



Endothelin alters the reactivity of vasa vasorum: mechanisms and implications for conduit vessel physiology and pathophysiology

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1 The walls of certain large blood vessels are nourished by the vasa vasorum, a network of microvessels that penetrate the adventitia and media of the vessel wall. The purpose of this study was to characterize endothelin-1 (ET-1)-mediated contraction of vasa and to investigate whether threshold concentrations of ET-1 alters the sensitivity to constrictors. Arterial vasa were dissected from the walls of porcine thoracic aorta and mounted in a tension myograph.

2 ET-1 and ET_B-selective agonist, sarafotoxin 6c (S6c), produced concentration-dependent contraction. ET_A receptor antagonist, BQ123 (10 μ M), caused a biphasic rightward shift of ET-1 response curves. ET_B receptor antagonist, BQ788 (1 μ M), produced a rightward shift of response curves to ET-1 and S6c of 5- and 80 fold respectively.

3 ET-1 responses were abolished in Ca²⁺-free PSS but unaffected by selective depletion of intracellular Ca²⁺ stores. Nifedipine (10 μ M), an L-type Ca²⁺ channel blocker, attenuated ET-1 responses by 44%. Inhibition of receptor-operated Ca²⁺ channels or non-selective cation entry using SKF 96365 (30 μ M) and Ni²⁺ (1 mM) respectively, attenuated ET-1 contractions by 60%.

4 ET-1 (1–3 nM) enhanced responses to noradrenaline (NA) (4 fold) but not to thromboxane A₂-mimetic, whilst K⁺ (10–20 mM) sensitized vasa to both types of constrictor.

5 Therefore, ET-1-induced contraction of isolated vasa is mediated by ET_A and ET_B receptors and involves Ca²⁺ influx through L-type and non-L-type Ca²⁺ channels. Furthermore elevation of basal tone of vasa vasorum alters the profile of contractile reactivity. These results suggest that ET-1 may be an important regulator of vasa vasorum reactivity.

Keywords: Endothelin; sarafotoxin; vasa vasorum; BQ123; BQ788

Abbreviations: BQ123, Cyclo-(D-Trp-D-Asp-Pro-D-Val-Leu); BQ788, N-cis-2,6-dimethylpiperidinocarbonyl- β -tBu-Ala-D-Trp(1-methoxycarbonyl)-D-Nle-OH; CPA, cyclopiazonic acid; EGTA, ethyleneglycol-bis-(β -aminoethyl-ether)-N,N,N',N'-tetraacetic acid; ET-1, endothelin-1; KPSS, 125 mM K⁺ equimolar substitution for Na⁺ in physiological salt solution; NA, noradrenaline; PSS, physiological salt solution; SKF 96365, 1-[β -(3-(4-methoxyphenyl)propoxy)-4-methoxyphenethyl]-1H-imidazole hydrochloride; S6c, sarafotoxin 6c; TXA₂, thromboxane A₂; U44069, 9,11-Dideoxy-9 α , 11 α -epoxy-methano prostaglandin F_{2 α}

Introduction

Large blood vessels are nourished by diffusion of oxygen and nutrients from the lumen and also by blood flow through the vasa vasorum. The vasa vasorum forms a network of microvessels that lie predominantly in the adventitia although in certain large arteries, veins and atherosclerotic arteries these microvessels also penetrate the media (for review see Williams & Heistad, 1996). Removal of periaortic fat containing the vasa vasorum (Stefanadis *et al.*, 1993) or occlusion of the vasa vasorum surrounding the abdominal aorta of dogs (Nakata & Shionoya, 1966) results in extensive medial necrosis and alters aortic reactivity *in vivo*. The application of a silastic collar to the adventitial surface of rabbit carotid arteries disrupts the vasa vasorum and induces neointima formation in the large vessel (Martin *et al.*, 1991). These studies support the hypothesis that the vasa vasorum is important in the maintenance of homeostasis of the parent vessel and that disruption of the control of blood flow through the vasa vasorum might contribute to disease states.

Several layers of contractile smooth muscle cells are oriented radially around the vasa indicating that blood flow through these vessels may be actively regulated, but unlike

the host vessel from which they are derived, these small vessels appear insensitive to potent arterial constrictors including noradrenaline (NA), thromboxane A₂-mimetic (TXA₂) U44069 and angiotensin II. Indeed, in our previous study, the only constrictor that produced marked contractile responses of porcine aortic vasa was endothelin-1 (ET-1) (Scotland *et al.*, 1999), similarly, ET-1 was also the only potent constrictor of isolated human aortic vasa vasorum (Sarsero *et al.*, 1998). These findings together with the observation that there is significant ET-1 binding in the vasa vasorum (Dashwood *et al.*, 1993) suggests that this mediator may be particularly important in the control of flow through the vasa vasorum.

However, in certain vessels contractile responses to agonists can be potentiated in the presence of a threshold concentration of a second agonist (see for example Stupecky *et al.*, 1986). In particular, synergy between ET-1 and several other constrictors has been reported in different vascular beds; subpressor doses of ET-1 enhance pressor responses to NA in perfused rat (Tabuchi *et al.*, 1989; 1991) and canine (Zhang *et al.*, 1996) small mesenteric arteries, and in human isolated arteries ET-1 potentiates contraction to NA and serotonin (Yang *et al.*, 1990; Okatani *et al.*, 1995). Thus overall reactivity of small blood vessels may be dependent upon the prevailing background concentrations of other

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constrictor agents, particularly ET-1. In the present study we have investigated the mechanisms involved in ET-1 induced contraction of isolated vasa and tested the hypothesis that threshold concentrations of ET-1 may alter the profile of reactivity of the vasa vasorum.

Methods

Porcine aorta were collected from an abattoir and placed immediately in cold (4°C) physiological salt solution (PSS) of the following composition (mM): NaCl 119, KCl 4.7, CaCl₂·2H₂O 2.5, MgSO₄·7H₂O 1.2, NaHCO₃ 25, KH₂PO₄ 1.2 and glucose 5.5. Small arteries of the vasa vasorum at the adventitial-medial border were dissected out of the large vessel wall and cleaned of surrounding tissue using micro-dissection as previously described (Scotland *et al.*, 1999).

Arteries were mounted between two stainless steel wires (40 µm in diameter) in an automated tension myograph (Danish Myo Technology, Aarhus, Denmark) for the measurement of isometric tension (Molvany & Halpern, 1977). Vessels were bathed in PSS gassed with 5% CO₂ in O₂ at 37°C. Arteries were stretched in a stepwise manner to determine the relationship between passive tension and internal circumference according to Laplace's equation. From this relationship the internal diameter was determined. Vessels were then stretched to 90% of the diameter achieved when the vessel was under an effective transmural pressure of 100 mmHg. Following the normalization procedure, vessels were contracted with high K⁺ (125 mM) PSS (KPSS) repeatedly until contractions were constant. Those vessels which did not produce active tension responses at least equivalent to that produced in response to an effective transmural pressure of 100 mmHg were rejected.

Characterization of contractile responses to ET-1

Receptor antagonism study Cumulative concentration-response curves were constructed to ET-1 (0.01–300 nM) or the ET_B-selective receptor agonist, sarafotoxin 6c (S6c, 0.01–30 nM) (Williams *et al.*, 1991). To determine whether the response to ET-1 was mediated by ET_A or ET_B receptors some vessels were pretreated with either the selective ET_A receptor antagonist BQ123 (10 µM) (Ihara *et al.*, 1992a,b), the ET_B receptor antagonist BQ788 (1 µM) (Ishikawa *et al.*, 1994) or a combination of both antagonists for 30 min. Concentration-response curves were also constructed to S6c in the presence of BQ788.

Calcium mobilization To investigate the mechanisms involved in ET-1-induced contractions vasa were pretreated with inhibitors of intracellular or extracellular Ca²⁺ flux, at concentrations previously shown to be maximally effective. Contractile response curves to ET-1 were constructed in the absence or presence of (i) Ca²⁺-free PSS containing 2 mM EGTA, (ii) L-type Ca²⁺-channel blocker, nifedipine (10 µM, 30 min), (iii) purported inhibitor of receptor-operated Ca²⁺ channel, SKF 96365 (30 µM, 30 min) (Merritt *et al.*, 1990), or (iv) an inhibitor of non-selective cation entry, nickel (1 mM, 60 min) (Shetty & DelGrande, 1994).

To investigate the role of Ca²⁺ release from intracellular stores, some vessels were pretreated with cyclopiazonic acid (CPA; 10 µM, 30 min), an inhibitor of sarcoplasmic reticulum CaATPase (Seidler *et al.*, 1989). Control experiments in the absence of the antagonist or inhibitors were carried out at the same time on paired rings from the same vessel.

Potential of contractile responses

To investigate whether raising tone with threshold concentrations of ET-1 alters the vasoconstrictor reactivity profile of the vasa the following experiments were carried out.

Concentration-response curves were constructed to NA (1–100,000 nM) or thromboxane A₂-mimetic (U44069, 0.1–1000 nM) in the absence and then in the presence of ET-1 (1–3 nM) or K⁺ (10–20 mM). In each case the concentration of ET-1 or K⁺ used was sufficient to precontract the vessel to approximately 10% of the initial KPSS response. The responses to NA and U44069 following precontraction were compared to responses to those agents in time-matched control vessels not precontracted with ET-1 or K⁺.

Data and statistical analysis

Contractile responses to agonists are expressed as a percentage of the response to KPSS (125 mM K⁺). pD₂ values were calculated using non-linear regression analysis for each vessel and expressed as arithmetic mean ± s.e.mean. Biphasic concentration-response curves were fitted to a modified four parameter logistic equation for two site binding using non-linear curve fitting analysis program (GraphPad Prism 2.0) to estimate two pD₂ values (McLean & Coupar, 1996). Statistical significance was determined using Student *t*-test. For multiple comparisons one way analysis of variance followed by Tukey's test was used. Differences were considered significant when *P* < 0.05. 'n' represents the number of animals.

Drugs

Noradrenaline bitartrate, U44069, nifedipine, nickel sulphate and EGTA were all purchased from Sigma Chemical Co., Poole, U.K. Endothelin-1, BQ123 and BQ788 were purchased from Bachem, U.K. S6c was purchased from Novabiochem, Nottingham, U.K. Cyclopiazonic acid and SKF 96365 were purchased from Calbiochem. Noradrenaline was made in saline. Stock solutions of endothelin-1 and SKF 96365 were made in sterile water. Stock solutions of sarafotoxin 6c were made in 0.1% acetic acid. Stock solutions of U44069, BQ123, nifedipine and cyclopiazonic acid were made in DMSO. Stock solutions of BQ788 were made in 50% methanol. All stock solutions were stored frozen except SKF 96365, which was stored at room temperature. Nifedipine and noradrenaline were made on day of use. All dilutions were made in saline. The maximum concentration of DMSO or methanol in the bath at any time was 0.005% and 0.0025% respectively.

Results

The mean diameter of the vessels studied was 142.0 ± 3.0 µm (130 vessels).

Characterization of contractile responses to ET-1

Receptor antagonism study ET-1 produced slowly developing and sustained concentration-dependent contractions of isolated arterial vasa (pD₂ = 7.8 ± 0.1 and max. response = 138.3 ± 2.3%, *n* = 32). Concentration-response curves to ET-1 were shifted to the right and became biphasic in the presence of BQ123 (10 µM, *n* = 5) (see Figure 1) consisting of a high potency (pD₂ = 8.1 ± 0.4, max. response = 54.3 ± 4.8%)

response and a low potency ($pD_2 > 5.8$, max. response $> 120\%$) response.

BQ788 ($1 \mu M$, $n=7$) produced a significant ($P < 0.01$) rightward shift (approximately 5 fold) of the ET-1 concentration-response curve (see Figure 1) giving pD_2 values of 7.8 ± 0.1 and 7.2 ± 0.2 in the absence and presence of the antagonist respectively. Pretreatment with a combination of both receptor antagonists ($n=4$) produced a significant ($P < 0.001$) rightward shift (approximately 20 fold) (see Figure 1) giving pD_2 values of 7.8 ± 0.2 and > 6.4 in the absence and presence of the antagonists respectively.

Similarly to ET-1, S6c produced potent concentration-dependent contractions ($pD_2 = 8.5 \pm 0.1$ ($n=7$)) and a maximum response of $87.4 \pm 1.6\%$ which were significantly ($P < 0.01$) shifted to the right (approximately 80 fold) by BQ788 ($pD_2 = 6.5 \pm 0.1$, $n=6$) (see Figure 2).

Calcium mobilization In Ca^{2+} -free PSS the contractile response to ET-1 was abolished ($n=4$). In the presence of nifedipine ($10 \mu M$, $n=5$) ET-1 caused contractions with a maximum response approximately 44% lower than in untreated tissues but without a shift in the curve ($pD_2 = 7.6 \pm 0.1$, see Figure 3). SKF 96365 ($30 \mu M$, $n=5$) or Ni^{2+} ($1 mM$, $n=4$) pretreatment attenuated ET-1-mediated contractions causing 61 and 55% suppression of the maximum response respectively (see Figure 3) and giving pD_2 values of

7.7 ± 0.1 and 7.2 ± 0.2 in the absence and presence of Ni^{2+} respectively. Combination of nifedipine with SKF 96365 ($n=5$) had a greater effect than either inhibitor alone attenuating the maximum response to ET-1 by 75% (see Figure 3). Maximum contractions to S6c were also attenuated in the presence of nifedipine by 55% ($n=7$) (see Figure 2). The contractile response to ET-1 that remained following BQ123 treatment was significantly ($P < 0.05$) suppressed by addition of nifedipine ($n=7$, see Figure 4) whilst that remaining following BQ788 was not. The pD_2 values in the presence of BQ788 were 7.8 ± 0.1 and 8.0 ± 0.1 ($n=5$) producing maximum contractions of 135.9 ± 16 and $110.2 \pm 20.3\%$ in the absence and presence of nifedipine respectively (not significantly different).

The contractions to ET-1 were not altered in the presence of CPA ($10 \mu M$, $n=5$), an inhibitor of sarcoplasmic reticulum CaATPase ($pD_2 = 7.6 \pm 0.2$ and 7.8 ± 0.1 in the absence and presence of CPA respectively).

Potential of contractile responses

ET-1 ($1-3 nM$) induced contraction of $7.5 \pm 0.9\%$ ($n=11$) and potentiated the responses to NA ($n=7$, Figure 5a) but not to U44069 ($n=4$, Figure 6a). Maximum responses to NA of $8.7 \pm 0.7\%$ and $32.1 \pm 0.8\%$ were achieved in the absence and presence of ET-1 respectively whilst the potency of NA was not

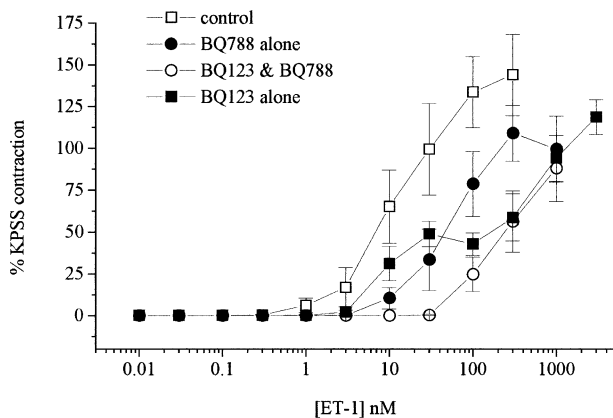


Figure 1 Concentration-response curves to ET-1 in the absence and presence of BQ123 ($10 \mu M$), BQ788 ($1 \mu M$) or BQ123 & BQ788. All values shown are means \pm s.e.mean.

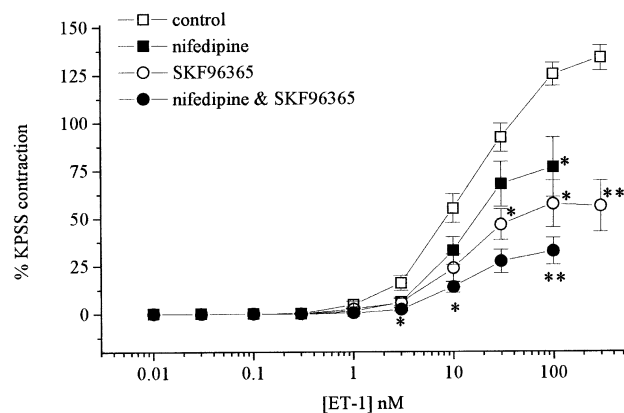


Figure 3 Concentration-response curves to ET-1 in the absence and presence of nifedipine ($10 \mu M$), SKF 96365 ($30 \mu M$) or SKF 96365 with nifedipine. All values shown are means \pm s.e.mean.

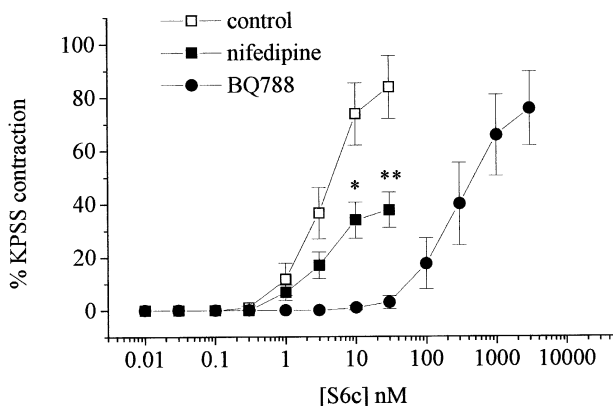


Figure 2 Concentration-response curves to sarafotoxin 6c in the absence and presence of nifedipine ($10 \mu M$) or BQ788 ($1 \mu M$). All values shown are means \pm s.e.mean. Significance is shown as * $P < 0.05$ or ** $P < 0.01$.

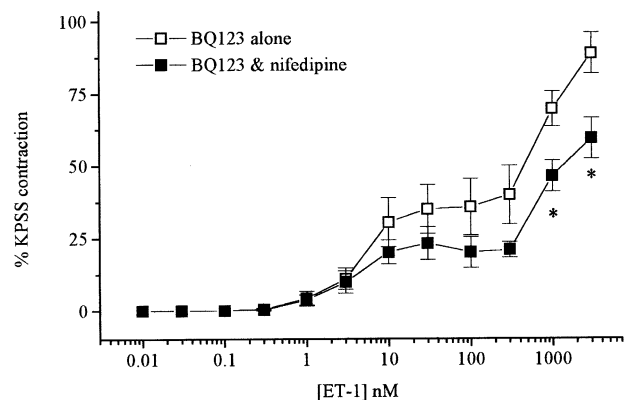


Figure 4 Concentration-response curves to ET-1 in the presence of BQ123 alone or BQ123 and nifedipine. All values shown are means \pm s.e.mean. Significance is shown as * $P < 0.05$.

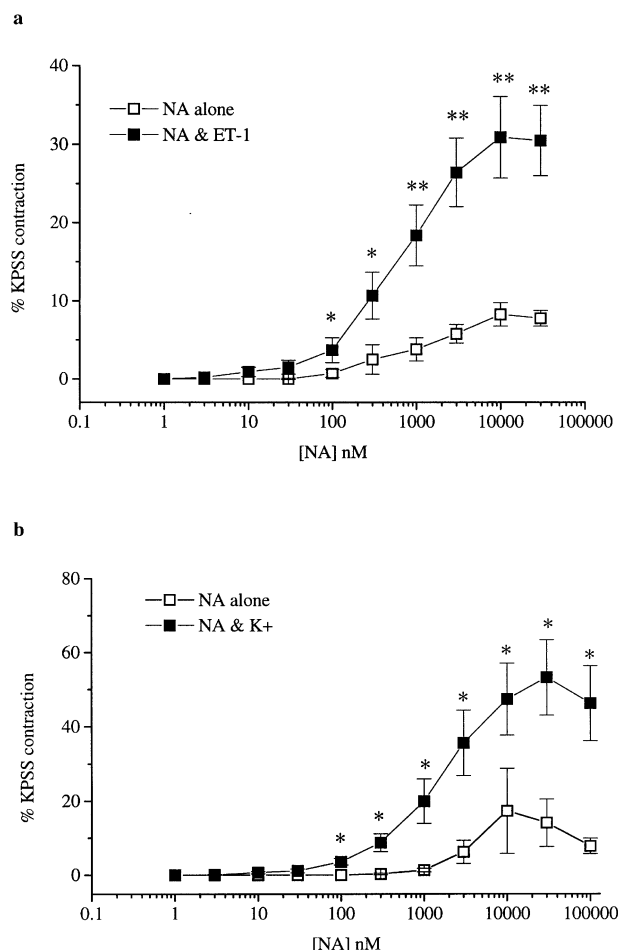


Figure 5 Responses to NA in the absence and presence of (a) ET-1 (1–3 nM) and (b) K^+ (10–20 mM). All values shown are mean \pm s.e.mean. Significant differences between responses to ET-1 in the presence of inhibitors compared to control are shown as * $P < 0.05$ or ** $P < 0.01$.

significantly altered (pD_2 values of 6.0 ± 0.2 and 6.2 ± 0.2 in the absence and presence of ET-1 respectively). The potentiated responses to NA were blocked following pretreatment with nifedipine ($n = 4$) but were unaffected by CPA. The pD_2 values in the presence of ET-1 were 6.1 ± 0.6 and 6.4 ± 0.2 ($n = 4$) in the absence and presence of CPA respectively.

In contrast raising basal tone by $8.2 \pm 0.6\%$ ($n = 11$) using K^+ (10–20 mM) significantly ($P < 0.01$) enhanced the responses to both NA ($n = 6$, Figure 5b) and U44069 ($n = 5$, Figure 6b). K^+ -induced precontraction produced a 4 fold increase in maximum response to NA without a significant change in the pD_2 values (5.6 ± 0.5 in the absence and 5.8 ± 0.2 in the presence of K^+). In the absence of K^+ U44069 had no contractile effect but in the presence of K^+ the TXA_2 -mimetic produced potent concentration-dependent contraction with a pD_2 of 7.2 ± 0.1 and a maximum effect of $83.6 \pm 3.8\%$. The potentiated response to U44069 was unaffected by pretreatment with CPA ($n = 7$). The pD_2 values in the presence of K^+ were 7.0 ± 0.2 ($n = 6$) in the absence and presence of CPA.

Discussion

ET-1 is a potent constrictor of arterial vasa isolated from porcine aorta. The results of this study indicate that the

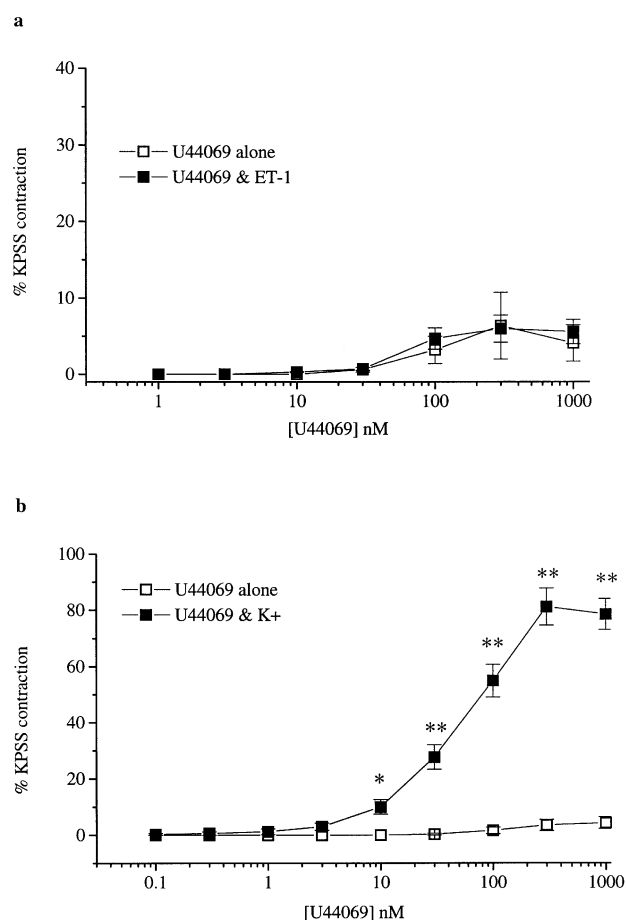


Figure 6 Responses to U44069 in the absence and presence of (a) ET-1 (1–3 nM) and (b) K^+ (10–20 mM). All values shown are mean \pm s.e.mean. Significance is shown as * $P < 0.05$ or ** $P < 0.01$.

contractions are mediated by two receptor subtypes and involve the influx of calcium through L-type and non-L-type Ca^{2+} channels. Furthermore low concentrations of ET-1 enhance the contractile responses to NA.

Characterization of contractile responses to ET-1

Receptor antagonism study Contraction of vascular smooth muscle to ET-1 is mediated by at least two distinct receptor subtypes: ET_A and ET_B . The distribution of receptor subtype and the contribution that they make to ET-1-induced contraction varies between vascular beds (for review see Bax & Saxena, 1994). In the present study ET-1 contracted isolated porcine vasa with a pD_2 similar to that reported in other porcine small arteries (Awane-Igata *et al.*, 1997) and human small arteries (Pierre & Davenport, 1998), and the contractile response to ET-1 was mediated by both ET_A and ET_B receptors.

Blockade of ET_B receptors using the selective ET_B antagonist BQ788 significantly shifted the ET-1 response curve to the right indicating that ET_B receptors mediate at least part of the ET-1 induced contraction of vasa. Confirmation of the presence of ET_B receptors was provided by the demonstration that S6c, the selective ET_B agonist, produced potent concentration-dependent contraction that was significantly shifted to the right by BQ788. Blockade of ET_A receptors, using the selective ET_A antagonist BQ123, also shifted the concentration-response curve to ET-1 to the right demonstrating that ET_A receptors are involved in ET-1

induced contractions. The response to ET-1 became biphasic in the presence of BQ123, such that responses to high concentrations of ET-1 were shifted to the right whilst the response to low concentrations were BQ123-resistant. Similar sensitivity to BQ123 has also been reported in other small resistance arteries (Mickley *et al.*, 1997). The persisting ET-1-induced contraction in the presence of ET_A antagonism, which was of a comparable magnitude to that to S6c, may be ET_B-mediated since BQ788 abolished the BQ123-resistant component of the ET-1 response. Combined blockade of ET_A and ET_B receptors resulted in a parallel monophasic shift of the concentration-response curve. Curiously, however this shift did not equate to an additive effect of BQ123 and BQ788, since the shift of the ET-1 curve was no greater than the effect of BQ123 alone on the upper portion of the response curve. The reasons for this anomaly are unclear but are consistent with a permissive role of ET_B in mediation of ET-1 contraction at the higher concentrations although further studies are required to test this hypothesis. Nevertheless, our results indicate that in the vasa vasorum of the porcine aorta ET-1-induced contraction is produced by activation of both ET_A and ET_B receptors.

Calcium mobilization The mechanisms of ET-1-stimulated Ca²⁺ influx have been studied extensively (for review see Decker & Brock, 1998) but the results are contradictory. The contractile response of the vasa vasorum to ET-1 is clearly dependent on Ca²⁺ entry since bathing the vessels in a Ca²⁺-free solution abolished the response, whereas depletion of intracellular Ca²⁺ stores with CPA had no effect. Our results indicate that voltage-gated and receptor-operated Ca²⁺ channels are important in mediating the Ca²⁺ entry in response to ET-1 in these vessels.

In untreated vessels ET-1 produced slowly developing, sustained contractions which were attenuated in the presence of nifedipine, an inhibitor of L-type Ca²⁺ channels. Other studies have suggested that ET-1 causes a biphasic increase in intracellular Ca²⁺ in vascular smooth muscle (Somlyo & Somlyo, 1994), suggesting the involvement of at least two different mechanisms of calcium mobilization. To test whether ET-1 stimulates an increase in Ca²⁺ through non-L-type channels we explored the effects of agents that block Ca²⁺ entry through receptor-operated channels. SKF 96365, a purported inhibitor of receptor-operated Ca²⁺ channels (Merritt *et al.*, 1990), inhibited contractions to ET-1 by about 50%. Ni²⁺, a non-selective inhibitor of cation entry (Shetty & DelGrande, 1994), produced a similar inhibition of ET-1-induced contractions. Combination of nifedipine with SKF 96365 produced almost complete blockade of ET-1 responses supporting a role for nifedipine-insensitive channels in mediating the responses to ET-1.

Since both BQ123 and BQ788 modified ET-1-induced contractions we investigated the possibility that the activation of two distinct Ca²⁺ mobilization pathways reflects the activation of two distinct receptors. Contractions to the selective ET_B agonist S6c were partially inhibited following pretreatment with nifedipine whilst ET-1-induced contractions seen in the presence of ET_B antagonism were resistant to nifedipine. These findings would be consistent with the possibility that in porcine vasa vasorum ET_B receptors are coupled to voltage-sensitive L-type channels whilst the ET_A-mediated component of ET-1 contractions is likely to be predominantly due to influx of Ca²⁺ through non-L-type channels. However, in the presence of BQ123 nifedipine suppressed both phases of the resulting contractions and further studies are required

to determine the mechanisms of Ca²⁺ mobilization following ET_A or ET_B activation in these vessels.

Potential of contractile responses

ET-1 not only produced potent contraction but also sensitized arterial vasa to the constrictor effects of NA such that in the presence of ET-1 the maximum contraction to NA increased 4 fold. Similar ET-1-induced enhancement of contractile responses to α -adrenergic agonists or adrenergic nerve stimulation has been reported in rat (Tabuchi *et al.*, 1989; 1991), and canine (Zhang *et al.*, 1996) isolated mesenteric arteries. Nifedipine abolished ET-1-induced potentiation of NA responses implicating influx of extracellular Ca²⁺ through L-type channels in mediation of this enhanced response to NA. Similar effects of inhibition of L-type channels have been seen in human internal mammary arteries (Yang *et al.*, 1990) and perfused rat mesenteric arteries (Tabuchi *et al.*, 1989). In contrast to its effects on NA-induced contractions, ET-1 did not potentiate responses to U44069. However, depolarization of smooth muscle with K⁺, which would also be expected to open voltage-gated Ca²⁺ channels, significantly potentiated the responses to both NA and TXA₂-mimetic. The reasons for the differences in potentiation of responses by ET-1 or K⁺ are not clear. Both stimuli increased basal tension to the same degree (10%) and both would be expected to open voltage-gated Ca²⁺ channels. Further studies will be needed to explore the mechanisms underlying these differential responses.

Clinical significance

The finding that low levels of ET-1 alters the responses to NA may be of considerable physiological and pathophysiological importance. The isolated vasa vasorum is normally resistant to NA-induced contraction and we have argued previously that this might be important to allow maintenance of vessel wall perfusion when the host conduit vessel is contracting to sympathetic stimulation (Scotland *et al.*, 1999). Our present findings suggest that an increase in ET-1 levels would expose reactivity to NA and that depolarization would enhance responses to both NA and TXA₂, which may be released from platelets.

Circulating plasma levels of ET-1 are elevated in several diseases including atherosclerosis, hypertension, congestive heart failure, asthma and diabetes (for review see Brooks *et al.*, 1998). Furthermore, radioimmunoassay studies have demonstrated a significant increase in endogenous ET in human aorta with atheromatous plaques (Bacon *et al.*, 1996). ET-1 synthesis (Bodi *et al.*, 1995) and release in both resistance (Rakugi *et al.*, 1990) and conduit (Pape *et al.*, 1997) vessels can also be stimulated by hypoxia or thrombin (Marsen *et al.*, 1995). Hypoxia may also lead to depolarization through inhibition of K⁺ channels (Grote *et al.*, 1988; Yuan *et al.*, 1993). The results of our study suggest therefore that various pathophysiological states might significantly alter the reactivity of the vasa vasorum which in turn may lead to underperfusion of the host vessel wall. In this respect endothelin receptor antagonists might have useful effects to preserve or restore patency of the vasa vasorum.

In conclusion, we have demonstrated that the porcine vasa vasorum contracts to ET-1. This contraction is mediated by ET_A and ET_B receptors and involves the influx of Ca²⁺ through L-type and non-L-type channels. Furthermore threshold concentrations of ET-1 alter the reactivity of vasa to NA. Together our findings suggest that ET-1 may have an

important role in the regulation and modulation of nutrient blood flow through the vasa vasorum.

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