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Inhibitory effects of TAK-044 on endothelin induced vasoconstriction in various canine arteries and porcine coronary arteries: a comparison with selective ET_A and ET_B receptor antagonists

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1 The inhibitory effects of the endothelin (ET) receptor antagonist, TAK-044, on ET-induced vasoconstriction in various canine arteries and porcine coronary arteries were studied and were compared to those of selective ET_A and ET_B receptor antagonists.

2 ET-1 (0.1 nM – 0.3 μM) caused vasoconstriction in canine coronary, femoral, renal, mesenteric and basilar arteries, and the strongest responses were obtained in coronary and basilar arteries. TAK-044 (10 nM, 100 nM) inhibited this ET-1-induced vasoconstriction except in the case of mesenteric arteries. The strongest inhibitory effects were obtained in coronary arteries; an EC_{50} value for ET-1 was 5.2 ± 0.77 nM ($n=12$) in the control and 24 ± 3.8 nM ($n=4$) in the presence of TAK-044 at 10 nM. BQ-123 (1 μM) inhibited the vasoconstriction in coronary and femoral arteries but did not in renal, mesenteric or basilar arteries.

3 TAK-044 (10 – 100 nM) inhibited the ET-1-induced vasoconstriction in porcine coronary arteries to a degree similar to that in canine coronary arteries. In contrast, BQ-123 (10 μM) did not inhibit the contraction completely, and a BQ-123-insensitive component was identified. Although BQ-788 (1 μM) did not modify the concentration-response curve at all, it abolished the BQ-123-insensitive component when applied together with BQ-123 (10 μM).

4 Sarafotoxin S6c (10 pM – 30 nM) caused vasoconstriction in porcine coronary arteries with the maximum amplitude of the contraction being 39% of that with ET-1. Both TAK-044 (10 nM, 100 nM) and BQ-788 (1 μM) inhibited this vasoconstriction, while BQ-123 (3 μM, 10 μM) did not.

5 Vasoconstriction induced by ET-3 (0.1 nM – 0.3 μM) in porcine coronary arteries showed a concentration-response curve with two distinct phases in contrast to that seen with sarafotoxin S6c. TAK-044 (0.3 nM – 10 nM) inhibited both phases in a concentration-dependent manner. BQ-123 (1 μM, 3 μM) inhibited only the second phase, while BQ-788 (1 μM) inhibited the first phase.

6 We concluded that the inhibitory effects of TAK-044 on ET-1-induced vasoconstriction were the strongest in coronary arteries among the canine arteries examined. In addition, we showed that both ET_A and ET_B receptors mediate vasoconstriction in porcine coronary arteries and TAK-044 inhibits the vasoconstriction mediated by both of these receptors.

Keywords: Endothelin receptor antagonist; TAK-044; porcine coronary arteries; ET_A receptors; ET_B receptors; endothelin isopeptide; sarafotoxin S6c; canine arteries

Introduction

Endogenous endothelin (ET) has been speculated to play an important role in various pathophysiological conditions (Masaki *et al*., 1992). Among them, we have demonstrated that an increase in tissue and plasma ET levels, in particular ET-1 levels, is one of the causes of the extension of myocardial infarct size in rats and rabbits (Watanabe *et al*., 1991; Kusumoto *et al*., 1993) and the induction of acute renal failure after renal ischaemia-and-reperfusion (Shibouta *et al*., 1990) by use of a monoclonal antibody against ET-1 and by measuring tissue and plasma ET-1 levels.

Recently, we found a new ET receptor antagonist, TAK-044, which is a competitive antagonist for both ET receptor subtypes, ET_A and ET_B receptors (Masuda *et al*., 1996), and inhibits phenomena induced by activation of either receptor subtype (Ikeda *et al*., 1994). In rat and dog models of cardiac ischaemia-and-reperfusion, TAK-044 inhibited the extension of myocardial infarct size (Kojima *et al*., 1995; Watanabe *et al*., 1995) and improved contractile function in the stunned myocardium (Kitayoshi *et al*., 1996). In contrast to the cardioprotective effects of TAK-044, it is controversial whether ET_A receptor selective antagonists can abbreviate damage related to ischaemic conditions of the heart. For example, BQ-123 has been shown to reduce myocardial infarct size (Grover *et al*., 1993), while FR139317 (McMurdo *et al*., 1994) and PD156707 (Mertz *et al*., 1996) did not show such an effect. Since endogenous ET-1 plays a significant role in the extension of the infarct size (Watanabe *et al*., 1991) and ET-1 is equally effective at both receptor subtypes (Masaki *et al*., 1992), both of which mediate vasoconstriction (Warner, 1994), one of the plausible explanations for these discrepancies is differences in the potency for ET-1-induced vasoconstriction in coronary arteries and the inhibitory profile for both ET-receptor subtypes, that is selective or non-selective.

In the present study, therefore, the inhibitory effects of TAK-044 on ET-1-induced vasoconstriction in various canine arteries were studied and were compared to those of BQ-123. In addition, the involvement of ET_A and ET_B receptors in vasoconstriction of porcine coronary arteries was investigated by use of TAK-044, selective ET_A and ET_B receptor antago-

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nists and ET_B receptor agonists. The results should provide a better insight into the cardioprotective effects of TAK-044 from the veiwpoint of its vascular action.

Methods

Experiments were performed in accordance with the guidelines of the Takeda Experimental Animal Care and Use Committee.

Tissue preparations

Canine arteries A total of 14 male beagle dogs (9–14 kg) were used in the present study. After the dogs had been anaesthetized with sodium pentobarbitone (30 mg kg^{-1}, i.v.), femoral, renal and mesenteric arteries were excised. Hearts and brains were then removed to obtain left anterior descending coronary arteries and basilar arteries, respectively. The excised arteries were placed in a modified Krebs solution (composition in mM: NaCl 113, KCl 4.6, $CaCl_2$ 1.2, $NaHCO_3$ 22, NaH_2PO_4 3.5, $MgCl_2$ 1.2 and dextrose 10) gassed with 95%O_2–5%CO_2 (pH 7.4) at room temperature. After adherent fat and connective tissue had been removed the arteries were cut to make ring preparations 3 mm in length: the diameter of the arteries was approximately 1, 2, 3, 3 and 1 mm for coronary, renal, femoral, mesenteric and basilar arteries, respectively. Two or three ring preparations were made from each artery obtained from one animal.

Porcine coronary arteries Porcine hearts were obtained from a local slaughter house and were transported to our laboratory under cold conditions (5–10°C) within 5 h of death. Left anterior descending coronary arteries were excised to make two to four ring preparations of approximately 3 mm in length and 1.5 mm in diameter, by use of methods similar to those used for canine arteries.

Experimental setup and procedures

Ring preparations were mounted horizontally in an organ bath by a pair of stainless wires (0.2 mm in diameter): one was fixed to the bottom of the bath chamber and the other was tied to a force transducer (FD pickup, Nihon Koden, Japan) with a silk suture. The organ bath was filled with 20 ml of the Krebs solution which was continuously gassed with 95%O_2–5%CO_2 (pH 7.4) and was heated to maintain a temperature of 37°C. Vasoconstriction was measured isometrically with the transducer connected to a polygraph system (360, NEC Medical Systems, Japan) and output to a pen recorder (RECTI-HORIZ, NEC Medical Systems, Japan).

Ring preparations were equilibrated for 90 min with a resting load of 5 g for canine renal, femoral and mesenteric arteries, 2 g for canine and porcine coronary arteries and 1 g for canine basilar arteries and were washed every 30 min with fresh Krebs solution. Preliminary experiments indicated that these resting tensions provided sub-maximum responses to 60 mM KCl. After the stabilization period, 60 mM KCl was added to the Krebs solution to induce stable vasoconstriction for 15 min, and the amplitude of the obtained contraction was used as a reference for each preparation to normalize data (see Data Analysis in detail). Preparations were then washed with normal solution and allowed to recover for at least 60 min before the following experiments were started.

Concentration-response curves were obtained by adding ET-1, ET-3 or sarafotoxin S6c to the bath solution in a cumulative manner at logarithmic increments. Ring preparations were exposed to each concentration until a plateau was reached before the next concentration was applied. Effects of ET receptor antagonists on the concentration-response curves were examined by adding an antagonist or saline 30 min before the cumulative addition of an agonist. Only one concentration-response curve was obtained for each ring preparation because vasoconstriction induced by ET-1, ET-3 or sarafotoxin S6c was difficult to wash-out which prevented the repetitive application of these agonists. Therefore, each preparation was assigned to control or drug-treatment groups as follows. One of the preparations from each dog or heart was used for the control experiments, and the remaining ring preparations were assigned to different groups avoiding duplication from the same animal.

Data analysis

Amplitudes of vasoconstriction were measured from the chart of the pen-recorder and were expressed in terms of percentage of the reference contraction obtained with 60 mM KCl which was defined as 100% contraction for each individual ring prepartion. A best fitted concentration-response curve was obtained individually based on the measured amplitude for each ring preparation with a least-squares method by use of a personal computer (IBM, U.S.A.). Each curve was expressed as

$$Y = Y_{max}/(1 + a * \exp(-bX)),$$

where Y is amplitude of the induced vasoconstriction, Y_{max} is the maximum amplitude of the response, a and b are arbitrary factors and X is the agonist concentration. An EC_{50} value, the concentration at 50% of the Y_{max}, was calculated from the fitted equation.

Statistical analyses of concentration-response curves between canine arteries were achieved with a one-way analysis of variance and a Tukey test and those between control and antagonist-treated groups were achieved with a one-way analysis of variance and Dunnett's test for EC_{50} and Y_{max} values in both cases. Values of $P<0.05$ were considered statistically significant. All data in the present study are expressed as a mean ± s.e.mean, with n as the number of ring preparations which was equal to the number of dogs or porcine hearts involved. Concentration-response curves in the figures were drawn based on mean values of the contraction at each concentration with linear interpolation for presentation purposes.

Drugs

TAK-044 (cyclo[D-α-aspartyl-3-[(4-phenyl-piperazin-1-yl)carbonyl] L-alanyl-L-α-aspartyl-D-2-(2-thienyl)glycyl-L-leucyl-D-tryptophyl] disodium salt, Kikuchi *et al*., 1994) and BQ-123 were dissolved in distilled water to make solutions of 1 mM and 10 mM, respectively, before the experiments were started each day. ET-1, ET-3 and sarafotoxin S6c were dissolved in distilled water with 0.1% acetic acid and BQ-788 was dissolved in dimethylsulphoxide (DMSO) to make stock solutions (0.01 mM and 0.1 mM) which were kept in a freezer until the day of the experiments. TAK-044 and BQ-123 were synthesized in our laboratories, and all other peptide chemicals were purchased (Peptide Institute, Japan). The total volume of all drug solutions added to the bath solution was less than 0.9%. A preliminary experiment showed that DMSO and acetic acid in the volume required in the present experiment design, had no significant effects on the concentration-response curves of ET-1, ET-3 or sarafotoxin S6c.

Results

ET-1 caused vasoconstriction in arteries obtained from various canine organs (Figure 1). In control preparations, the maximum amplitude of the induced contraction was significantly ($P<0.01$) higher in coronary (178 ± 15%, $n=12$) and basilar (150 ± 10%, $n=12$) arteries than that in mesenteric (94 ± 8.0%, $n=8$), femoral (77 ± 5.1%, $n=12$) and renal (77 ± 4.4%, $n=12$) arteries. The threshold concentration of ET-1 was 0.1 nM in the coronary artery, while the renal, the femoral and the mesenteric arteries required a higher concentration (1 nM).

In contrast to these arteries, the basilar artery showed vasoconstriction even at the lowest concentration of ET-1 (0.1 nM). ET-1 caused stronger vasoconstriction in coronary and basilar arteries than the other arteries in terms of EC_{50} values (Table 1). The EC_{50} value of ET-1 in the coronary artery was significantly ($P<0.01$) lower than that in the femoral, the renal and the mesenteric arteries but similar to that in the basilar artery.

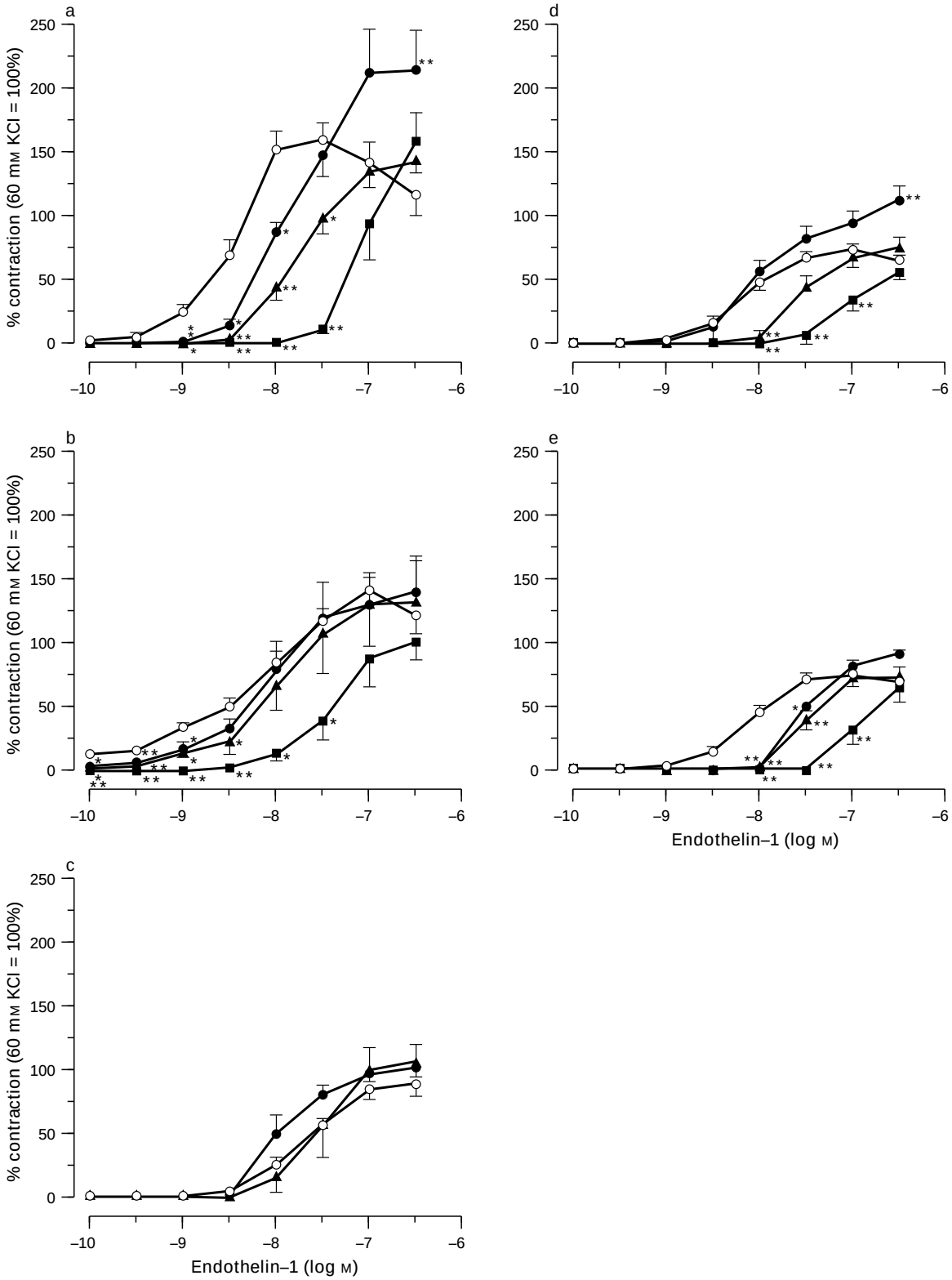

Figure 1 Concentration-response curves for endothlin-1 in various canine arteries in the presence and absence of TAK-044 and BQ-123. Endothelin-1 caused vasoconstriction in (a) coronary, (b) basilar, (c) mesenteric, (d) renal and (e) femoral arteries, and the degree of contraction varied between the arteries. TAK-044 inhibited the vasoconstriction in a concentration-dependent manner, and the strongest inhibition was obtained in coronary arteries. BQ-123 also inhibited the vasoconstriction but not as potently as TAK-044. It should be noted that the two antagonists had different inhibitory profiles. Control (○), $n=12$, except for the mesenteric artery ($n=8$), and $n=4$ for TAK-044 (10 nM (▲) and 100 nM (■)) and BQ-123 (1 μM (●)) except for the basilar artery with BQ-123 ($n=5$). $^{*}P<0.05$; $^{**}P<0.01$.

Pretreatment with TAK-044 inhibited the ET-1-induced vasoconstriction in a concentration-dependent manner in all arteries except the mesenteric artery (Figure 1). Although the degree of inhibition varied between the arteries, it was not related to the maximal contractile amplitudes, the EC_{50} values or the threshold concentrations. The strongest inhibition was observed in the coronary artery (Table 1). TAK-044 shifted the concentration-response curve to the right by a factor of 4.6 based on the ratio of EC_{50} values, (TAK-044 at 10 nM)/(control). Weaker but significant ($P<0.01$) inhibition was observed in the femoral and the renal arteries: their concentration-response curves were shifted by a factor of 3.3 and 3.6, respectively (Table 1). Although TAK-044 at 10 nM did not affect the EC_{50} values obtained in the mesenteric or the basilar arteries, the vasoconstriction induced by lower concentrations of ET-1, below 3 nM, in the basilar artery was significantly inhibited. Inhibitory effects of TAK-044 100 nM in the various arteries were similar to those at 10 nM (Table 1): the shift in the EC_{50} value was again the largest in the coronary artery.

The ET_A receptor antagonist BQ-123 (1 μM) also inhibited the ET-1-induced vasoconstriction in various canine arteries, but the inhibitory profile and the degree of inhibition were different from those seen with TAK-044 (Figure 1). The degree of inhibition in the femoral artery with BQ-123 1 μM was similar to that with TAK-044 10 nM: a factor of 3.6 for BQ-123 (the ratio of EC_{50} values, (BQ-123 at 1 μM)/(control)) vs. 3.3 for TAK-044 (Table 1). Vasoconstriction in the coronary artery was also inhibited by BQ-123 to a degree similar to that in the femoral artery. BQ-123 did not change the EC_{50} values in the renal, the mesenteric or the basilar arteries (Table 1). However, the vasoconstriction induced by low concentrations of ET-1, below 1 nM, in the basilar artery was significantly inhibited by BQ-123 similarly to the result with TAK-044 10 nM.

The inhibitory effects of TAK-044 and BQ-123 in coronary arteries were studied in detail in porcine coronary artery. ET-1 caused vasoconstriction in porcine coronary artery similar to that obtained in the canine coronary artery (Figure 2a). In control preparations, the threshold concentration, EC_{50} value and maximum amplitude of the contraction were 0.3 nM, 6.4 ± 0.62 nM and $192\pm6.9\%$ ($n=36$) respectively. Pretreatment with TAK-044 inhibited the ET-1-induced vasocon-

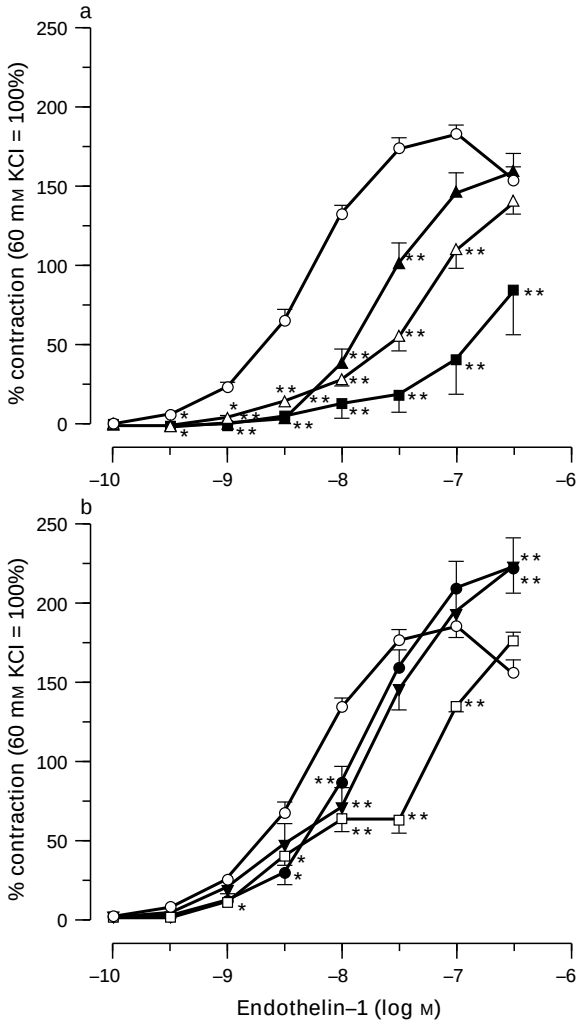

Figure 2 Concentration-response curves for endothelin-1 in porcine coronary arteries in the presence and absence of TAK-044 (a) and BQ-123 (b). Pretreatment with TAK-044 inhibited the endothelin-1-induced vasoconstriction in a concentration-dependent manner. Note the small hump with 30 or 100 nM TAK-044 at lower concentrations of endothelin-1. The results following pretreatment with BQ-123 revealed the hump, a BQ-123-insensitive component, clearly. Control (○), $n=36$; TAK-044: $n=8$ at 10 nM (▲), $n=6$ at 30 nM (△) and $n=7$ at 100 nM (■), and BQ-123: $n=7$ at 1 μM (●), $n=8$ at 3 μM (▼) and $n=13$ at 10 μM (□). $^*P<0.05$; $^{**}P<0.01$.

Table 1 EC_{50} values for ET-1-induced vasoconstriction in various canine arteries with TAK-044 (10 nM, 100 nM) or BQ-123 (1 μM)

		TAK-044		*BQ-123*
Artery	*Control*	*10 nM*	*100 nM*	*1 μM*
Coronary	5.2 ± 0.77	24 ± 3.8*	87 ± 13*	21 ± 0.62*
Femoral	14 ± 1.8†	46 ± 6.8*	157 ± 59*	50 ± 3.6*
Renal	13 ± 1.5†	47 ± 8.0*	98 ± 24*	20 ± 4.5
Mesentery	29 ± 4.5†	42 ± 14	ND	25 ± 3.5
Basilar	7.8 ± 1.8	12 ± 1.8	62 ± 19*	13 ± 3.1

Data shown are means$\pm$s.e.mean. $^*P<0.01$ vs. control in each artery preparation. †$P<0.05$ vs. coronary or basilar artery in the control group. ND; not determined.

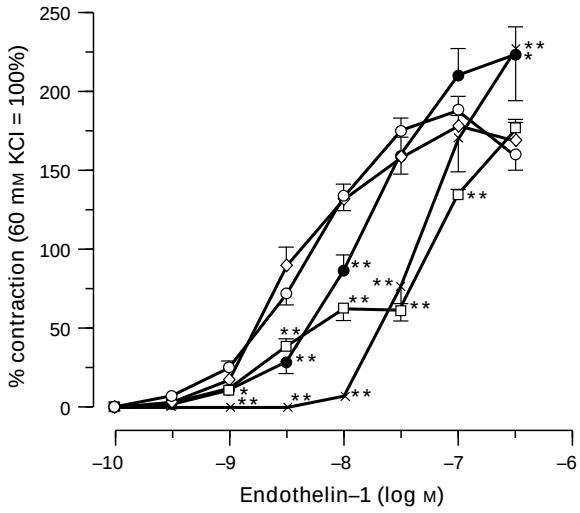

Figure 3 Inhibitory effects of selective endothelin receptor antagonists in porcine coronary arteries. The ET_A receptor antagonist BQ-123 inhibited the endothelin-1-induced vasoconstriction partially, and additional application of the ET_B receptor antagonist BQ-788 completely inhibited the BQ-123-insensitive component. BQ-788 alone did not inhibit the vasoconstriction. Control (○), $n=27$; BQ-123: $n=7$ at 1 μM (●) and $n=13$ at 10 μM (□); BQ-788: $n=5$ at 1 μM (◇); $n=4$ for BQ-123 at 10 μM with BQ-788 at 1 μM (×). $^*P<0.05$; $^{**}P<0.01$.

striction in a concentration-dependent manner (Figure 2a). It shifted the concentration-response curve without changing the maximum amplitude of the contraction: the EC_{50} value was changed significantly ($P<0.01$) to 26 ± 2.8 nM ($n=8$), 46 ± 7.7 nM ($n=6$) and 101 ± 29 nM ($n=7$) with TAK-044 at 10 nM, 30 nM and 100 nM, respectively. Although it was not clearly noticeable, the curve obtained with TAK-044 deviated slightly from the expected curve at low concentrations of ET-1 suggesting involvement of another receptor subtype in the ET-1-induced vasoconstriction. This 'hump' was clearly seen with the application of BQ-123 10 μM (Figure 2b).

BQ-123 also inhibited the ET-1-induced vasoconstriction in porcine coronary artery but was less potent than TAK-044 (Figure 2b). The results with higher concentrations of BQ-123 (3 μM, 10 μM) indicated the existence of a BQ-123-insensitive component of the vasoconstriction or response (the hump in the curve). BQ-123 inhibited the vasoconstriction when the ET-1 concentration was greater than 10 nM, while a significant contraction was still observed at ET-1 concentrations of less than 30 nM even with BQ-123 10 μM. The maximum amplitude of the vasoconstriction in the preparations treated with BQ-123 was augmented significantly ($P<0.01$) at 0.3 μM ET-1. The involvement of ET_B receptors in the BQ-123-insensitive component was studied with an ET_B receptor antagonist BQ-788 (Figure 3). BQ-788 1 μM alone did not modify the concentration-response curves of ET-1. However, application of both BQ-123 (10 μM) and BQ-788 (1 μM) together shifted the curve to the right significantly ($P<0.01$), and the BQ-123-insensitive component was abolished completely.

The role of ET_B receptors in ET-1-induced vasoconstriction in porcine coronary artery was studied further by using ET_B receptor agonists sarafotoxin S6c and ET-3. Sarafotoxin S6c caused vasoconstriction in the porcine coronary artery with a threshold concentration and an EC_{50} value of 30 pM and 1.2 ± 0.19 nM ($n=21$), respectively (Figure 4). The maximum amplitude of the contraction ($74\pm6.2\%$) was significantly ($P<0.01$) less than that obtained with ET-1, that is, only 39% of that with ET-1. Pretreatment with TAK-044 inhibited the

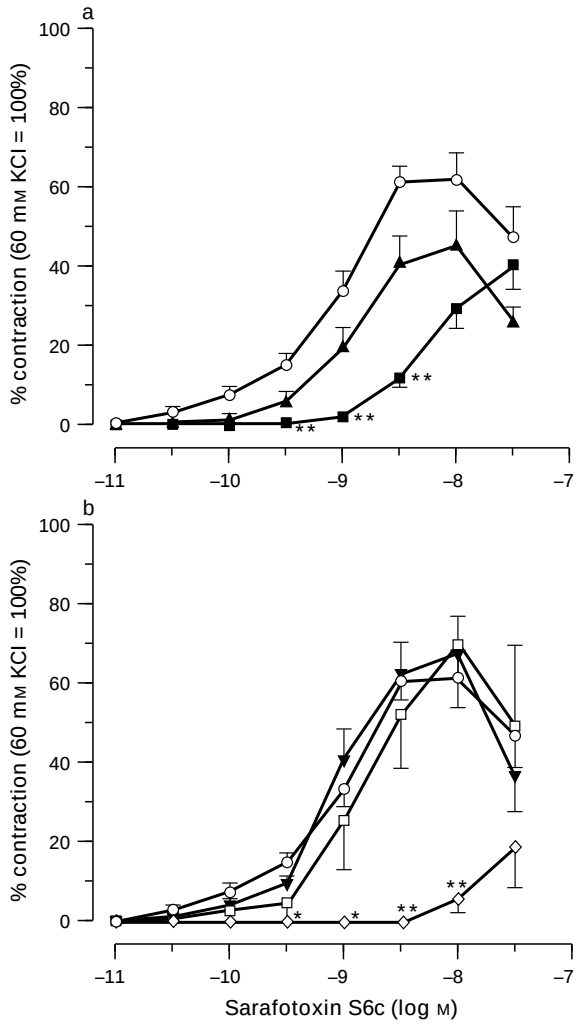

Figure 4 Concentration-response curves for sarafotoxin S6c in porcine coronary arteries in the presence and absence of TAK-044 (a) and selective endothelin receptor antagonists (b). Pretreatment with TAK-044 inhibited the sarafotoxin S6c-induced vasoconstriction in a concentration-dependent manner. BQ-123 did not inhibit the vasoconstriction, while BQ-788 completely inhibited it. Control (○), $n=21$; TAK-044: $n=6$ at 10 nM (▲) and 100 nM (■); BQ-123: $n=5$ at 3 μM (▼) and $n=6$ at 10 μM (□), and BQ-788; $n=4$ at 1 μM (◇). $^*P<0.05$; $^{**}P<0.01$.

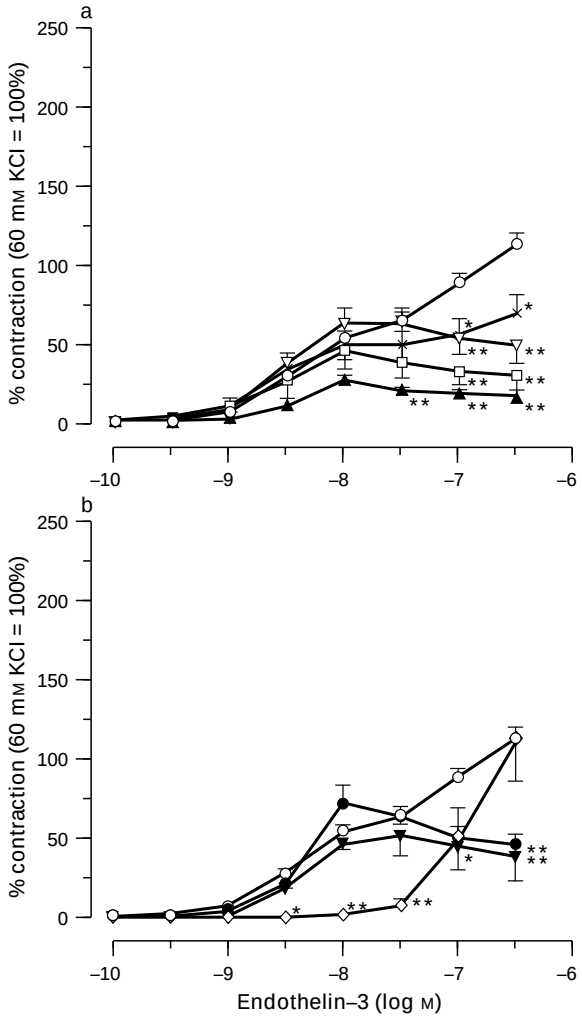

Figure 5 Concentration-response curves for endothelin-3 in porcine coronary arteries in the presence and absence of TAK-044 (a) and selective endothelin receptor antagonists (b). Responses in two distinct phases were obtained, and pretreatment with TAK-044 inhibited both phases in a concentration-dependent manner. BQ-123 did not inhibit the vasoconstriction induced by lower concentrations of ET-3, while it inhibited contraction in the second phase. BQ-788 showed just the opposite effect. Control (○), $n=27$; TAK-044: $n=7$ at 0.3 nM (×), $n=10$ at 1 nM (▽), $n=5$ at 3 nM (□) and $n=4$ at 10 nM (▲); BQ-123: $n=4$ at 1 μM (●) and 3 μM (▼); BQ-788: $n=4$ at 1 μM (◇). $^*P<0.05$; $^{**}P<0.01$.

sarafotoxin S6c-induced vasoconstriction in a concentration-dependent manner (Figure 4a). BQ-788 (1 μM) also inhibited the vasoconstriction significantly, while BQ-123 (3, 10 μM) did not (Figure 4b).

In contrast to sarafotoxin S6c, a concentration-response curve with two distinct phases was obtained to ET-3 (Figure 5): the first phase was between 0.3 nM and 10 nM, and the second phase was over 10 nM. A clear maximum response was not observed with ET-3, even at 0.3 μM, unlike with ET-1 and sarafotoxin S6c. This biphasic nature of the dose-response curve to ET-3 is most likely explained as follows. ET-3 as a non-selective agonist acts at both ET_A and ET_B receptors in contrast to sarafotoxin S6c which, as a selective agonist, acts at only ET_B receptors; therefore, low concentrations of ET-3 activated ET_B receptors, and higher concentrations activated ET_A receptors (Fukuroda *et al*., 1992). This hypothesis is supported by the following results obtained with various ET receptor antagonists.

TAK-044 at 0.3 and 1 nM showed little inhibitory effect on the ET-3-induced vasoconstriction in the first phase, while TAK-044 inhibited that in the second phase in a concentration-dependent manner (Figure 5a). Higher concentrations of TAK-044, 3 nM and 10 nM, inhibited both phases of the vasoconstriction. BQ-123 at 1 μM and 3 μM did not inhibit the vasoconstriction in the first phase, but that in the second phase was inhibited significantly, similar to the results with TAK-044 at 1 nM (Figure 5b). In contrast to BQ-123, BQ-788 at 1 μM inhibited the contraction in the first phase only.

Discussion

In the present study it was shown that ET-1 induced vasoconstriction in various canine arteries being most potent in coronary and basilar arteries. TAK-044 inhibited the ET-1-induced vasoconstriction and was most potent in the coronary artery. In addition, we demonstrated that the ET-1-induced vasoconstriction in the porcine coronary artery was mediated by activation of both the ET_A- and the ET_B-receptor subtypes and that TAK-044 inhibited the ET-1-induced vasoconstriction irrespective of the receptor subtype involved.

ET-1 caused vasoconstriction in various canine arteries, and the strongest contraction was obtained in coronary and basilar arteries. A plausible explanation for this heterogeneous vasoconstriction is differences in ET receptor density between the various arteries. The coronary and basilar arteries showed higher amplitude contractions than the other arteries, suggesting a larger number of ET receptors in these arteries. In addition, it is possible that the existence of ET_B receptors in addition to ET_A receptors is responsible for stronger vasoconstriction in the coronary arteries (Fukuroda *et al*., 1992), while this may not be the case in cerebral arteries which have been shown to possess only ET_A receptors (Willette *et al*., 1994). The higher sensitivity of these arteries was particularly noticeable in the canine arteries, and this possibly implies some physiological or pathophysiological significance of ET-1. Although, the underlying mechanisms for the high sensitivity are likely to be due to some mechanism other than the ratio of ET receptor subtypes.

Since TAK-044 showed regional-related differences in inhibitory effects on ET-1-induced vasoconstriction and the strongest effects were obtained in the coronary artery, any deterioration induced by coronary vasoconstriction is expected to be lessened by TAK-044. Indeed, we have shown that TAK-044 ameliorated damage induced by ischaemic conditions in the heart in various animal models, such as models of myocardial infarction, stunned myocardium and acute cardiac dysfunction (Watanabe & Fujino, 1996; unpublished observations). In contrast, although TAK-044 reduced damage due to acute renal failure after renal occlusion and reperfusion, it required high doses compared to those effective in the ischaemic heart models (Kusumoto *et al*., 1994). It is, thus, possible that the heterogeneous inhibition among the various arteries by TAK-044 is partially responsible for the differences between the two pathophysiological models. However, it should be remembered that the pathophysiological conditions mentioned above cannot be explained solely by the vasoconstriction of the arteries which supply these organs (heart, kidney). Moreover, relatively large conduit arteries were used in the present study which may not reflect responses in small arterioles or venula, because different responses to ET-1 have been found between large and small arteries (Miyauchi *et al*., 1996), between veins and arteries (Miller *et al*., 1989) and between regions of the coronary arteries (Godfraind, 1993).

Although the distribution and proportion of ET_A and ET_B receptors have been found to be heterogeneous in species and organ arteries (Masaki *et al*., 1994), there is little doubt that ET_A receptors play a role in the ET-1-induced vasoconstriction. It has been speculated that an additional receptor subtype other than ET_A receptors is involved in the vasoconstriction in porcine (Fukuroda *et al*., 1992; Harrison *et al*., 1992) and canine (Teerlink *et al*., 1994) coronary arteries. Although the most likely candidate for the other receptor subtype is ET_B receptors, because only two receptor subtypes have been determined in mammals (Masaki *et al*., 1994), pharmacological studies with various ET-related peptides in porcine coronary arteries have suggested the involvement of receptor subtypes other than ET_A and ET_B subtypes (Harrison *et al*., 1992). In the present study, we have shown that vasoconstriction in the porcine coronary artery can be caused by the activation of both ET_A and ET_B receptor subtypes (Figures 2 and 4) as has been found previously (Fukuroda *et al*., 1992). Since a re-classification of ET receptor subtypes has been proposed from a pharmacological point of view (Bax & Saxena, 1994), further studies are required to identify the type of receptor involved other than the classical subtypes in the coronary arteries.

It is important to discuss the types of receptors responsible for ET-1-induced vasoconstriction in the human coronary artery. Although the existence of ET_A receptors in human arteries has been clearly shown, the role of ET_B receptors is still a controversial issue. Vasoconstriction induced by ET_A receptors has been demonstrated to be a main factor in the human coronary artery by use of selective ET_A and ET_B receptor antagonists *in vitro* (Riezebos *et al*., 1994; Maguire & Davenport, 1995). However, the existence of both ET_A and ET_B receptors in smooth muscles of human coronary arteries has also been demonstrated (Davenport *et al*., 1995). In contrast, the involvement of receptor subtypes other than ET_A receptors in the ET-1-induced vasoconstriction in human coronary arteries has been demonstrated (Godfraind, 1993). Although it is difficult to explain these discrepancies, differences in subtype distribution related to the size and part of the coronary arteries may be responsible (Godfraind, 1993: Riezebos *et al*., 1994).

The role of ET_B receptor subtypes in human coronary arteries is controversial as discussed above. However, it has been clearly shown that ET_B receptors mediate vasoconstriction in other human arteries *in vitro* (Seo *et al*., 1994) as well as *in vivo* (Haynes *et al*., 1995). Moreover, a role for ET_B receptors in pathophysiological conditions has been deduced from binding studies. An increase in ET_B receptors following myocardial ischaemia (Sargent *et al*., 1994), upon vascular cell proliferation after injuries (Eguchi *et al*., 1994) or in acute renal failure (Nambi *et al*., 1993) has been obtained. Therefore, it should be beneficial to inhibit both ET_A and ET_B receptor-induced phenomena in pathophysiological conditons, as we have demonstrated previously with TAK-044 in various animal models as well as in the present study.

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