potentiation by L-NAME suggested that NO release may attenuate K^+ -induced tension development, and the effect of L-NAME upon K^+ concentration-response curves was therefore further investigated. A cumulative K^+ concentration-response curve was constructed and then repeated in the presence of L-NAME. Since a depolarizing potassium stimulus may lead to neurotransmitter release from adrenergic terminals (Vanhoutte *et al.*, 1981) the above protocol was repeated in the presence of phentolamine (10^{-5} M). The involvement of possible β -receptor stimulation was also investigated by the construction of two consecutive concentration-response curves to K^+ in the presence of phentolamine (10^{-5} M), and phentolamine plus propranolol (10^{-6} M) respectively. As a time control, two consecutive K^+ concentration-response curves were carried out in separate arteries. The effect of endothelial removal on the response to K^+ was also studied.

Statistical analysis

Relaxations to agonists are expressed as a percentage of the precontraction and are given as mean $\pm$ s.e.mean. Mean tensions for precontractions to K^+ and NA ($mN\ mm^{-1}$) are given for the initial isoprenaline experiments and were of similar magnitude for all subsequent protocols. Concentration-response curves for agonist-induced relaxation and contraction were fitted by the Graphpad package (GraphPAD Software Inc., San Diego, U.S.A.) to obtain maximum effect and pEC_{50} ($-\log$ of molar concentration producing 50% of maximum response). In the experiments in which K^+ concentration responses were investigated, results are expressed as percentages of the maximum response to NAK for each artery. Differences between means were assessed either by paired or unpaired t test as appropriate. For multiple comparisons ANOVA (Statview SE, Abacus Concepts Inc.) followed by an unpaired t test was used.

Chemicals

The chemicals used in this study were acetylcholine chloride, L-NAME hydrochloride, L-arginine (free base), forskolin, and propranolol hydrochloride (Sigma, Poole, UK); D-NAME hydrochloride (Calbiochem-Novabiochem, Nottingham, UK); noradrenaline tartrate (Winthrop, Guildford, U.K.); isoprenaline hydrochloride (Pharmax, Bexley, U.K.); dobutamine hydrochloride (Eli Lilly & Co. Ltd, Basingstoke, U.K.); salbutamol sulphate (Allen & Hanburys Ltd, Greenford, U.K.); phentolamine mesylate (CIBA Laboratories, Horsham, U.K.). All other chemicals (Analar grade) were obtained from BDH, Poole, U.K.

Results

Response to β -agonists and the effect of NO synthase inhibitors

Isoprenaline

Isoprenaline relaxed rat mesenteric resistance arteries pre-contracted with K^+ (initial tension $2.18 \pm 0.24\ mN\ mm^{-1}$) in

Figure 1 Relaxation of rat mesenteric resistance arteries to isoprenaline ($10^{-9}\ M$ – $10^{-5}\ M$): control (○); propranolol $10^{-6}\ M$ (▽); N^m -nitro-L-arginine methyl ester (L-NAME, $10^{-4}\ M$) (●). Results expressed as percentage relaxation (mean $\pm$ s.e.mean) of K^+ pre-contraction. Significant difference of maximum relaxation v control indicated by * $P < 0.05$.

a concentration-dependent manner (pEC_{50} (M) 8.03 ± 0.40 ; maximum relaxation $66.79 \pm 2.43\%$, $n = 7$). Subsequent incubation with L-NAME ($10^{-4}\ M$) reduced the maximum relaxation to isoprenaline (initial tension $2.28 \pm 0.06\ mN\ mm^{-1}$, maximum relaxation $27.64 \pm 7.64\%$, $n = 7$, $P < 0.001$, paired t test). Two successive isoprenaline concentration-response curves, carried out as a control, showed no change in sensitivity (pEC_{50} 7.72 ± 0.2 v 7.63 ± 0.21 , $n = 6$, $P = 0.42$ paired t test) but maximum relaxation in the second was less than the first ($51.14 \pm 4.68\%$ v $60.96 \pm 3.11\%$, $n = 6$, $P < 0.005$, paired t test). When results for this second isoprenaline concentration-response curve were compared to those for isoprenaline + L-NAME, the difference in the maximum response remained significant ($n = 7$, $P < 0.05$, unpaired t test) (Figure 1). The relaxation to isoprenaline of arteries pre-contracted with K^+ was completely inhibited by propranolol ($10^{-6}\ M$) (Figure 1). Isoprenaline also led to relaxation of arteries pre-contracted with NA, the addition of L-NAME again reducing the maximum relaxation to isoprenaline ($73.50 \pm 9.74\%$, control, v $40.89 \pm 7.04\%$, L-NAME, $n = 6$, $P < 0.001$, paired t test). Time controls in which arteries were pre-contracted with K^+ without the addition of agonists showed $11.5 \pm 4.8\%$ maximum relaxation without L-NAME and $4.8 \pm 5.5\%$ with L-NAME ($n = 6$, $P = 0.38$, paired t test) (Figure 2).

Salbutamol

Salbutamol also relaxed arteries pre-contracted with K^+ (maximum relaxation $35.93 \pm 2.93\%$), but L-NAME did not reduce the relaxation (maximum relaxation $33.05 \pm 4.11\%$, $n = 6$, $P = 0.70$, paired t test) (Figure 2).

Dobutamine

The β_1 -agonist dobutamine led to the relaxation of arteries pre-contracted with K^+ (maximum relaxation ($10^{-5}\ M$))

Figure 2 Relaxation of rat mesenteric resistance arteries to salbutamol ($10^{-9}\ M$ – $10^{-5}\ M$): control (○) and N^m -nitro-L-arginine methyl ester (L-NAME, $10^{-4}\ M$) (●). Data also shown for tension development to K^+ over the time course of the experiment, with (▽) and without (●) L-NAME ($10^{-4}\ M$). Results expressed as percentage relaxation (mean $\pm$ s.e.mean) of K^+ pre-contraction.

65.1 ± 4.2%, $n = 9$). The maximum relaxation was completely inhibited by L-NAME (maximum relaxation 6.52 ± 3.2%, $n = 6$, $P < 0.001$ v control unpaired t test) but was not attenuated by D-NAME (10^{-4} M) (Figure 3). Preincubation with L-arginine (10^{-3} M) partially reversed the inhibition by L-NAME (Figure 3). Propranolol prevented relaxation to dobutamine and mechanical removal of the endothelium attenuated relaxation to dobutamine (Figure 4).

Effect of L-NAME on forskolin-induced relaxation

Forskolin relaxed arteries pre-contracted with K^+ in a concentration-dependent manner (pEC_{50} 7.32 ± 0.18; maximum relaxation 94.3 ± 2.1%, $n = 6$). L-NAME led to a slight increase in sensitivity to forskolin which did not reach significance (pEC_{50} 7.76 ± 0.08; $P = 0.06$ v control; maximum relaxation 98.6 ± 0.6%, $n = 6$, $P = 0.08$ v control, unpaired t test) (Figure 5).

Modification of K^+ contraction by NO

L-NAME increased both the sensitivity to K^+ (pEC_{50} 1.37 ± 0.01, control v 1.48 ± 0.03, L-NAME; $n = 6$, $P < 0.02$, paired t test) and the maximum response (65.82 ± 1.02% of contraction to NAK, control, v 78.44 ± 3.41%, L-NAME, $n = 6$, $P < 0.005$, paired t test). The maximum response between two successive concentration-response curves to K^+ in the absence of L-NAME was unaltered (75.1 ± 2.0% of NAK v 75.35 ± 4.6%, $n = 6$, $P = 0.72$, paired t test, $n = 6$). There was, however, a small but significant reduction in sensitivity to K^+ for the second curve (pEC_{50} 1.32 ± 0.03 v 1.27 ± 0.02, $n = 6$, $P < 0.05$ paired t test). Mechanical removal of the endothelium also increased the sensitivity to K^+ (pEC_{50} 1.37 ± 0.01; control, v 1.52 ± 0.03, without endothelium; $n = 6$, $P < 0.001$, unpaired t test) and the maximum response (65.8 ± 1.0% of NAK, control, v 90.4 ± 2.2% without endothelium, $n = 6$, $P < 0.001$, unpaired t test) (Figure 6a). The maximum tension development to NAK was 4.68 ± 0.17 mN mm $^{-1}$ for the arteries used in the investigation of L-NAME, and 3.87 ± 0.17 mN mm $^{-1}$ for those in which the endothelium was removed, the difference being attributable to slightly

Figure 3 Relaxation of rat mesenteric resistance arteries to dobutamine (10^{-9} M– 10^{-5} M): control (○); D $^{\alpha}$ -nitro-L-arginine methyl ester (D-NAME, 10^{-4} M) (●); L-NAME (10^{-4} M) (▼); L-NAME (10^{-4} M) + L-arginine 10^{-3} M (▽). Results expressed as percentage relaxation (mean ± s.e.mean) of K^+ pre-contraction. Significant differences in maximum relaxation v control indicated by *** $P < 0.001$.

Figure 4 Relaxation of rat mesenteric resistance arteries by dobutamine (10^{-9} M– 10^{-5} M): control (○); propranolol (10^{-6} M) (●); minus endothelium (▽). Results are expressed as percentage relaxation (mean ± s.e.mean) of K^+ pre-contraction. Significant difference of maximum relaxation v control indicated by *** $P < 0.001$.

Figure 5 Relaxation of rat mesenteric resistance arteries to forskolin (10^{-8} M– 10^{-5} M): control (○); N $^{\omega}$ -nitro-L-arginine methyl ester (L-NAME, 10^{-4} M) (●). Results expressed as percentage relaxation (mean ± s.e.mean) of K^+ pre-contraction.

larger arteries being used for the L-NAME study ($328 \pm 9.1 \mu\text{m}$ v $289 \pm 7.5 \mu\text{m}$).

Phentolamine reduced K^+ induced tension ($65.8 \pm 1.0\%$ of NAK, control v $31.1 \pm 6.07\%$ phentolamine, $n = 6$, $P < 0.001$ unpaired t test). Despite this α -adrenoceptor inhibition, L-NAME again led to potentiation of K^+ -induced tension, affecting both the maximum response ($31.1 \pm 6.1\%$ of NAK, control v $68.2 \pm 3.73\%$ L-NAME, $n = 6$, $P < 0.01$ paired t test) and the sensitivity (pEC_{50} 1.47 ± 0.06, control, v 1.56 ± 0.04, L-NAME, $n = 6$, $P < 0.05$ paired t test) (Figure 6b). β -Blockade with propranolol did not affect the maximum response to K^+ in the presence of phentolamine ($20.2 \pm 1.1\%$ of NAK, control, v $23.14 \pm 3.4\%$, propranolol, $n = 4$,

Figure 6 (a) Tension development to K^+ in rat mesenteric resistance arteries: control (○); $N^ω$ -nitro-L-arginine methyl ester (L-NAME, 10^{-4} M) (●), - endothelium (▽). (b) Tension development to K^+ in the presence of phentolamine (10^{-5} M) with (●) and without (○) L-NAME (10^{-4} M). Results expressed as percentage (mean $\pm$ s.e.mean) of contraction to NAK ($5 \mu\text{M}$ NA; 125 mM K^+). Significant difference of maximum contraction v control indicated by ** $P < 0.005$ and *** $P < 0.001$.

$P < 0.35$, paired t test), or significantly alter sensitivity (pEC_{50} 1.48 ± 0.01 , control, v 1.39 ± 0.03 , propranolol, $n = 4$, $P = 0.10$, paired t test).

Discussion

The isoprenaline-induced relaxation observed in rat mesenteric resistance arteries is in agreement with an earlier report by Heeson & De Mey (1990) in which this non-selective β -agonist was found to relax small arteries from the same circulation. In the present study, relaxation to isoprenaline was abolished by propranolol, confirming the involvement of β -adrenoceptors. Relaxation to isoprenaline was also inhibited, but not completely, by the NO synthase inhibitor, L-NAME, suggesting that in this preparation non-selective β -agonists may act partially through the release of NO. Isoprenaline-induced relaxation and the inhibitory effect of

L-NAME were similar in arteries pre-contracted with NA or depolarizing potassium solution.

In order to investigate further the involvement of NO release by β -agonists, the effects of agonists specific to β_1 - and β_2 -adrenoceptors were determined. A relatively poor relaxation was found to the β_2 -agonist salbutamol, which was unaffected by L-NAME. This modest relaxation to salbutamol was similar (as a percentage of the original tension) to the relaxation that Fujimoto *et al.* (1987) reported in strips of rat mesenteric artery pre-contracted with potassium. The lack of inhibition by L-NAME on β_2 -agonist-induced relaxation agrees with the *in vivo* study of Gardiner *et al.* (1991) in which L-NAME did not attenuate the small vasodilatation to salbutamol observed in the rat mesenteric circulation. It is of interest that L-NAME reduced the hyperaemic vasodilatation to salbutamol in the rat hindlimb (Gardiner *et al.*, 1991) suggesting that the mechanism of relaxation to β -agonists may vary from circulation to circulation.

In contrast to salbutamol, the β_1 -agonist dobutamine led to substantial relaxation of arteries pre-contracted with potassium. This relaxation was completely blocked by propranolol at all concentrations of dobutamine. This surprisingly effective inhibition is of interest as it suggests a very high affinity of propranolol for β -adrenoceptors in this preparation. Indeed, similarly profound inhibition of relaxation to a mixed β -agonist was found by Tsujimoto & Hoffman (1985) in the same circulation of the rat, but in arteries of larger diameter (3 mm). Whereas, L-NAME had no effect on β_2 -induced relaxation, it effectively blocked the relaxation to dobutamine, suggesting that β_1 -agonists led to relaxation in this circulation via the release of NO. The D-enantiomer of L-NAME (D-NAME) had no effect on dobutamine-induced relaxation, indicating that the inhibition of relaxation by L-NAME was due to a specific effect on NO synthase. The NO synthase substrate, L-arginine, only partially reversed the effects of L-NAME on dobutamine-induced relaxation. In larger arteries L-arginine completely reversed the effect of NO synthase inhibition (Palmer *et al.*, 1988; Rees *et al.*, 1989; Moore *et al.*, 1990), but this study agrees with others in resistance arteries which have also demonstrated partial reversal of nitric oxide inhibition. These include a study by Moore *et al.* (1990) using various NO synthase inhibitors in the perfused rat mesenteric bed, and another from our laboratory in human resistance arteries in which L-arginine only partially reversed the inhibitory effect of L-NAME monomethyl-arginine (L-NMMA) on ACh-induced relaxation (Woolfson & Poston, 1990). There is no obvious explanation for this observation, but there is increasing evidence that L-arginine and NO synthase inhibitors may not interact in an entirely competitive manner. This is supported by the frequent observation that greater concentrations of L-arginine than those calculated have often been required to achieve complete or even partial reversal of NO synthase inhibition (Amezcuca *et al.*, 1989; Vallance *et al.*, 1989; Moore *et al.*, 1990; Woolfson & Poston, 1990).

The relaxation to dobutamine was also inhibited by mechanical removal of the endothelium, providing evidence that it is the endothelium which is the source of the NO released by β_1 -activation. Little information is available concerning β -adrenoceptors in cells of the vascular endothelium, and the mechanism whereby β_1 -adrenoceptor activation leads to NO release must be speculative. It could be suggested that nitric oxide is produced in response to β_1 -induced elevation of cyclic AMP in the endothelial cell, but Kuhn *et al.* (1991) have shown that experimental elevation of cyclic AMP or cyclic GMP does not affect nitric oxide release from bovine cultured aortic endothelial cells. In vascular smooth muscle (Sadoshima *et al.*, 1988), β -adrenoceptor activation led to activation of calcium-activated potassium channels. The consequent hyperpolarization would lead to a fall in cell calcium and, in vascular smooth muscle, to relaxation, but in the endothelial cell hyperpolarization leads to an increase in cell calcium, the stimulus for NO release (Luckhoff & Busse,

1990). Whilst potassium channel activation and hyperpolarization would be an attractive hypothesis for endothelial cell NO release by β -agonists, it is an unlikely explanation for the present findings since β_1 -induced relaxation occurred in arteries pre-contracted with a depolarizing potassium solution.

The relaxation of rat mesenteric resistance arteries to forskolin, a direct activator of adenylate cyclase, was not attenuated by L-NAME. In fact, there was a small but non-significant increase in sensitivity to forskolin in the presence of L-NAME. These experiments were carried out to determine whether L-NAME affected cyclic AMP dependent relaxation, since β -agonists are conventionally held to effect relaxation through this pathway in vascular smooth muscle. The results obviate any suggestion of a non-specific inhibitory effect of L-NAME on cyclic AMP-dependent relaxation, but cannot discount an effect of forskolin on the endothelium itself.

Further interesting observations to emerge from this study are the effects of L-NAME and endothelial removal on K^+ -induced contraction, both of which increased the sensitivity and maximum response to K^+ . This indicates that K^+ -induced contraction is modified by release of NO. Modification by NO of NA-induced contraction has been previously reported in arteries from this vascular bed (Dohi *et al.*, 1990), and Martin *et al.* (1986) have come to similar conclusions from experiments in the rat aorta. They concluded that this was due to spontaneous release of NO and

was not mediated via α -adrenoceptors. Because adrenergic nerve terminals may liberate noradrenaline when arteries are stimulated with potassium (Vanhoutte *et al.*, 1981) we investigated the effect of α -adrenoceptor blockade together with NO synthase inhibition. Indeed, phentolamine substantially attenuated K^+ -induced contraction, but we found that L-NAME still potentiated the K^+ response in the presence of phentolamine. We may also conclude therefore that α -adrenoceptor-induced release of NO is not involved. Neither would it appear that β -adrenoceptor activation of NO is involved, as propranolol did not affect the response to potassium in the presence of phentolamine. It may therefore be the case that NO is released in direct response to tension development; indeed stretch activated calcium channels have been described in endothelial cells (Lansman *et al.*, 1987) and could provide a mechanism for release.

In conclusion, this is the first extensive study of β -adrenoceptor-mediated NO release in resistance arteries. As detailed in the introduction, several studies in conduit arteries have implied a facilitatory role for the endothelium and/or NO in β -adrenoceptor-mediated relaxation although not all are in agreement. This investigation demonstrates that β -agonist-induced relaxation of rat mesenteric resistance arteries can be mediated via endothelial release of NO and that, in this circulation, this is due to β_1 -adrenoceptor stimulation. This study therefore suggests that β -adrenoceptors can produce relaxation via pathways other than those mediated via cyclic AMP.

References

- AMEZCUA, J.L., PALMER, R.M.J., DE SOUZA, B.M. & MONCADA, S. (1989). Nitric oxide synthesized from L-arginine regulates vascular tone in the coronary circulation of the rabbit. *Br. J. Pharmacol.*, **96**, 418–424.
- DE MEY, J.G. & VANHOUTTE, P.M. (1982). Heterogeneous behaviour of the canine arterial and venous wall: importance of the endothelium. *Circ. Res.*, **51**, 439–447.
- DOHI, Y., THIEL, M.A., BUHLER, F.R. & LUSCHER, T.F. (1990). Activation of endothelial L-arginine pathway in resistance arteries. Effect of age and hypertension. *Hypertension*, **15**, 170–179.
- FUJIMOTO, S., DOHI, Y., AOKI, K., ASANO, M. & MATSUDA, T. (1987). Diminished β -adrenoceptor-mediated relaxation of arteries from spontaneously hypertensive rats before and after development of hypertension. *Eur. J. Pharmacol.*, **136**, 317–324.
- GARDINER, S.M., KEMP, P.A. & BENNETT, T. (1991). Effects of N^G -nitro-L-arginine methyl ester on vasodilator responses to acetylcholine, 5'-N-ethylcarboxyaminoadenosine or salbutamol in conscious rats. *Br. J. Pharmacol.*, **103**, 1725–1732.
- GRAVES, J. & POSTON, L. (1992). Effect of inhibiting nitric oxide synthase on relaxations to isoprenaline in isolated rat mesenteric resistance arteries. *Br. J. Pharmacol.*, **106**, 23P.
- GRAY, D.W. & MARSHALL, I. (1991). Isoprenaline relaxation of rat thoracic aorta is endothelium-dependent, releases nitric oxide and raises cyclic GMP and cyclic AMP. *Br. J. Pharmacol.*, **102**, 125P.
- HEESON, B.-J. & DE MEY, J.G.R. (1990). Effect of cyclic AMP-affecting agents on contractile reactivity of isolated mesenteric and renal resistance arteries of the rat. *Br. J. Pharmacol.*, **101**, 859–864.
- KAMATA, K., MIYATA, N. & KASUYA, Y. (1989). Involvement of endothelial cells in relaxation and contraction responses to isoproterenol in naive and streptozotocin-induced diabetic rats. *J. Pharmacol. Exp. Ther.*, **249**, 890–894.
- KONISHI, M. & SU, C. (1983). Role of the endothelium in dilator responses of spontaneously hypertensive rat arteries. *Hypertension*, **5**, 881–886.
- KUHN, M., OTTEN, A., FROLICH, J.C. & FORSTERMANN, U. (1991). Endothelial cyclic GMP and cyclic AMP do not regulate the release of endothelium-derived relaxing factor/nitric oxide from bovine aortic endothelial cells. *J. Pharmacol. Exp. Ther.*, **256**, 677–682.
- KUKOVETZ, W.R., POCH, G. & HOLZMANN, S. (1981). Cyclic nucleotides and relaxation of vascular smooth muscle. In *Vasodilatation*. eds. Vanhoutte, P.M. & Leuzen, I. pp. 339–353. New York: Raven Press.
- LANSMAN, J.B., HALLAM, T.J. & RINK, T.J. (1987). Single stretch activated ion channels in vascular endothelial cells as mechanotransducers? *Nature*, **325**, 811–813.
- LUCKHOFF, A. & BUSSE, R. (1990). Calcium influx into endothelial cells and formation of endothelium-derived relaxing factor is controlled by the membrane potential. *Pflügers Arch.*, **416**, 305–311.
- MARTIN, W., FURCHGOTT, R.F., VILLANI, G.M. & JOTHIANDAN, D. (1986). Depression of contractile responses in rat aorta by spontaneously released endothelium-derived relaxing factor. *J. Pharmacol. Exp. Ther.*, **237**, 529–538.
- MONCADA, S., REES, D.D., SCHULTZ, R. & PALMER, R.M.J. (1991). Development and mechanisms of a specific supersensitivity to nitrovasodilators after inhibition of vascular nitric oxide synthesis in vivo. *Proc. Natl. Acad. Sci. USA*, **88**, 2166–2170.
- MOORE, P.K., AL-SWAYEH, O.A., CHONG, N.W.S., EVANS, R.A. & GIBSON, A. (1990). L- N^G -nitroarginine (L-NOARG), a novel, L-arginine reversible inhibitor of endothelium-dependent vasodilatation in vitro. *Br. J. Pharmacol.*, **99**, 408–412.
- MULVANY, M.J. & HALPERN, W. (1977). Contractile properties of small arterial resistance vessels in spontaneously hypertensive and normotensive rats. *Circ. Res.*, **41**, 19–26.
- PALMER, R.M.J., REES, D.D., ASHTON, D.S. & MONCADA, S. (1988). L-arginine is the physiological precursor for the formation of nitric oxide in endothelium dependent relaxation. *Biochem. Biophys. Res. Commun.*, **153**, 1251–1256.
- REES, D.D., PALMER, R.M.J., HODSON, H.F. & MONCADA, S. (1989). A specific inhibitor of nitric oxide formation from L-arginine attenuates endothelium-dependent relaxation. *Br. J. Pharmacol.*, **96**, 418–424.
- RUBANYI, G. & VANHOUTTE, P.M. (1985). Endothelium-removal decreases relaxations of canine coronary arteries caused by β -adrenergic agonists and adenosine. *J. Cardiovasc. Pharmacol.*, **7**, 139–144.

- SADOSHIMA, J.-I., AKAIKE, N., KANAIDE, H. & NAKAMURA, M. (1988). Cyclic AMP modulates Ca-activated K channel in cultured smooth muscle cells of rat aortas. *Am. J. Physiol.*, **255**, H754–H759.
- TSUJIMOTO, G. & HOFFMAN, B.B. (1985). Desensitization of β -adrenergic receptor-mediated vascular smooth muscle relaxation. *Mol. Pharmacol.*, **27**, 210–217.
- VALLANCE, P., COLLIER, J. & MONCADA, S. (1989). Effects of endothelium-derived nitric oxide on peripheral arterial tone in man. *Lancet*, **ii**, 997–999.
- VANHOUTTE, P.M., VERBEUREN, T.J. & WEBB, R.C. (1981). Local modulation of adrenergic neuroeffector interaction in the blood vessel wall. *Physiol. Rev.*, **61**, 151–247.
- WOOLFSON, R.G. & POSTON, L. (1990). Effect of N^G-monomethyl-L-arginine on endothelium-dependent relaxation of human resistance arteries. *Clin. Sci.*, **79**, 273–278.

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