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Functional role of endothelin ET_A and ET_B receptors in venous and arterial smooth muscle

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Abstract

The functional importance of endothelin ET_A and ET_B receptors in selected arterial and venous smooth muscle preparations was characterized. Endothelin-1 induced force in the saphenous and jugular veins is normally mediated by endothelin ET_B-like receptors. However, desensitization or pharmacological block of these receptors reveals an endothelin ET_A receptor population that is of sufficient size to mediate full endothelin-1-evoked force. Block of either endothelin ET_A or endothelin ET_B receptors alone is insufficient to antagonize endothelin-1-evoked force in saphenous vein. Endothelin-1-induced force in hamster aorta may also be mediated by activation of both endothelin ET_A and ET_B receptors. However, activation of endothelin ET_B-like receptors alone is insufficient to generate a full endothelin-1 response. Sarafotoxin S6c treatment, to desensitize endothelin ET_B receptors, failed to affect the responses of rat aorta and rabbit carotid artery to endothelin-1 or endothelin ET_A receptor antagonists. These findings indicate that selective endothelin receptor antagonists will vary enormously in their efficacy against endothelin-induced force in different vascular beds.

Keywords: Endothelin; Smooth muscle; Vascular; Endothelin receptor

1. Introduction

The recent discovery of an endothelin ET_B-like constrictor receptor in airways and vascular smooth muscle (e.g. Hay, 1992; LaDouceur et al., 1993; Lodge and Halaka, 1993; Moreland et al., 1992) raised the possibility that both endothelin ET_A and ET_B receptors may mediate vasoconstriction. We have previously shown that sarafotoxin S6c is a potent agonist in rabbit jugular vein. Moreover, endothelin-1 evoked responses in this preparation were refractory to inhibition by the selective endothelin ET_A receptor antagonists, BQ-123 and FR139317 (Lodge and Halaka, 1993). Nevertheless, the concentration-response curve for endothelin-3 was shallow and somewhat biphasic suggesting the presence of more than one endothelin receptor subtype in the jugular vein. A similar situation was found in the rabbit saphenous vein where sarafotoxin S6c elicited contractions with a potency and efficacy similar to that of endothelin-1 (Moreland et al., 1994). Furthermore, the endothelin ET_A receptor-selective antagonist BQ-123 had no effect on the endothelin-1 induced contractions even at concentrations as high as 300 nM (Moreland et al., 1994). Thus, it appears that endothelin-1 induced force in the jugular and saphenous veins is normally mediated primarily by activation of endothelin ET_B-like receptors.

The present study provides evidence for the presence of endothelin ET_A receptors, in addition to the endothelin ET_B-like vasoconstrictor receptors, on both the jugular and saphenous veins. In addition, the study reveals that endothelin ET_A and ET_B-like vasoconstrictor receptor subtypes coexist in many, but not all, blood vessels. The vasoconstrictor effect of endothelin-1 is apparently mediated by both receptors. The receptors were pharmacologically characterized using endothelin-1 (an endothelin ET_A/ET_B non-selective agonist), sarafotoxin S6c (an endothelin ET_B selective agonist), BMS-182874, FR139317, and BQ-123 (endothelin ET_A selective antagonists), Ro 46-2005 and SB

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209670 (endothelin ET_A/ET_B non-selective antagonists) and BQ-788 (an endothelin ET_B selective antagonist). In addition, the effects of endothelin-1 were also assessed following the desensitization of endothelin ET_B receptors.

2. Materials and methods

Tissue preparation: 12 week old male hamsters and male Sprague-Dawley rats (250–300 g) were killed by CO_2 inhalation and the thoracic aorta was quickly removed. Male New Zealand white rabbits (2.5–3.5 kg) were killed by injection of Brevital sodium (75 mg) in the ear vein. The external jugular veins, the saphenous veins and carotid arteries were then removed. All vessels were rinsed of blood, placed in ice-cold physiological salt solution (PSS), and carefully cleaned of connective tissue. Rings of 4 mm in length were cut and the endothelium was gently rubbed off. The rings were mounted for isometric force recording on wires in 10 ml tissue baths filled with PSS at 37°C. The PSS was bubbled with 5% CO_2 in O_2 to maintain a pH of 7.4 and was of the following composition, in mM: 118.4 NaCl, 4.7 KCl, 1.2 $MgSO_4$, 1.2 KH_2PO_4, 2.5 (rabbit jugular and saphenous veins, rat aorta) or 1.8 (hamster aorta, rabbit carotid arteries) $CaCl_2$, 25 $NaHCO_3$, 10.6 D-glucose, and 0.023 Na_2EDTA.

Experimental protocol: rings were gradually stretched to 0.5 (hamster aorta, rabbit saphenous vein), 1 (rabbit jugular vein), 2 (rat aorta) or 4 (rabbit carotid artery) g preload over an equilibration period of 60–90 min. The rings were then contracted with 1 μM phenylephrine (hamster and rat aorta, rabbit carotid artery) or 1 μM bradykinin (rabbit jugular and saphenous veins) to test for viability. The removal of endothelium was confirmed by the lack of relaxation upon addition of 1 μM acetylcholine or carbachol.

For measurement of the antagonistic activity of a test compound, the rings were first incubated with various concentrations of the compound for 20 (hamster aorta, rabbit carotid artery) or 30 (rabbit jugular and saphenous veins, rat aorta) min. Concentration-response curves were constructed by adding endothelin-1, endothelin-3, or sarafotoxin S6c cumulatively to the baths in half-log increments. At each concentration, the force was allowed to increase to a steady state level before the addition of next higher concentration.

To eliminate the effect of the constrictor endothelin ET_B-like receptors, the tissues were incubated with 100 nM sarafotoxin S6c for 60–180 min. The addition of the sarafotoxin S6c produced a transient contraction that declined to a steady state level over the incubation time. The activity of specific endothelin receptor antagonists was then assessed as described above.

Endothelin ET_B-receptor mediated relaxation was measured in endothelium-intact rings of rat aorta. Rings were gradually stretched to 5 g preload over an equilibrium period of 90 min, then contracted with 30 nM norepinephrine to test for viability. The integrity of the endothelium was tested by observation of a substantial (> 80%) relaxation upon addition of 1 μM acetylcholine. For measurement of the antagonistic activity of BQ-788, the rings were first incubated with various concentrations of the compound for 10 min. The rings were then contracted with 40 nM norepinephrine($\sim EC_{80}$) and the contractions allowed to reach a steady state level (approximately 15 min). Sarafotoxin S6c concentration-relaxation curves were constructed by adding sarafotoxin S6c cumulatively in half-log increments.

Stock solutions of BMS-182874 [5-(dimethylamino)-*N*-(3,4-dimethyl-5-isoxazolyl)-1-naphthalenesulfonamide], FR139317 [(*R*)-α-[N-[N-[(hexahydro-1*H*-azepin-1-yl)carbonyl]-L-leucyl]-1-methyl-D-tryptophyl]-2-pyridinepropanoic acid], Ro 46-2005 [4-*tert*-butyl-*N*-[6-(2-hydroxyethoxy)-5-(3-methoxy-phenoxy)-4-pyrimidinyl]-benzenesulphonamide], SB 209670 [(+)-(1*S*, 2*R*,3*S*)-3-(2-carboxymethoxy-4-methoxyphenyl)-1-(3,4-methylenedioxyphenyl)-5-(prop-1-yloxy)indane-2-carboxylic acid], BQ-123 [(*cyclo*)-D-Trp-D-Asp-Pro-D-Val-Leu] and BQ-788 [*N-cis*-2,6-dimethylpiperidinocarbonyl-L-γ-methylleucyl-D-1-methycarbonyltryptophanyl-D-norleucine] were prepared in dimethylsulfoxide.

Data analysis: the data are plotted as the means ± S.E.M. of at least four rings from different animals. Force is expressed as a percentage of the response to the reference agonist. Apparent K_B values were calculated from the following equation: $K_{Bapp} = [\text{test compound}]/((EC'_{50}/EC_{50}) - 1)$, assuming the test compound was a competitive antagonist. Here the EC'_{50} and EC_{50} were calculated from concentration-response curves in the presence of the test compound or vehicle, respectively. K_B is expressed as the mean ± S.E.M. of all apparent K_B values obtained from the above equation using at least three concentrations of antagonist. pA_2 values were determined from Schild plots.

3. Results

3.1. Rabbit saphenous vein

Endothelin-1-evoked force in the rabbit saphenous vein was insensitive to the endothelin ET_A-selective receptor antagonists BMS-182874 (10 μM) (Stein et al., 1994) and FR139317 (3 μM); endothelin-1 EC_{50} values for control and BMS-182874 were 2.4 ± 0.4 and 1.4 ± 0.5 nM ($n = 4$) and for control and FR139317

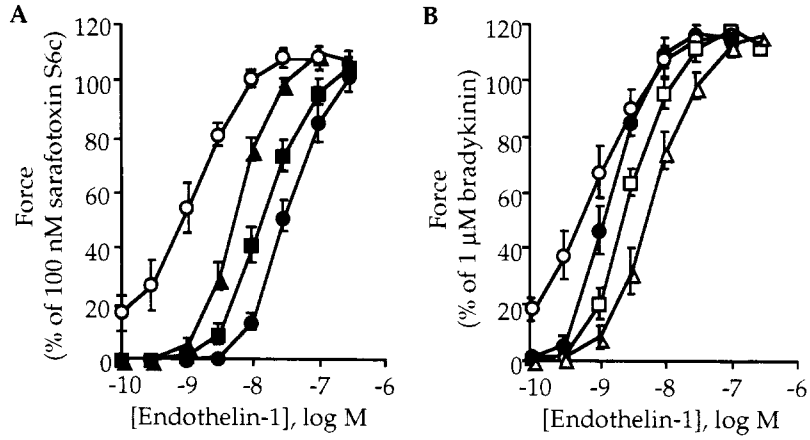

Fig. 1. Cumulative concentration-response curves for endothelin-1 in rabbit saphenous vein following prolonged exposure to sarafotoxin S6c (A) and in time-matched controls (B). Contractions were elicited in the presence of vehicle (open circles) and 0.03 (filled triangles), 0.1 (filled squares), 0.3 (filled circles), 1 (open squares), or 3 (open triangles) μM SB 209670. $n = 6$ rings from different animals.

were 5.2 ± 1.4 and 4.9 ± 3.5 nM ($n = 4$), respectively. However, prolonged exposure of the vein to 100 nM sarafotoxin S6c, a procedure believed to desensitize endothelin ET_B receptors (LaDoucer et al., 1993), rendered the subsequent endothelin-1-evoked force susceptible to inhibition by endothelin ET_A-selective antagonists. Sarafotoxin S6c (100 nM) caused a contraction that faded slowly, reaching near basal levels after 3 h. The subsequent cumulative addition of endothelin-1, in the continued presence of sarafotoxin S6c, produced contractile responses that were of similar magnitude to those of time-matched controls (i.e. non-sarafotoxin S6c-treated); maximum force was 8.7 $\pm$ 0.9 g ($n = 4$) in control and 9.6 ± 0.3 g ($n = 4$) in sarafotoxin S6c-treated tissues. Endothelin-1 potency was actually somewhat greater in the sarafotoxin S6c-treated tissues ($EC_{50} = 0.7 \pm 0.1$ nM, $n = 4$) than in time-matched controls ($EC_{50} = 2.4 \pm 0.5$ nM, $n = 4$).

In sarafotoxin S6c-treated tissues, BMS-182874 exhibited a K_{Bapp} of 5.7 ± 1.4 μM ($n = 4$) and FR139317 a K_{Bapp} of 1.0 ± 0.29 μM ($n = 4$). Similarly, the potency of the relatively non-selective endothelin receptor antagonist SB 209670 (Ohlstein et al., 1994), was also improved following sarafotoxin S6c pretreatment; its pA_2 value was increased from 6.67 (slope = 0.82; $K_B = 370 \pm 70$ nM) in control tissues to 8.29 (slope = 0.82; $K_B = 12 \pm 3.4$ nM) in sarafotoxin S6c-desensitized tissues, an approximately 40-fold increase in potency ($n = 6$; Fig. 1). For comparison, the potency of these antagonists against endothelin-1-induced force in the rabbit carotid artery, a vessel in which endothelin-1-evoked force appears to be mediated primarily by endothelin ET_A receptors, was as follows (K_B values): FR139317, 51 ± 10 nM (Moreland et al., 1994); BMS-182874, 520 ± 99 nM (Stein et al., 1994) and SB 209670, 1.5 ± 0.2 nM ($n = 4$).

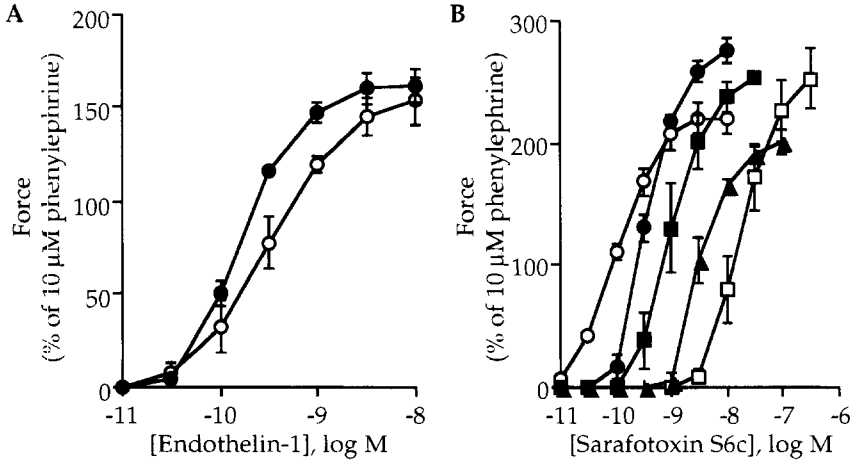

Fig. 2. (A). Concentration-response curves for endothelin-1 in saphenous vein in the presence of vehicle (open circles) or 10 μM BQ-788 (filled circles). (B) Concentration-response curves for sarafotoxin S6c in saphenous vein in the presence of vehicle (open circles) and 0.3 (filled circles), 1 (filled squares), 3 (filled triangles), or 10 (open squares) μM BQ-788. $n = 4$ rings from different animals.

Endothelin-1-induced responses in non-sarafotoxin S6c-treated tissues were also insensitive to inhibition by the selective endothelin ET_B antagonist BQ-788 (Fig. 2A). However, exposure of tissues to BQ-788 improved the potency of both BMS-182874 (K_{Bapp} = 4.2 ± 1.0 μM, n = 4) and SB 209670 (K_{Bapp} = 11 ± 1.9 nM, n = 4) against endothelin-1-evoked force. This increased potency was of similar magnitude to that observed following prolonged exposure to sarafotoxin S6c. Force evoked directly by sarafotoxin S6c was readily antagonized by BQ-788, yielding a pA_2 of 6.82 (slope = 1.2; K_B = 0.11 ± 0.02 nM; Fig. 2B); a value similar to that reported for its potency against force generated by the endothelin ET_B selective agonist BQ-3020 in the rabbit pulmonary artery (Ishikawa et al., 1994). [The mechanism underlying the increase in sarafotoxin S6c-induced maximum force observed in the presence of BQ-788 is unknown (Fig. 2B)].

Sarafotoxin S6c-induced endothelin ET_B receptor desensitization was reversible upon sarafotoxin S6c washout. The recovery time course was assessed as follows. Rings of saphenous vein were exposed to 10 nM sarafotoxin S6c for 1 h during which time force increased to a maximum and then began to fade. After 1 h of continuous exposure to 10 nM sarafotoxin S6c, addition of 100 nM sarafotoxin S6c produced no further increase in force. Recovery from desensitization was assessed, following various periods of sarafotoxin S6c washout, by measuring the force produced by a single administration of 10 nM sarafotoxin S6c (i.e. one measurement per tissue). Recovery began almost immediately upon washout of sarafotoxin S6c, proceeding with a half-time of approximately 55 mins.

3.2. Rabbit jugular vein

BMS-182874, at concentrations of ≤ 30 μM, had little effect on endothelin-1 induced force in the rabbit jugular vein, confirming that selective endothelin ET_A receptor antagonists do not inhibit endothelin-1-evoked force in this preparation under normal conditions (EC_{50} values for control and 30 μM BMS-182874 were 0.20 ± 0.07 and 0.17 ± 0.07 nM, n = 4).

As with the saphenous vein, exposure of the jugular vein to a single high concentration of sarafotoxin S6c (100 nM) generated a rapid development of force which then faded to near basal levels in 3 h. Subsequent addition of endothelin-1 generated 93% of that force produced by the time-matched endothelin-1 controls that were not exposed to sarafotoxin S6c. The potency of endothelin-1 was unaffected by this procedure; control EC_{50} = 0.11 ± 0.1 nM, following sarafotoxin S6c = 0.12 ± 0.11 nM (n = 4). However, the sensitivity of endothelin-1-induced force to inhibition by endothelin ET_A receptor antagonists was increased remarkably following sarafotoxin S6c exposure. FR139317 gave a pA_2 for inhibition of 8.15 (slope = 1.0; K_B = 8.7 ± 1.4 nM, n = 4; Fig. 3A) and BMS-182874 a K_{Bapp} of 330 ± 90 nM (n = 4). Endothelin-1-evoked force in time-matched controls, that were not treated with sarafotoxin S6c, was unaffected by FR139317 (Fig. 3B).

3.3. Rat aorta and rabbit carotid artery

The effects of sarafotoxin S6c exposure on the potency of endothelin ET_A-selective antagonists was also assessed in the rat aorta and rabbit carotid artery. Endothelin-1-evoked force in both of these preparations is thought to be mediated primarily by endothelin ET_A receptors (Moreland et al., 1992, 1994). Exposure of the aorta to 100 nM sarafotoxin S6c for 1 h failed to produce any increase in force development. The potency of FR139317 (pA_2 = 6.81, slope 1.4; n = 4) against the subsequent addition of endothelin-1, in the continued presence of sarafotoxin S6c, was the same as that observed in non-sarafotoxin S6c-treated tissues (pA_2 = 6.89, slope = 1.0; K_B = 170 ± 26 nM; n = 4). Similar results were obtained with the rabbit carotid

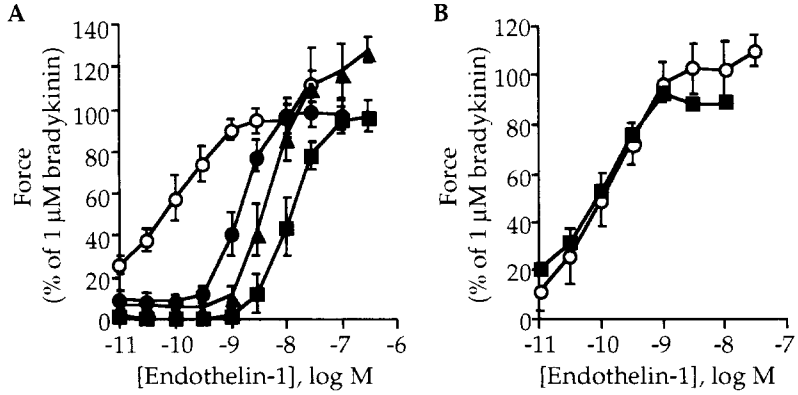

Fig. 3. Cumulative concentration-response curves for endothelin-1 in rabbit jugular vein following prolonged exposure to sarafotoxin S6c (A) and in time-matched controls (B). Contractions were elicited in the presence of vehicle (open circles) and 0.3 (filled circles), 1 (filled triangles), or 3 (filled squares) μM FR139317. n = 4 rings from different animals.

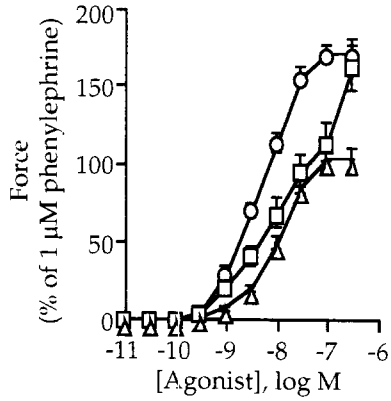

Fig. 4. Concentration-response curves for endothelin-1 (open circles), endothelin-3 (open squares), and sarafotoxin S6c (open triangles) in hamster aorta. $n = 4$ rings from different animals.

artery where prolonged exposure to sarafotoxin S6c failed to contract the vessel or alter the potency of BMS-182874 against endothelin-1-evoked force. Consistent with these data, the endothelin ET_B-selective antagonist BQ-788 was relatively ineffective against endothelin-1-evoked force in the carotid artery ($K_{Bapp} = 5.9 \pm 2.1$ μM, $n = 4$). As a comparison, BQ-788 antagonized the sarafotoxin S6c-induced relaxation of the norepinephrine-contracted endothelium-intact rat aorta in a competitive manner yielding a pA_2 of 8.3 (slope = 0.9; $K_B = 10 \pm 2.3$ nM, $n = 4$).

3.4. Hamster aorta

Endothelin-1, endothelin-3, and sarafotoxin S6c were approximately equipotent in the hamster aorta yielding EC_{50} values of 4.8 ± 0.8 nM, 19 ± 4.5 nM, and 11 ± 1.5 nM, respectively (Fig. 4). However, sarafotoxin S6c was less efficacious than either endothelin-1 or endothelin-3 (maximum force produced by sarafotoxin S6c was ~ 60% of that produced by either endothelin-1 or endothelin-3).

In the presence of the endothelin ET_A selective antagonist BMS-182874 the endothelin-1 concentration-response curve became clearly biphasic (Fig. 5A); that is, BMS-182874 was relatively more effective against contractions produced by higher concentrations of endothelin-1 than those produced by lower concentrations. Force initially plateaued at endothelin-1 concentrations between 10 and 100 nM and then increased again at concentrations above 100 nM. The initial plateau was observed at 2.3 ± 0.4 g of force which was similar to the maximum force induced by sarafotoxin S6c (2.3 ± 0.1 g; Fig. 4). Similar biphasic endothelin-1 concentration-response curves were also obtained from tissues incubated with 30 μM BQ-123 ($n = 4$; not shown). These data suggest the presence of two endothelin vasoconstrictor receptor subtypes in hamster aorta: an endothelin ET_B-like subtype primarily responsible for contractions induced by lower concentrations of endothelin-1 and an endothelin ET_A subtype contributing to force elicited by higher concentrations of endothelin-1. Force induced by sarafotoxin S6c stimulation of the endothelin ET_B-like receptor population was competitively blocked by the non-selective endothelin receptor antagonist Ro 46-2005 yielding a K_{Bapp} of 22 ± 2.3 μM ($n = 4$).

Exposure of the hamster aorta to a single large concentration of sarafotoxin S6c (100 nM) produced a contraction that gradually returned to baseline over a 60 min incubation period. Following this procedure, endothelin-1 generated a monophasic concentration-response curve that was shifted to the right in parallel fashion in the presence of BMS-182874 ($K_{Bapp} = 430 \pm 37$ nM, $n = 4$; Fig. 5B). Maximum endothelin-1-induced force was the same in the sarafotoxin S6c-treated and control tissues.

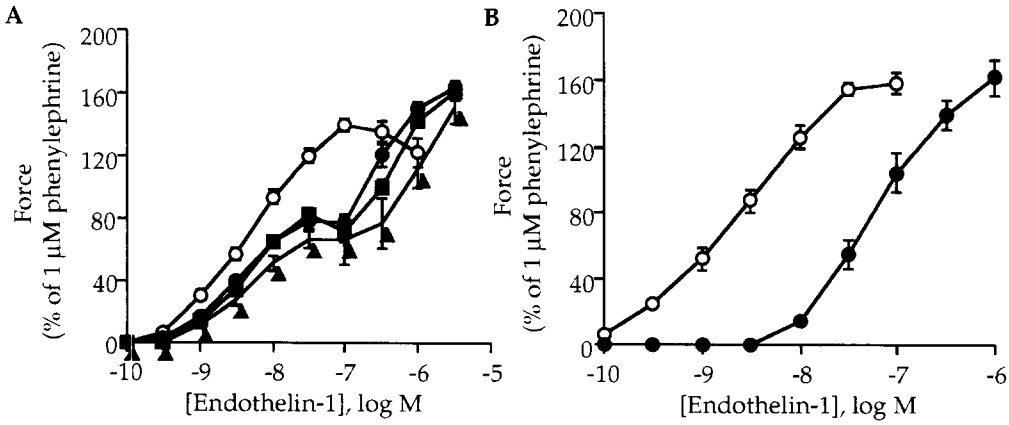

Fig. 5. (A). Concentration-response curves for endothelin-1 in hamster aorta in the presence of vehicle (open circles) and 30 (filled circles), 60 (filled squares), or 100 (filled triangles) μM BMS-182874. (B) Effect of sarafotoxin S6c pre-incubation of hamster aorta on concentration-response curves to endothelin-1 in the presence of vehicle (open circles) or 10 μM BMS-182874 (filled circles). $n = 4$ rings from different animals.

4. Discussion

Our results indicate that the saphenous vein possesses an endothelin ET_A-like receptor population in addition to its endothelin ET_B-like receptors. However, the sensitivity of the endothelin ET_A-like receptors to standard endothelin ET_A antagonists is approximately 10-fold lower than would have been expected. Nevertheless, the rank order of potency of the structurally distinct antagonists SB 209670 > FR139317 > BMS-182874 is the same in the sarafotoxin S6c-treated saphenous vein as it is in the untreated carotid artery, suggesting that the vein contains typical endothelin ET_A receptors. Thus, it appears that endothelin-1-induced force is normally evoked by activation of endothelin ET_B-like receptors in the saphenous vein. However, desensitization or pharmacological block of these receptors reveals an endothelin ET_A receptor population that is of sufficient size to mediate full endothelin-1-evoked force development. Evidence for both endothelin ET_A and endothelin ET_B receptors on this vein has also been provided by Karaki et al. (1994) based on functional studies and by Webb et al. (1993), based on radioligand binding studies.

Selective desensitization of endothelin ET_B-like receptors by prolonged exposure to sarafotoxin S6c rendered both the saphenous and jugular veins sensitive to antagonism by endothelin ET_A-selective antagonists. Moreover, endothelin-1-induced force in these desensitized vessels was approximately equivalent in amplitude to that in the non-desensitized preparations. Thus, these data indicate that the rabbit saphenous and jugular veins are endowed with a sufficiently large population of endothelin ET_A receptors to generate force of equivalent magnitude to that normally produced by activation of endothelin ET_B-like receptors. Our data also indicate that the potency of endothelin-1 at both endothelin ET_A- and ET_B-like receptors is similar. Thus, endothelin-1 should bind equally well to both endothelin ET_A and ET_B-like receptors under normal (i.e. non-desensitized) conditions. Consequently, endothelin-1-induced force in these venous preparations is refractory to selective endothelin ET_A receptor antagonists because maximal endothelin-1-induced force may be mediated by the endothelin ET_B-like receptor population. Similarly, endothelin ET_A receptors mediate endothelin-1-induced force in the face of selective endothelin ET_B receptor blockade. An alternative explanation for the failure of endothelin ET_A antagonists to prevent endothelin-1-induced force in these veins would be to assume that activation of endothelin ET_B-like receptors by endothelin-1 functionally uncouples endothelin ET_A receptors thereby rendering the vein insensitive to endothelin ET_A antagonists. Thus, following desensitization of the endothelin ET_B-like receptor population by prolonged exposure to sarafotoxin S6c, endothelin ET_A receptors would become functionally active and mediate force development in response to endothelin-1.

Endothelin-1-evoked force in the hamster aorta may also be mediated by activation of both endothelin ET_A and ET_B receptors. Treatment of this tissue with the endothelin ET_A antagonists BMS-182874 or BQ-123 produced biphasic endothelin-1 concentration-response curves with greater antagonism manifested at higher endothelin-1 concentrations. The antagonists were ineffective against force activated by lower endothelin-1 concentrations indicating that a non-endothelin ET_A subtype was primarily responsible for this portion of the endothelin-1 response. In addition, the sarafotoxin S6c-induced contractions in the aorta were moderately blocked by the endothelin ET_A/ET_B non-selective antagonist Ro 46-2005 with K_B values similar to those obtained in rabbit saphenous vein, a tissue containing endothelin ET_B-like vasoconstrictor receptors. However, in contrast to the saphenous and jugular vein preparations, the partial antagonism of the endothelin-1 concentration-response curve by endothelin ET_A antagonists indicates that activation of the endothelin ET_B-like receptor population alone is insufficient to generate a full endothelin-1 response. In contrast to the hamster aorta, we failed to show any evidence for a role of smooth muscle endothelin ET_B receptors in endothelin-1-mediated force in rat aorta or in the rabbit carotid artery.

In summary, the data presented in this study show that both endothelin ET_A- and endothelin ET_B-like receptors mediate force development in vascular smooth muscle. Both receptors can be present in the same tissue preparation and both can be activated in parallel to elicit contraction. In some cases, such as the hamster aorta, evidence for both receptors is apparent even under standard tissue bath conditions. In other tissues, such as the rabbit jugular and saphenous veins, the participation of endothelin ET_A receptors is not clearly observed until the endothelin ET_B-like receptors have been desensitized or blocked. There are also vessels, such as the rat aorta and rabbit carotid artery, in which the functional consequences of endothelin receptor activation are apparently mediated only by endothelin ET_A receptors. To date we have not found a vascular tissue which contracts only to endothelin ET_B-like, and not to endothelin ET_A receptor stimulation. The presence of two receptor subtypes in differing proportions on different vascular tissue types suggests that selective endothelin receptor antagonists will vary enormously in their efficacy against endothelin-induced force in these different preparations. Those tissues in which endothelin-1 force is mediated primarily by endothelin ET_A receptors, such as the rat aorta, are clearly susceptible to inhibition by endothelin ET_A-selective and -non-selective (endothelin ET_A/

ET_B) endothelin antagonists. In those tissues that have a mixture of both endothelin ET_A- and ET_B-like receptors, the efficacy of selective endothelin ET_A antagonists will vary with the relative proportion of each receptor subtype. In the case of vessels such as the saphenous and jugular veins, the presence of sufficiently high numbers of endothelin ET_B receptors renders these tissues refractory to selective endothelin ET_A antagonists. The high level of endothelin ET_A receptors on these venous tissues also leaves them insensitive to selective endothelin ET_B antagonists. Thus, endothelin-induced force in some vascular beds will be effectively antagonized only by non-selective endothelin antagonists.

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