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Activation of endothelin ET_A receptors masks the constrictor role of endothelin ET_B receptors in rat isolated small mesenteric arteries

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1 Endothelin-1 (ET-1) produces constriction of the rat mesenteric vascular bed *in vivo* via ET_A and ET_B receptor subtypes. The aim of this study was to investigate the relative roles of these receptor subtypes in rat isolated, endothelium-denuded, small mesenteric arteries, under pressure, by use of ET-1; the ET_A receptor antagonist, BQ-123; the ET_B receptor selective agonist, sarafotoxin S6c (SRTX S6c); the ET_B receptor selective antagonist, BQ-788; and the ET_A/ET_B antagonist, TAK-044.

2 In 3rd generation mesenteric arteries, ET-1 ($10^{-13}-10^{-7}$ M) produced concentration-dependent contractions (pD_2 9.86). SRTX S6c ($10^{-12}-10^{-7}$ M) also induced concentration-dependent contractions in 53% of arteries studied, although the E_{max} was much less than that obtained with ET-1 ($10.7\pm2.9\%$ vs $101.9\pm2.6\%$ of the 60 mM KCl-induced contraction).

3 Neither ET_B receptor desensitization, by a supra-maximal concentration of SRTX S6c (10^{-7} M), nor incubation with BQ-788 (3×10^{-8} M), had any significant effect on the ET-1 concentration-response curve, although both treatments tended to enhance rather than inhibit responses to ET-1.

4 In the presence of BQ-123 (10^{-6} M), responses to low concentrations of ET-1 (up to 10^{-10} M) were unaffected but responses to concentrations of ET-1 above 10^{-10} M were significantly inhibited.

5 SRTX S6c desensitization followed by incubation with BQ-123 (10^{-6} M) or co-incubation with BQ-788 (3×10^{-8} M) and BQ-123 caused inhibition of responses to all concentrations of ET-1, resulting in a rightward shift of the ET-1 concentration-response curve. The same effect was obtained by incubation with TAK-044 (10^{-8} M and 3×10^{-7} M).

6 Thus, responses of rat small mesenteric arteries to ET-1 are mediated by both ET_A and ET_B receptors. The relative role of ET_B receptors is greater than that predicted by the small responses to SRTX S6c or by resistance of ET-1-induced contraction to ET_B receptor desensitization or BQ-788. The effect of ET_B receptor desensitization or blockade is only revealed in the presence of ET_A receptor blockade, suggesting the presence of a 'crosstalk' mechanism between the receptors. These results support the concept that dual receptor antagonists, like TAK-044, may be required to inhibit completely constrictor responses to ET-1.

Keywords: Endothelin-1; sarafotoxin S6c; ET_A receptors; ET_B receptors; BQ-123; BQ-788; TAK-044

Introduction

It is now well established that the vasoactive effects of the peptide endothelin-1 (ET-1) are mediated via both ET_A (Arai *et al*., 1990) and ET_B receptors (Sakurai *et al*., 1990). Administration of ET-1 to anaesthetized or conscious rats leads to a brief decrease, followed by a long lasting increase, in blood pressure (Yanagisawa *et al*., 1988) that is accompanied by increased resistance in virtually all vascular beds studied (Gardiner *et al*., 1994; Allcock *et al*., 1995). Prior administration of an ET_A receptor antagonist, e.g. BQ-123 or FR 139317, enhances the initial depressor effect of ET-1 (an ET_B receptor-mediated effect) and reduces the pressor effect (McMurdo *et al*., 1993; Gardiner *et al*., 1994). However, the pressor and regional constrictor effect of ET-1 is not fully inhibited by ET_A receptor antagonists, even with high doses, implying that ET_B receptors may also have a vasoconstrictor role (McMurdo *et al*., 1993). Consistent with this possibility, the ET_B receptor selective agonist, sarafotoxin S6c (SRTX S6c) was found to produce vasoconstriction in pithed rats (Williams *et al*., 1991; Clozel *et al*., 1992).

In vitro experiments have also demonstrated ET_A receptor antagonist-resistant responses to ET-1 (Ihara *et al*., 1992; Sumner *et al*., 1992; Fukuroda *et al*., 1994b) and constrictions to SRTX S6c (Moreland *et al*., 1992; Sumner *et al*., 1992; La Douceur *et al*., 1993; Gray *et al*., 1994). As a consequence of these *in vitro* data, it has been suggested that constrictor ET_B receptors have a role only in large calibre vessels and in the venous circulation (Moreland *et al*., 1992; Davenport & Maguire, 1995). However, in the conscious rat (Gardiner *et al*., 1994) and the anaesthetized ganglion-blocked rat (Allcock *et al*., 1995), ET-1-induced reduction of blood flow to the mesenteric resistance bed is partly resistant to ET_A receptor inhibition. Reduction of regional blood flow in response to SRTX S6c is also most marked in the mesenteric bed of the pithed rat (Clozel *et al*., 1992). In man, ET-1 constrictions in upper limb blood vessels are also partly resistant to BQ-123 and constrictions to SRTX S6c can be seen (Haynes *et al*., 1995; Strachan *et al*., 1995). Thus, there may be an important role for constrictor ET_B receptors in mediating vascular resistance and blood pressure. Indeed, the recently described non-peptide ET_B receptor antagonist, Ro 46-8443, causes a reduction in blood pressure in anaesthetized, normotensive rats (Clozel & Breu, 1996).

In contrast to the evidence for ET_B receptor-mediated constriction of the rat mesenteric bed *in vivo*, *in vitro* studies of perfused mesenteric beds or human and rat isolated mesenteric arteries mounted in wire or perfusion myographs have led to the conclusion that constrictor ET_B receptors have little (Tschudi & Luscher, 1994; Takase *et al*., 1995; Deng *et al*., 1995; Touyz *et al*., 1995) or no role (D'Orleans-Juste *et al*., 1993) in this vascular bed. All of these studies have based their conclusions on inhibition of ET-1-induced contraction by ET_A

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receptor antagonists, or responses to ET_B selective agonists. The aim of the present study was to investigate further the role of ET_B receptors in mediating constriction in rat mesenteric arteries pressurized by use of ET-1, the ET_A receptor antagonist, BQ-123 (Ihara *et al*., 1992), the ET_B selective agonist SRTX S6c (Williams *et al*., 1991), the ET_B receptor selective antagonist, BQ-788 (Ishikawa *et al*., 1994) and the ET_A/ET_B antagonist, TAK-044 (Kikuchi *et al*., 1994).

Some of this work has been presented to the British Pharmacological Society (Mickley *et al*., 1995).

Methods

Male Wistar rats (10–16 weeks old) were killed by exsanguination and the mesenteric bed immediately excised and placed into cold, oxygenated Krebs-Henseleit solution. Third order branches of the mesenteric artery (internal diameter 150–350 μm) were dissected (~3 mm length) and mounted between two glass microcannulae in a small vessel arteriograph (Living Systems Instrumentation Inc., Burlington, U.S.A.). The vessel was constantly superfused with warmed (37°C), oxygenated (95% O_2; 5% CO_2) Krebs-Henseleit solution (composition, in mM: NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25 and glucose 5.5). The intraluminal pressure of the vessel was raised to 60 mmHg and maintained at this pressure with a pressure servo unit without further intraluminal perfusion. Luminal diameter was measured with a video dimension analyser (Living Systems Instrumentation Inc., U.S.A.) and by hand, with a calibrated micrometer, when the optical dimension analyser was unable to detect differences in optical density at smaller lumen diameters. After an equilibration period of 60 min, the vessels were exposed twice to modified Krebs-Henseleit solution containing 60 mM KCl (equimolar replacement of NaCl by KCl) in order to produce maximum constriction. KCl induced a reduction in lumen diameter but never to the level where the lumen was completely occluded (see Table 1). The endothelium was removed by passing an air bubble through the lumen of the vessel (Falloon *et al*., 1993; Smith, 1996) and complete denudation was confirmed by addition of acetylcholine (ACh 10^{-6} M) to vessels pre-constricted with phenylephrine (PE 10^{-5} M). In all vessels, the relaxation induced by ACh before the passage of an air bubble (usually back to resting diameter), was completely abolished after endothelial denudation. After washing, a closed system with a total volume of 30 ml of Krebs-Henseleit solution was constantly superfused at a constant flow rate of 5 ml min^{-1}. It was this reservoir of Krebs-Henseleit solution to which the agonists and antagonists were applied, keeping the volume at 30 ml by removing one ml of Krebs and adding one ml of the drug in a stepwise fashion (as previously described, Smith *et al*., 1995). Responses were recorded 5 min after addition of each agonist concentration, which was sufficient time for an equilibrium response. All of the following studies were carried out in random order and only one concentration response curve to ET-1 or SRTX S6c was performed per tissue. None of the drug treatments resulted in complete occlusion of the vessel lumen within the concentration range studied (see Table 1).

ET-1 and SRTX S6c study

In the first set of experiments cumulative concentration-response curves to ET-1 ($10^{-13}-3\times10^{-8}$ M, $n=10$) or SRTX S6c ($10^{-12}-10^{-7}$ M, $n=17$) were obtained as described above.

Receptor antagonism study

In the second set of experiments, vessels were exposed to either BQ-123 (10^{-6} M, $n=8$), BQ-788 (3×10^{-8} M, $n=8$), TAK-044 (10^{-8} and 3×10^{-7} M, $n=4$ and 8 respectively), BQ-123 + BQ-788 (concentrations as before, $n=8$) or vehicle ($n=8$) for 30 min, before concentration-response curves to ET-1 ($10^{-13}-3\times10^{-8}$ M) were obtained. For these experiments, agonists were prepared in a solution of antagonist so that addition to the perfusion circuit did not dilute the antagonist solution superfusing the tissue. In some experiments, the vessels were exposed for 30 min to SRTX S6c (10^{-7} M) twice (with a wash out period of 10 min between each exposure), in order to desensitise the ET_B receptor before commencement of the ET-1 concentration-response curve. This was carried out both in the absence and in the presence of BQ-123 ($n=8$ each). In all experiments, the time-course of the protocol was the same; 2 h after verification of the removal of the endothelium, the concentration-response curve to ET-1 was begun.

Data analysis

The results are calculated as a percentage of maximum constriction obtained with the second exposure to 60 mM KCl Krebs solution and are expressed as mean ± s.e.mean. Where a maximum response to the agonist was obtained, the negative log of the concentration causing half-maximal contraction (pD_2) was calculated by linear regression analysis and compared by unpaired one-tailed t test. The concentration-response curves were compared by one-way ANOVA followed by Fisher's least significant difference test. Significance was taken at $P<0.05$.

Materials

ET-1 and SRTX S6c were purchased from Novabiochem (Nottingham, U.K.) and BQ-788 (N-cis-2,6-dimethylpiperidinocarbonyl-L-γ-MeLeu-D-Trp($COOCH_3$)-D-Nle, sodium salt) from Neosystems (Strasbourg, France), all were reconstituted in 50 : 50 methanol : distilled water. BQ-123 (cyclo[D-Trp-D-Asp-L-Pro-D-Vel-L-Leu]) from Neosystems (France) and TAK-044 (cyclo[D-α-Asp-3-[(phenylpiperazin-1-yl)carbonyl]-L-Ala-α-Asp-D-2-(2-thienyl)-Gly-L-Leu-D-Trp] disodium salt) synthesised by Takeda Chemical Industries (Osaka, Japan) were reconstituted in 0.9% saline, placed in aliquots and stored frozen at −20°C until use. All peptide agonists and antagonists were diluted in Krebs-Henseleit solution containing 0.1% bovine serum albumin (BSA: Sigma, Poole, U.K.). In

Table 1 Mean resting lumen diameters and lumen diameters after exposure to 60 mM KCl solution or after the maximum concentration of endothelin-1 (ET-1) or sarafotoxin S6c (SRTX S6c) in each experimental group

	ET-1 control	*SRTX S6c*	*+ BQ-123*	*+ BQ-788*	*+ SRTX S6c desens*	*+ BQ-123 + BQ-788*	*+ BQ-123 + SRTX S6c desens*	*+ TAK-044* (10^{-8} M)	*+ TAK-044* (3×10^{-7} M)
Resting diameter	277 ± 15	300 ± 9	261 ± 13	287 ± 15	281 ± 7	273 ± 21	304 ± 19	300 ± 12	301 ± 12
+60 mM KCl diameter	51 ± 3	48 ± 2	53 ± 3	51 ± 1	48 ± 3	55 ± 2	50 ± 3	45 ± 3	50 ± 2
Max ET-1/SRTX S6c diameter	47 ± 3	273 ± 12	56 ± 7	50 ± 2	48 ± 3	64 ± 8	118 ± 27	118 ± 39	233 ± 31

Data shown are mean ± s.e.mean.

all antagonist experiments the ET-1 concentrations were diluted in 0.1% BSA Krebs-Henseleit solution with the appropriate antagonist. ACh (chloride salt, Sigma, Poole, U.K.) and PE (hydrochloride salt; Fisons, U.K.) were prepared in saline at stock concentration of 10^{-2} M, placed in aliquots, and stored at $-20°C$ until use when diluted in Krebs-Henseleit solution.

Results

Effects of 60 mM KCl

In all experiments 60 mM KCl superfusion constricted the arteries, an effect which was reversible, back to initial resting diameter, on washout (Table 1). The initial diameter remained constant until agonist-induced constriction was generated.

Effects of ET-1 and SRTX S6c

ET-1 constricted the arteries in a concentration-dependent manner (Figure 1, pD_2 9.86, E_{max} $101.9 \pm 2.6\%$ KCl induced contraction at 10^{-8} M ET-1, $n=10$). SRTX S6c also produced a concentration-dependent contraction (Figure 1), but the response was extremely variable, the maximum response obtained with 3×10^{-8} M SRTX S6c ranging from 0 to 39% of KCl contraction (mean response $= 10.7 \pm 2.9\%$, $n=17$). In fact, only 9 of the 17 vessels (53%) responded to SRTX S6c.

Effect of ET_A receptor blockade

Incubation with BQ-123 (10^{-6} M) before and during exposure to ET-1 (Figure 2) had no effect on contractile responses to low concentrations of ET-1 (10^{-13} to 10^{-10} M) but resulted in inhibition of responses to concentrations of ET-1 between 10^{-10} and 3×10^{-8} M. Incubation with BQ-123 significantly inhibited the constrictions to 10^{-9} and 3×10^{-9} M ET-1 ($P=0.006$ and 0.01, respectively) when compared by ANOVA. However, the effect of BQ-123 on the overall pD_2 of the ET-1 concentration-response curve did not reach statistical significance (pD_2 9.15 ($n=8$) vs 9.86 ($n=10$) NS, $P=0.094$).

Effect of ET_B receptor desensitization or blockade

Exposure to a supra-maximal concentration of SRTX S6c (10^{-7} M), to achieve ET_B receptor desensitization, produced an initial constriction in 4 out of the 8 vessels studied (mean response $= 8.1 \pm 3.5\%$ KCl constriction). The vessel diameter returned to the initial resting value during the first 30 min exposure to SRTX S6c. No constriction was seen, in any of the vessels studied, during the second exposure to SRTX S6c confirming that tachyphlaxis had occurred. The ET-1 concentration-response curve was not significantly altered by either ET_B receptor desensitisation (Figure 3a, $pD_2=9.88$, $n=8$) or following incubation with the selective ET_B receptor antagonist, BQ-788 (3×10^{-8} M, Figure 3b, $pD_2=10.02$, $n=8$), although both treatments tended to shift the ET-1 concentration-response curve to the left ($P=0.5$ and 0.34, respectively).

Effect of combined ET_A and ET_B receptor blockade

Co-incubation of vessels with BQ-123 (10^{-6} M) and BQ-788 (3×10^{-8} M) resulted in a parallel shift of the ET-1 concentration-response curve to the right (Figure 4, $n=8$). Incubation with BQ-123 (10^{-6} M) following desensitization of ET_B receptors with 10^{-7} M SRTX S6c caused a similar rightward shift (Figure 4, $n=8$). Incubation of vessels with the ET_A/ET_B receptor antagonist, TAK-044 (Figure 5, 10^{-8} M, $n=4$ and 3×10^{-7} M, $n=8$) also caused a parallel concentration-dependent shift to the right of the ET-1 concentration-response curve. As the maximum response to ET-1 was not reached within the concentration range studied it was not possible to calculate pD_2 values for ET-1 in experiments with BQ-123 plus either BQ-788 or SRTX S6c desensitization, or with TAK-044 (both concentrations).

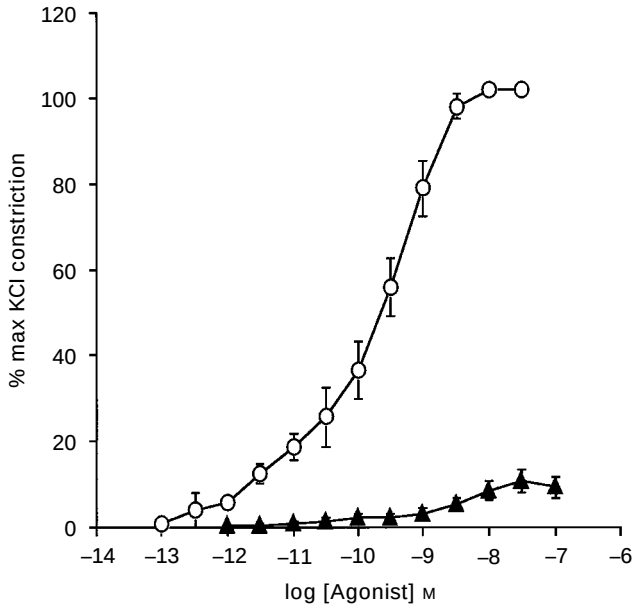

Figure 1 Comparison of the contractile responses to endothelin-1 (ET-1, ○) and sarafotoxin S6c (SRTX S6c, ▲) in rat small mesenteric arteries. ET-1 ($n=10$) produced a maximal constriction of similar proportion to 60 mM KCl at 3×10^{-9} M. SRTX S6c ($n=17$) induced small constrictions at the highest concentrations, suggesting a small population of ET_B receptors present on the smooth muscle of the resistance arteries. All values are mean and vertical lines show s.e.mean.

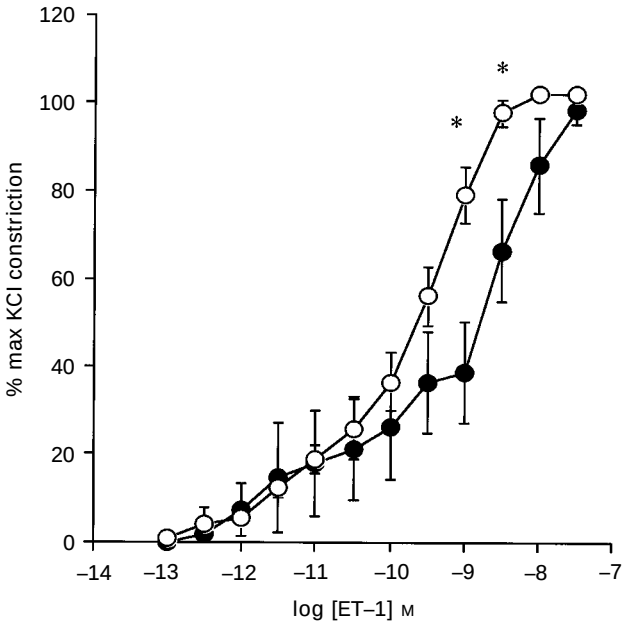

Figure 2 Effect of the ET_A receptor antagonist BQ-123 on the endothelin-1 (ET-1) concentration-response curve in rat small mesenteric arteries. Pre-incubation with BQ-123 (10^{-6} M) for 30 min (●, $n=8$) shifted the responses to the higher concentrations of ET-1 in a parallel fashion to the right. All values are mean and vertical lines show s.e.mean. $^*P<0.05$ compared to control (○) ET-1 responses.

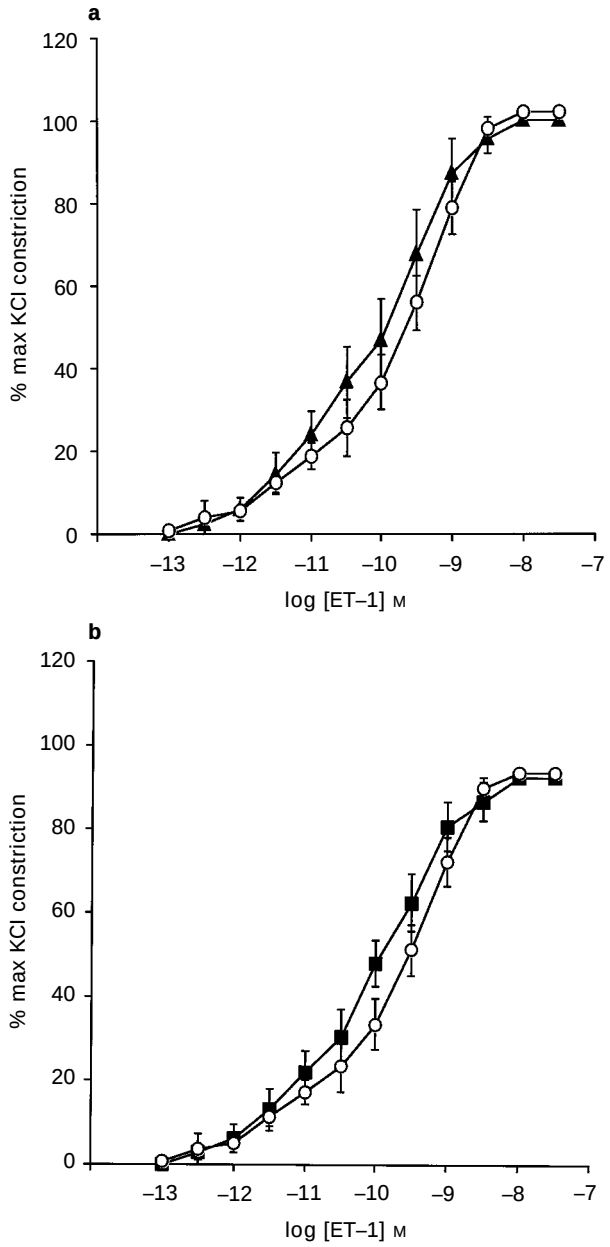

Figure 3 The effects of selective ET_B receptor blockade on endothelin-1 (ET-1)-induced constrictions in rat small mesenteric arteries. The vessels were exposed to either (a) sarafotoxin S6c (SRTX S6c; 10^{-7} M, ▲, $n=8$) twice before addition of ET-1 or (b) BQ-788 (3×10^{-8} M, ■, $n=8$) pre-incubated for 30 min before the start of the ET-1 concentration-response curve. In both treatments the ET-1 concentration-response curves tended to be shifted slightly to the left as compared to control (○), (though not significant, $P=0.54$ and 0.42, respectively, as compared by ANOVA). All values are mean and vertical lines show s.e.mean.

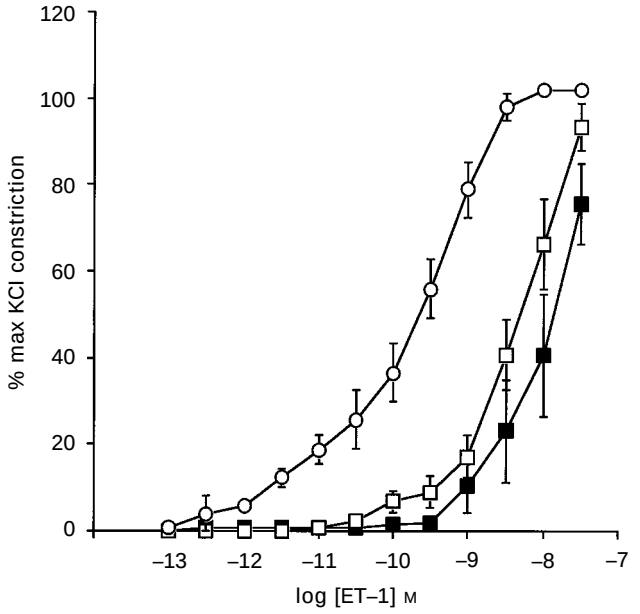

Figure 4 The effects of non-selective ET_A/ET_B combination treatment on endothelin-1 (ET-1)-induced constrictions in rat small mesenteric arteries. The vessels were exposed to either vehicle (○), BQ-123 plus BQ-788 (10^{-6} M and 3×10^{-8} M, □, $n=8$) or pre-incubated with sarafotoxin S6c twice (each 10^{-7} M) plus BQ-123 (10^{-6} M, ■, $n=8$). Both treatments significantly shifted the ET-1 concentration-response curve to the right in a parallel fashion ($P=0.0001$ for both). All values are mean and vertical lines show s.e.mean.

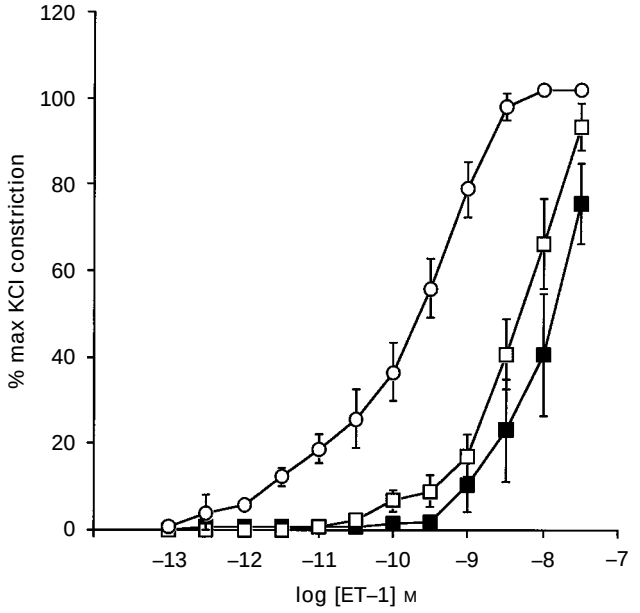

Figure 5 The effects of the non-selective ET_A/ET_B receptor antagonist TAK-044 on endothelin (ET-1)-induced constrictions in rat small mesenteric arteries. The vessels were pre-incubated for 30 min with either 10^{-8} M (△, $n=4$) or 3×10^{-7} M (▲, $n=8$) TAK-044. Both treatments significantly inhibited the ET-1 concentration-response curve ($P=0.0002$ and 0.0001 respectively) as compared to control (○). All values are mean and vertical lines show s.e.mean.

Discussion

Previous *in vivo* studies have clearly indicated a role for ET_B receptors in mediating vasoconstriction in resistance beds, but their role has been difficult to demonstrate in isolated resistance vessels. In the present study, we show that a role for ET_B receptors in rat isolated mesenteric arteries emerges when both ET_A and ET_B receptors are blocked, whereas blockade of ET_A receptors alone only partially inhibited ET-1-induced contraction and inhibition of ET_B receptors alone had no effect. This phenomenon is similar to previous observations in rabbit pulmonary artery (Fukuroda *et al*., 1994c), rat trachea (Clozel & Gray, 1995) and human bronchus (Fukuroda *et al*., 1996), and may be explained by the existence of a 'crosstalk' mechanism between the ET_A and ET_B receptors.

In initial experiments we used the highly selective ET_B receptor agonist SRTX S6c (Williams *et al*., 1991) to investigate the presence of ET_B receptors in pressurised mesenteric arteries. SRTX S6c produced concentration-dependent con-

striction but the maximum constriction reached only ~10% of that routinely seen with ET-1, much less than would have been predicted from previous *in vivo* experiments (Clozel *et al*., 1992). However, the magnitude of responses to SRTX S6c is in agreement with responses obtained by Takase *et al*. (1995) and Deng *et al*. (1995), in rat mesenteric arteries studied in the perfusion and wire myograph, respectively. Interestingly, in all three studies, the contractions of SRTX S6c occurred at relatively high concentrations (10 nM). The ET_B receptor agonists, BQ-3020 and IRL 1620, were equally ineffective in the rat perfused mesenteric bed at concentrations up to 1 nM (D'Orleans-Juste *et al*., 1993). This is quite different to the ET_B agonist responses induced in large blood vessels, which are generally larger and occur at lower concentrations (Moreland *et al*., 1992, Sumner *et al*., 1992; LaDouceur *et al*., 1993; Gray *et al*., 1994). Another interesting feature of our results, not mentioned by previous investigators, is the variability in responsiveness to SRTX S6c. While some vessels failed to respond, others gave up to ~40% of the maximum contraction obtained with ET-1. This might be explained by differential distribution of ET_B receptors in the mesenteric bed, although 3rd generation branches of the main mesenteric artery were routinely used for these studies. Another possibility is variation in intrinsic myogenic tone that these vessels can develop when under pressure. In a separate experiment, in which vessels mounted in the wire myograph were studied, we found that no responses were obtained to STRX S6c until some tone was introduced by a low concentration of the stable thromboxane analogue, U46619 (Mickley *et al*., 1995).

An alternative approach for the investigation of the role of ET_B receptors is to remove the influence of ET_B receptors, either by desensitization (LaDouceur *et al*., 1993) or by use of a selective ET_B receptor antagonist, like BQ-788 (Ishikawa *et al*., 1994). In the present study, neither of these interventions inhibited ET-1 induced contraction, a result which would support the view that ET_B receptors have little or no role in rat mesenteric arteries. Interestingly, both desensitization and BQ-788 treatment seemed to potentiate responses to ET-1 slighty, although this effect was not significant. Seo (1996) recently found a similar potentiation of ET-1-induced constriction by the ET_B receptor antagonist, Res 701-1 in human gastroepiploic arteries. There are several possible explanations for these observations. Potentiation of contractions by ET_B receptor antagonists would be expected in the presence of the vascular endothelium due to blockade of endothelial ET_B receptor-mediated release of relaxing factors by ET-1. However, this is an unlikely explanation for the present results as the endothelium was effectively removed by passing of an air bubble through the lumen of the vessels, as evidenced by the loss of relaxant responses to acetylcholine. Previous histological studies in our laboratory have also shown complete removal of the endothelium by this method (Smith, 1996). The experiments of Seo (1996) were also conducted in endothelium-denuded vessels. Alternatively, potentiation might have been caused by displacement of ET-1 from low affinity ET_B clearance receptors (Fukuroda *et al*., 1994a) by BQ-788, but this would not account for the similar effect of receptor desensitisation. Another alternative, suggested by Seo (1996), is the presence of sensitive ET_B receptors on smooth muscle which inhibit or negatively modulate ET_A receptor-mediated constrictions to ET-1.

From the results obtained with SRTX S6c, BQ-788, and desensitization alone, one would predict that blockade of ET_A receptors, by use of a selective competitive antagonist, like BQ-123 (Ihara *et al*., 1992), would cause a parallel rightward shift of the ET-1 concentration-response curve. However, in the presence of BQ-123 the ET-1 concentration-response curve in mesenteric arteries under pressure was biphasic, only responses to high concentrations of ET-1 being shifted to the right in a parallel manner by BQ-123, consistent with competitive antagonism at the ET_A receptor. Interestingly, the BQ-123-resistant, possibly ET_B-mediated, responses to ET-1 were at the lower end of the dose-response curve, consistent with the presence of a high affinity ET_B receptor. Takase *et al*. (1995) obtained similar results with the ET_A receptor antagonist, FR139317 in rat mesenteric arteries, although in that case the ET_A-resistant component was smaller than seen here. Takase *et al*. perfused the vessels at a pressure of 30 mmHg, half of that used in the present study. Given our observation that increased tone may reveal constrictor ET_B receptors, as implied by the responses to SRTX S6c (Mickley *et al*., 1995), the lower pressure used by Takase *et al*. (1995) may account for the smaller ET_A receptor antagonist-resistant element of the ET-1 curve. The results of the present study are consistent with the ET_A receptor antagonist resistant reduction in mesenteric blood flow induced by ET-1 *in vivo* found by Gardiner *et al*. (1994) and Allcock *et al*. (1995).

In order to investigate whether the residual ET_A antagonist resistant portion of the ET-1 response is mediated by ET_B receptors, we used combined treatment with BQ-123 and either desensitization or BQ-788. Both of these combination treatments resulted in a parallel shift of the ET-1 concentration-response curve. In fact, the BQ-123-sensitive portion was moved further to the right than with BQ-123 alone, in agreement with Fukuroda *et al*. (1996) who described a similar phenomenon in human bronchi. Responses to ET-1 were also inhibited, in a concentration-dependent manner, by TAK-044, a peptide antagonist with similar potency at both ET_A and ET_B receptors (Kikuchi *et al*., 1994).

These results demonstrate a clear role for ET_B receptors in mediation of constrictor responses to ET-1 in small mesenteric arteries that is only revealed when ET_A receptors, in addition to ET_B receptors, are blocked. The lack of effect of ET_B receptor blockade or desensitization alone seems to indicate that ET_A receptors can somehow compensate for the inactivation of ET_B receptors. Similar observations have been obtained in vascular (Fukuroda *et al*., 1994c) and non-vascular (Clozel & Gray, 1995; Fukuroda *et al*., 1996) tissues. The concept of receptor 'crosstalk' has been proposed to explain these observations. The mechanism is not fully understood, although interactions at the second messenger level have been suggested, such that blockade of the ET_B receptor releases an inhibitory mechanism acting at the ET_A receptor (Fukuroda *et al*., 1996). Allosteric interactions between ET receptors have been suggested to account for the results of radioligand binding studies in rat heart (Sokolovsky, 1993). Further biochemical studies are required to elucidate the interactions between ET receptors co-existing in the same tissue and the mechanism of the apparent crosstalk phenomenon. Interestingly, similar interactions have been described between α_1- and α_2-adrenoceptors activated by noradrenaline (Daly *et al*., 1988).

In the rat, the mesenteric bed receives a high proportion of cardiac output and thus resistance in this bed is an important determinant of total peripheral resistance and of blood pressure. The present results show that simultaneous blockade of both ET_A and ET_B receptors is required for complete inhibition of constrictor responses to ET-1 in the rat mesentery *in vitro*. This agrees with observations that blockade of both receptors is required to inhibit ET-1-induced increases in blood pressure *in vivo* (McMurdo *et al*., 1993). The role of ET_B receptors in regulating constrictor responses to ET-1 might be even greater in human resistance vessels, where ET_B agonists have a greater direct effect than in other species *in vitro* (Takase *et al*., 1995, Mickley, unpublished observations) and *in vivo* (Haynes *et al*., 1995).

In some pathophysiological states associated with increased peripheral resistance and increased plasma concentrations of ET-1, there is evidence for an upregulation of smooth muscle ET_B receptors; most notably in heart failure in dogs (Cannan *et al*., 1996) and man (Love *et al*., 1996); in atherosclerosis (Winkles *et al*., 1993; Dagassan *et al*., 1996) and in hypertension (Kanno *et al*., 1993; Batra *et al*., 1993). The results of the present study suggest that blockade of both ET_A and ET_B receptors may be required for effective inhibition of ET-1-induced constriction in these diseases. This study was conducted

in vessels without endothelium. However, in the presence of endothelium, ET_B receptor blockade can actually enhance responses to ET-1 by blocking the release of nitric oxide and prostacyclin through endothelial ET_B receptor stimulation (De Nucci *et al*., 1988). Thus, the effectiveness of endothelin receptor blockade therapeutically will depend on the level of endothelial ET_B receptor stimulation and on the relative selectivity of the antagonist for endothelial and smooth muscle ET_B receptors, the ideal antagonist allowing ET-1 to act at the endothelial ET_B receptor while blocking its effects at smooth muscle ET_A and ET_B receptors.

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