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Endothelin receptor subtype antagonist activity of S-0139 in various isolated rabbit and canine arteries

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Abstract

Vascular responses to endothelin peptides have been proposed to be mainly mediated via subtypes of the endothelin receptor, endothelin ET_{A1}, endothelin ET_{B1}, and endothelin ET_{B2}. The antagonist activity of 27-*O*-3-[2-(3-carboxy-acryloylamino)-5-hydroxy-phenyl]acryloyloxy myricerone, sodium salt (S-0139) at these endothelin receptor subtypes was evaluated using isolated rabbit femoral, pulmonary, and mesenteric arteries. S-0139 competitively antagonized the endothelin-1-induced contraction mediated by the endothelin ET_{A1} receptor in endothelium-denuded rabbit femoral arteries with a pA_2 value of 8.6 ± 0.1. Endothelin ET_{B2} receptor-mediated contraction induced by sarafotoxin S6c in endothelium-denuded rabbit pulmonary arteries was also inhibited by S-0139 with a pA_2 value of 5.6 ± 0.1. The pA_2 value of S-0139 for the endothelin ET_{B1} receptor, evaluated from the endothelin-3-induced relaxant response in endothelium-intact rabbit mesenteric arteries, was 6.2 ± 0.2. In isolated canine basilar, coronary, mesenteric and renal arteries, endothelin-1 caused concentration-dependent contractions with EC_{50} values of 0.49 ± 0.07, 0.61 ± 0.25, 0.92 ± 0.21 and 1.18 ± 0.24 nM, respectively. S-0139 antagonized the endothelin-1-induced contraction in these arteries with pA_2 values of 8.0 ± 0.1, 7.6 ± 0.2, 7.6 ± 0.2 and 7.6 ± 0.1, respectively. These results suggest that S-0139 is a potent and selective endothelin ET_{A1} receptor antagonist, and that the contractions induced by endothelin-1 in canine basilar, coronary, mesenteric and renal arteries are mediated mainly via the endothelin ET_{A1} receptor subtype. © 2000 Published by Elsevier Science B.V.

Keywords: S-0139; Endothelin receptor subtype; Antagonist activity; Blood vessel, rabbit; Blood vessel, canine

1. Introduction

Endothelin is a 21-residue peptide which was initially identified as a potent vasoconstrictor substance released from vascular endothelial cells (Yanagisawa et al., 1988). Endothelin consists of three isoforms, termed endothelin-1, endothelin-2, and endothelin-3 (Inoue et al., 1989). At least two distinct receptors for endothelins, termed endothelin ET_A and endothelin ET_B, have been cloned (Arai et al., 1990; Sakurai et al., 1990). The endothelin ET_A receptor displays higher affinity for endothelin-1 and endothelin-2 than for endothelin-3, whereas the endothelin ET_B receptor shows equal affinity for all isoforms. Based on pharmacological studies, however, the existence of further subtypes of endothelin receptors has been reported in the vasculature (Bax and Saxena, 1994). The endothelin ET_A receptor-mediated vasocontraction induced by endothelin-1 is known to be mostly antagonized by cyclo(-D-Trp-D-Asp-Pro-D-Val-Leu-) (BQ-123) in various arteries (Bax and Saxena, 1994), although a BQ-123-resistant contraction has been reported in some blood vessels, such as the human saphenous vein (White et al., 1994; Nishiyama et al., 1995a) and the rabbit saphenous vein (Sudjarwo et al., 1994; Nishiyama et al., 1995b). Therefore, the existence of two subtypes of endothelin ET_A receptor, termed endothelin ET_{A1} (sensitive to BQ-123) and endothelin ET_{A2} (resistant to BQ-123), has been proposed (Bax et al., 1994; Sudjarwo et al., 1994; Nishiyama et al., 1995a,b). However, the physiological role of the endothelin ET_{A2} receptor subtype is not known, since the vasocontractions mediated by the endothelin ET_{A2} receptor subtype are caused by higher concentrations of endothelin-1. Activation of the endothelin ET_B receptor located in the vascular endothelium and smooth muscle by endothelins mediates vasorelaxation through the release of endothelium-derived relax-

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ing substances, such as nitric oxide and prostaglandin I_2 (De Nucci et al., 1988; Iwasaki et al., 1999). Endothelins also produce vasocontraction mediated by the endothelin ET_B receptor in several vascular smooth muscles, such as in the rabbit pulmonary artery (Hay et al., 1996), rat renal artery (Gellai et al., 1996), rabbit saphenous vein (Sudjarwo et al., 1994) and human saphenous vein (Nishiyama et al., 1995a). Ac-(3,3-D-diphenylalanine-L-Leu-L-Asp-L-Ile-L-Ile-L-Trp trifluoroacetate) (PD142893), an endothelin ET_A/ET_B receptor antagonist, or cyclic(Gly^1–Asp^9)(Gly-Asn-Trp-His-Gly-Thr-Ala-Pro-Asp-Trp-Phe-Phe-Asn-Tyr-Tyr-Trp) (RES-701-1), a selective endothelin ET_B receptor antagonist, has been reported to inhibit the endothelin ET_B receptor-mediated vasorelaxation, but not the endothelin ET_B receptor-mediated vasocontraction (Douglas et al., 1994). These observations have suggested that the endothelin ET_B receptor also exists as two subtypes, endothelin ET_{B1} (sensitive to PD142893 and RES-701-1) and endothelin ET_{B2} (resistant to PD142893 and RES-701-1) (Douglas et al., 1994; Sudjarwo et al., 1994).

It has been reported that 27-*O*-3-[2-(3-carboxy-acryloylamino)-5-hydroxyphenyl]acryloyloxy myricerone, sodium salt (S-0139) is a potent non-peptide endothelin ET_A receptor antagonist (Mihara et al., 1998). In this study, we evaluated the antagonist activity of S-0139 for endothelin ET_{A1}, endothelin ET_{B1}, and endothelin ET_{B2} receptors using various isolated rabbit arteries. We also analyzed the endothelin receptor subtype mediating contraction induced by endothelin-1 in isolated canine basilar, coronary, mesenteric and renal arteries using S-0139.

2. Materials and methods

2.1. Measurement of isometric tension

Male Japan White rabbits bred at Kitayama Labes Laboratories (Minowa, Japan), weighing 2.1–4.0 kg, and male Beagle dogs bred at Shionogi Aburahi Laboratories (Shiga, Japan) or Nihon Nosan Kougyo (Yokohama, Japan), weighing 10.5–13.0 kg, were anesthetized by intravenous injection of sodium pentobarbital (50 mg/kg) and killed by bleeding from the common carotid artery. Femoral, pulmonary, and mesenteric arteries from rabbits and basilar, coronary, mesenteric and renal arteries from the dogs were isolated and cut into rings approximately 3 mm in length. The endothelium in rabbit femoral and pulmonary arterial rings was removed by gently rubbing the intimal surface with a thin wooden stick. The rings were vertically fixed between hooks in 10-ml organ baths containing Krebs–Henseleit solution (pH 7.4), which was maintained at 37 ± 0.3°C and aerated with a mixture of 95% O_2 and 5% CO_2. The hooks anchoring the upper end of the rings were connected to the lever of a force-displacement transducer (TB-611T, Nihon Kohden, Tokyo, Japan). The resting tension of the preparations was adjusted to the value optimal for inducing maximal contraction: rabbit femoral artery 2 g, rabbit pulmonary artery 2 g, rabbit mesenteric artery 2 g, canine basilar artery 1 g, canine coronary artery 3.5 g, canine mesenteric artery 3.5 g and canine renal artery 3 g. The constituents of the solution were as follows (mM): NaCl 118, KCl 4.7, KH_2PO_4 1.2, $MgSO_4$ 1.2, $CaCl_2$ 2.5, $NaHCO_3$ 25 and glucose 11. The pH of the solution was 7.35 to 7.42. Before the start of the experiment, each ring was allowed to equilibrate for 60 to 90 min in the bathing medium, during which time the solution was replaced every 10 to 15 min.

Isometric contractions and relaxations were recorded on a polygraph recorder (WT-685G, Nihon Kohden). The contractile response to 50-mM K^+ was first obtained, and then the preparations were repeatedly washed with fresh media and equilibrated. The concentration–response curves for endothelin-1, endothelin-3, and sarafotoxin S6c were obtained by adding the agonists directly to the bathing media in cumulative concentrations. To test the relaxant response, rabbit mesenteric arteries were partially contracted with phenylephrine; the contraction was between 21% and 48% of that induced by 50-mM K^+. After the end of each experiment on the relaxant effect of agonist, 100-μM papaverine was added to obtain the maximal relaxation. The K^+ (50 mM)-induced contraction and the papaverine-induced relaxation were taken as 100% for contractile and relaxant responses to the test agonists, respectively. Preparations were treated for 30 min with vehicle (0.1% dimethyl sulfoxide) or endothelin receptor antagonists before the concentration–response curves for agonists were obtained. The responses of preparations treated with vehicle were compared with those of preparations treated with endothelin receptor antagonist. Removal of the endothelium of the preparation was confirmed by the disappearance of the relaxation induced by acetylcholine (0.1 μM) in rings precontracted with phenylephrine (1 μM). The concentration of an agonist causing 50% of the maximal response (EC_{50}) was calculated from each concentration–response curve using Probit analysis (Finney, 1953), after which the pA_2 value was calculated by Schild plot analysis (Arunlakshana and Schild, 1959).

2.2. Drugs and reagents

Drugs used were S-0139 and 4-*tert*-butyl-*N*-[6-(2-hydroxy-ethoxy)-5-(2-methoxy-phenoxy)-2,2′-bipyrimidin-4-yl]benzenesulfonamide (Bosentan) (Shionogi Research Laboratories, Osaka, Japan), BQ-123, and *N*-*cis*-2,6-dimethylpiperidinocarbonyl-L-γ-methylleucyl-D-1-methoxycarbonyltryptophanyl-D-norleucine (BQ-788) (Neosystem, Strabourg, France), endothelin-1, endothelin-3, sarafotoxin S6c, *N*-succinyl[Glu^9,$Ala^{11,15}$]endothelin-1(8–21) (IRL1620) and [Cys^{11}–Cys^{15}]endothelin-1(11–21) (IRL1038) (Peptide Institute, Osaka, Japan), phenylephrine hydrochloride and bovine serum albumin (Sigma, St. Louis,

MO), acetylcholine chloride (Daiichi Pharmacy, Tokyo, Japan), papaverine hydrochloride (Dainippon Pharmacy, Osaka, Japan) and sodium pentobarbital (Abbott, North Chicago, IL). Endothelin-1, endothelin-3, and sarafotoxin S6c were dissolved in physiological saline containing 0.1% bovine serum albumin.

2.3. Statistical analysis

The results shown in the text and figures are expressed as mean values ± S.E. Statistical analyses were done using Student's *t*-test and Tukey's method after one-way analysis of variance.

3. Results

3.1. Effect of S-0139 on the endothelin-1-induced contraction in endothelium-denuded rabbit femoral arteries

Endothelin-1 (0.01 to 10 nM) caused concentration-dependent contractions with an EC_{50} value of 0.27 ± 0.03 nM ($n = 5$) in isolated endothelium-denuded rabbit femoral arteries (Fig. 1). The concentration–contractile response curve of endothelin-1 in the arteries was shifted to the right by treatment with S-0139 (10 to 300 nM) in a concentration-dependent manner. Schild plot analysis of the inhibitory effects of S-0139 yielded a pA_2 value of 8.6 ± 0.1 ($n = 5$). The slope of the regression line was nearly equal to unity (0.97 ± 0.06, $n = 5$). The curves of endothelin-1 were shifted to the right by treatment with BQ-123 and Bosentan, another endothelin receptor antagonist, with pA_2 values of 7.7 ± 0.2 ($n = 6$) and 7.4 ± 0.03 ($n = 5$), respectively. Therefore, the antagonist activity of S-0139 was 11 and 15 times more potent than that of BQ-123 and Bosentan, respectively. In the arteries, en-

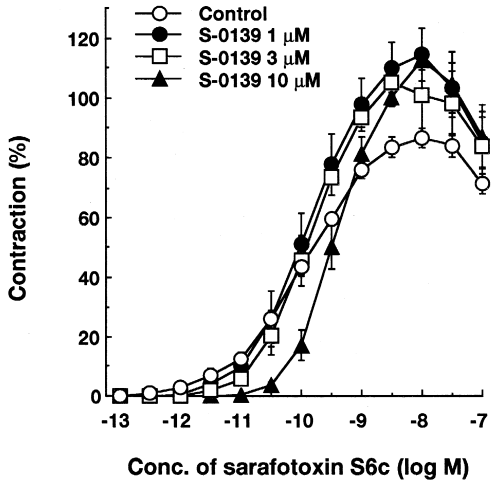

Fig. 2. Effect of S-0139 on the concentration–response curves for sarafotoxin S6c-induced contraction in isolated endothelium-denuded rabbit pulmonary arteries. S-0139 at a concentration of 1, 3, or 10 μM was added 30 min before the application of sarafotoxin S6c. Contractions caused by 50-mM K^+ were taken as 100% contraction; mean absolute values in control arteries and those treated with S-0139 at 1, 3, and 10 μM were 5.0 ± 0.4 g ($n = 5$), 5.2 ± 0.9 g ($n = 5$), 5.2 ± 0.5 g ($n = 5$) and 4.9 ± 0.4 g ($n = 5$), respectively. Values are means ± S.E.

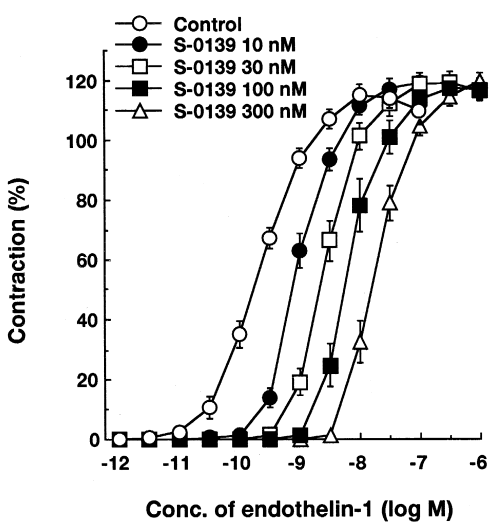

Fig. 1. Effect of S-0139 on the concentration–response curves for endothelin-1-induced contraction in isolated endothelium-denuded rabbit femoral arteries. S-0139 at a concentration of 10, 30, 100 or 300 nM was added 30 min before the application of endothelin-1. Contractions induced by 50-mM K^+ were taken as 100% contraction; mean absolute values in control arteries and those treated with S-0139 at 10, 30, 100 and 300 nM were 6.1 ± 0.2 g ($n = 5$), 5.8 ± 0.3 g ($n = 5$), 6.0 ± 0.4 g ($n = 5$), 5.6 ± 0.3 g ($n = 5$) and 5.6 ± 0.3 g ($n = 5$), respectively. Values are means ± S.E.

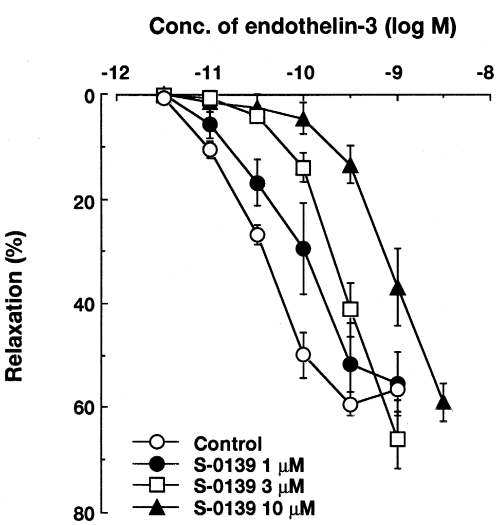

Fig. 3. Effect of S-0139 on the concentration–response curves for endothelin-3-induced relaxation in isolated endothelium-intact rabbit mesenteric arteries precontracted with phenylephrine. S-0139 at a concentration of 1, 3, or 10 μM was added 30 min before the application of endothelin-3. Relaxations induced by 100-μM papaverine were taken as 100% relaxation; mean absolute values in control arteries and those treated with S-0139 at 1, 3, and 10 μM were 1.0 ± 0.1 g ($n = 5$), 0.7 ± 0.04 g ($n = 5$), 1.0 ± 0.1 g ($n = 5$) and 0.9 ± 0.1 g ($n = 5$), respectively. Values are means ± S.E.

dothelin-3 at a concentration above 30 nM also caused weak contractions; the contraction induced by 1 μM endothelin-3 was 31.3 ± 6.1% ($n = 7$). However, sarafotoxin S6c, a selective endothelin ET_B receptor agonist, up to the concentration of 10 μM did not cause contractions in the arteries ($n = 4$).

3.2. Effect of S-0139 on the sarafotoxin S6c-induced contraction in endothelium-denuded rabbit pulmonary arteries

In isolated endothelium-denuded rabbit pulmonary arteries, sarafotoxin S6c (0.001 to 10 nM) induced concentration-dependent contractions with an EC_{50} value of 0.10 ± 0.02 nM ($n = 5$) and a maximal contraction of 86.6 ± 3.3% ($n = 5$) (Fig. 2). Endothelin-1 (0.001 to 10 nM) and IRL1620 (0.1 to 10 nM) also induced contractions in a concentration-dependent manner with an EC_{50} value and maximal contraction of 0.16 ± 0.03 nM ($n = 5$), 90.0 ± 3.6% ($n = 5$) and 0.62 ± 0.21 nM ($n = 3$), 85.0 ± 9.6% ($n = 3$), respectively. As shown in Fig. 2, S-0139 (1 to 10 μM) antagonized the concentration–contractile response to a low concentration of sarafotoxin S6c (up to 0.1 or 0.3 nM) in the arteries, although treatment with S-0139 augmented the contraction induced by a high concentration of sarafotoxin S6c (0.3 to 10 nM). The pA_2 value and slope calculated by Schild plot analysis of the inhibitory effects of S-0139 were 5.6 ± 0.1 ($n = 5$) and 1.00 ± 0.20 ($n = 5$), respectively. The contraction induced by sarafotoxin S6c was also antagonized by treatment with BQ-788 (100 to 1000 nM), an endothelin ET_{B1}/ET_{B2} receptor antagonist (Karaki et al., 1994), and Bosentan with pA_2 values of 7.8 ± 0.1 ($n = 6$) and 6.1 ± 0.1 ($n = 5$), respectively. The curve for sarafotoxin S6c was not affected by treatment with IRL1038 (10 μM), a selective endothelin ET_{B1} receptor antagonist (Sudjarwo et al., 1994) ($n = 5$) or BQ-123 (1 μM) ($n = 3$). In the arteries, endothelin-3 showed two distinct phases of the concentration–contractile response: the first phase was observed between 0.003 and 3 nM and

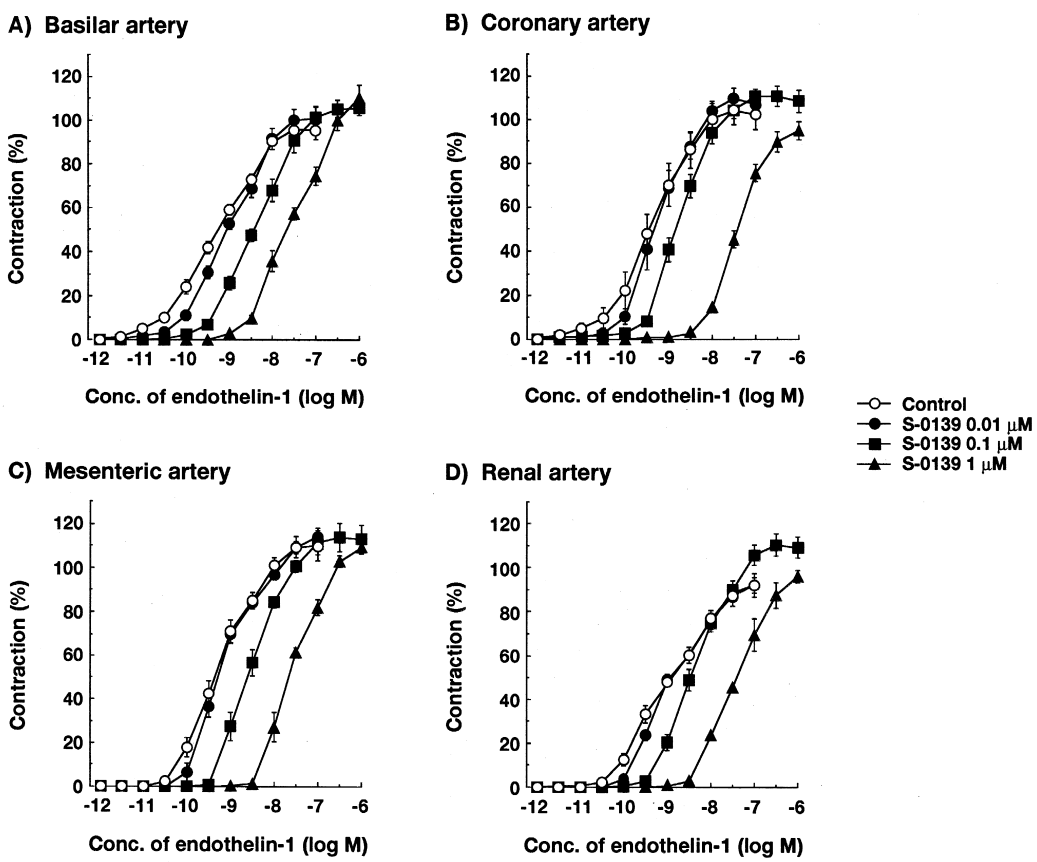

Fig. 4. Effect of S-0139 on the concentration–response curves for endothelin-1-induced contraction in isolated endothelium-intact canine basilar arteries (A), coronary arteries (B), mesenteric arteries (C) and renal arteries (D). S-0139 at a concentration of 0.01, 0.1, or 1 μM was added 30 min before the application of endothelin-1. Contractions induced by 50-mM K^+ were taken as 100% contraction; mean absolute values in control basilar arteries and those treated with S-0139 at 0.01, 0.1, and 1 μM were 2.5 ± 0.6 g ($n = 6$), 2.6 ± 0.7 g ($n = 6$), 2.1 ± 0.3 g ($n = 6$) and 2.1 ± 0.3 g ($n = 3$), respectively; mean absolute values in control coronary arteries and those treated with S-0139 at 0.01, 0.1, and 1 μM were 4.3 ± 0.2 g ($n = 6$), 4.3 ± 0.1 g ($n = 6$), 3.9 ± 0.3 g ($n = 6$) and 3.6 ± 0.5 g ($n = 3$), respectively; mean absolute values in control mesenteric arteries and those treated with S-0139 at 0.01, 0.1, and 1 μM were 5.3 ± 0.2 g ($n = 6$), 5.5 ± 0.3 g ($n = 6$), 4.9 ± 0.6 g ($n = 6$) and 5.0 ± 0.3 g ($n = 3$), respectively; and mean absolute values in control renal arteries and those treated with S-0139 at 0.01, 0.1, and 1 μM were 2.5 ± 0.3 g ($n = 6$), 2.8 ± 0.5 g ($n = 6$), 2.8 ± 0.3 g ($n = 6$) and 3.3 ± 0.6 g ($n = 3$), respectively. Values are means ± S.E.

the contraction at 3 nM was 59.9 ± 4.1% ($n = 4$), and the second phase was observed at above 3 nM and the contraction at 1 μM was 89.1 ± 4.2% ($n = 4$).

3.3. Effect of S-0139 on the endothelin-3-induced relaxation in endothelium-intact rabbit mesenteric arteries

As shown in Fig. 3, endothelin-3 (0. 1 to 3 nM) caused concentration-dependent relaxations with an EC_{50} value of 0.20 ± 0.04 nM ($n = 5$) in isolated endothelium-intact rabbit mesenteric arteries precontracted with phenylephrine. Treatment with S-0139 (1 to 10 μM) shifted the concentration–relaxant response curve of endothelin-3 to the right in a concentration-dependent manner. Schild plot analysis of the inhibitory effects of S-0139 yielded a pA_2 value of 6.2 ± 0.2 ($n = 5$) and a slope of 1.17 ± 0.19 ($n = 5$). Bosentan also inhibited the endothelin-3-induced relaxation with a pA_2 value of 6.7 ± 0.2 ($n = 5$).

S-0139 (10 μM) did not affect the concentration–contractile response to norepinephrine and 5-hydroxytryptamine in endothelium-denuded rabbit femoral arteries or the concentration–relaxant response to acetylcholine and prostaglandin I_2 in endothelium-intact rabbit mesenteric arteries ($n = 3$, respectively). In addition, application of S-0139 up to the concentration of 10 μM did not cause agonist activity in any preparation used in this study ($n = 5$).

3.4. Effect of S-0139 on the endothelin-1-induced contraction in canine basilar, coronary, mesenteric and renal arteries

Endothelin-1-induced contractions were observed at concentrations ranging from 0.003 to 100 nM in canine basilar and coronary arteries (Fig. 4A and B). Contractions induced by 0.01 and 0.03 nM endothelin-1 in basilar arteries were 5.0 ± 1.4% ($n = 6$) and 10.1 ± 1.9% ($n = 6$), and those in coronary arteries were 5.6 ± 1.9% ($n = 6$) and 9.9 ± 3.8% ($n = 6$), respectively. Although the maximal contractions induced by 100 nM in mesenteric and renal arteries were almost the same as in basilar or coronary arteries (Table 1), the contractions induced by endothelin-1 were observed at concentrations above 0.03 nM (Fig. 4C and D). Contractions induced by 0.03 nM endothelin-1 in mesenteric arteries were 2.2 ± 0.7% ($n = 6$) and those in renal arteries were 2.2 ± 0.8% ($n = 6$). The EC_{50} values and maximal responses for the concentration–contractile responses to endothelin-1 in these arteries are summarized in Table 1. The EC_{50} value in basilar arteries tended to be lower than that in mesenteric and renal arteries. Treatment with S-0139 (0.01 to 1 μM) competitively antagonized the contraction induced by endothelin-1 in either arteries. The pA_2 values obtained by Schild plot analysis of the inhibitory effects of S-0139 in basilar, coronary, mesenteric and renal arteries are summarized in Table 2. The slope of the regression line was nearly unity for either arteries (data not shown).

Table 1
EC_{50} values and maximal responses of endothelin-1-induced contractions in canine basilar, coronary, mesenteric and renal arteries

Preparation	n	Endothelin-1-induced contraction	
		EC_{50} (nM)	Maximal response (%)
Basilar artery	6	0.49 ± 0.07	95.6 ± 4.3
Coronary artery	6	0.61 ± 0.25	98.9 ± 7.5
Mesenteric artery	6	0.92 ± 0.21	110.1 ± 7.0
Renal artery	6	1.18 ± 0.24	92.0 ± 3.6

n = Number of experiments. Values are means ± S.E.

Table 2
Antagonist activities of S-0139 for endothelin-1-induced contractions in canine basilar, coronary, mesenteric and renal arteries

Preparation	n	pA_2 value
Basilar artery	6	8.0 ± 0.1
Coronary artery	6	7.6 ± 0.2
Mesenteric artery	6	7.6 ± 0.2
Renal artery	6	7.6 ± 0.1

n = Number of experiments. Values are means ± S.E.

4. Discussion

Functional studies have suggested that the endothelin ET_A and endothelin ET_B receptors located in the vasculature can be classified into four subtypes, endothelin ET_{A1} (sensitive to BQ-123), endothelin ET_{A2} (resistant to BQ-123), endothelin ET_{B1} (sensitive to PD142893, IRL1038 or RES-701-1) and endothelin ET_{B2} (resistant to PD142893, IRL1038 or RES-701-1) (Bax and Saxena, 1994; Sudjarwo et al., 1994; Nishiyama et al., 1995a,b). Receptor subtypes of endothelin ET_{A1}, endothelin ET_{A2}, and endothelin ET_{B2} have been reported to exist in vascular smooth muscle and to mediate endothelin-induced vasocontractions (Sudjarwo et al., 1994; Nishiyama et al., 1995a,b). However, since the vasocontractions mediated by the endothelin ET_{A2} receptor subtype are caused by higher concentrations of endothelin-1 (Sudjarwo et al., 1994; Nishiyama et al., 1995b), the physiological role of the endothelin ET_{A2} receptor subtype is not known. The endothelin ET_{B1} receptor subtype has been observed to exist in both vascular endothelial cells and vascular smooth muscle and to mediate vasorelaxation (Douglas et al., 1994; Iwasaki et al., 1999).

In rabbit endothelium-denuded femoral arteries, endothelin-1 (0.01 to 10 nM) caused concentration-dependent contraction with an EC_{50} value of 0.26 nM. Endothelin-3 at concentrations up to 30 nM did not cause any contraction in the arteries. Furthermore, sarafotoxin S6c, a selective endothelin ET_B receptor agonist, did not cause con-

traction in the arteries. Taken together with the results of a previous report (Doherty et al., 1993), these results suggest that endothelin-1 causes contraction via the endothelin ET_A receptor in rabbit femoral artery smooth muscles. In the present study, the endothelin-1-induced contraction in the arteries was competitively antagonized by BQ-123, a selective endothelin ET_A receptor antagonist (Sudjarwo et al., 1994), with a pA_2 value of 7.7. It has been reported that the pA_2 values of BQ-123 for the endothelin-1-induced contractions in the blood vessels expressing only endothelin ET_{A1} receptors, such as rat thoracic aorta and rabbit carotid artery, are in a range of 6.8–7.5 (Bax and Saxena, 1994), whereas those in the blood vessels expressing endothelin ET_{A2} receptors, such as human and rabbit saphenous veins, are in a range of 5.7–6.3 (Nishiyama et al., 1995a,b). The pA_2 value of BQ-123 for the endothelin-1-induced vasocontraction in endothelium-denuded rabbit femoral arteries is similar to that in rat thoracic aorta and rabbit carotid artery. Therefore, it appears that the endothelin-1-induced contraction is mediated via the endothelin ET_{A1} receptor subtype on the smooth muscle cells of the rabbit femoral artery. In contrast, endothelin-1, endothelin-3, sarafotoxin S6c and IRL1620 caused concentration-dependent contractions in endothelium-denuded rabbit pulmonary arteries. In the present studies, the sarafotoxin S6c-induced contraction in the arteries was antagonized by BQ-788, an endothelin ET_{B1}/ET_{B2} receptor antagonist (Karaki et al., 1994), but not by IRL1038, a selective endothelin ET_{B1} receptor antagonist (Sudjarwo et al., 1994), or BQ-123. As reported previously (Hay et al., 1996), our results also confirmed that the sarafotoxin S6c-induced contractions in endothelium-denuded rabbit pulmonary arteries are mediated via the endothelin ET_{B2} receptor subtype. We have already reported that endothelin ET_B receptor agonists, such as endothelin-3, sarafotoxin S6c or IRL1620, induce relaxation mediated via the endothelin ET_{B1} receptor subtype in isolated rabbit mesenteric arteries, whereas endothelin-1 does not cause contraction (Iwasaki et al., 1999). Based on these pharmacological analyses of endothelin receptor subtypes, we evaluated the antagonist activity of S-0139 for endothelin ET_{A1}, endothelin ET_{B1}, and endothelin ET_{B2} receptors using rabbit femoral, mesenteric, and pulmonary arteries, respectively.

Treatment with S-0139 antagonized the endothelin-1-induced contraction mediated by the endothelin ET_{A1} receptor with a pA_2 value of 8.6 in endothelium-denuded rabbit femoral arteries. Although S-0139 also inhibited the sarafotoxin S6c-induced contraction mediated by the endothelin ET_{B2} receptor in endothelium-denuded rabbit pulmonary arteries, the pA_2 value was almost 1000-fold greater than that for the endothelin ET_{A1} receptor. In addition, S-0139 antagonized the endothelin-3-induced relaxation mediated by the endothelin ET_{B1} receptor in endothelium-intact rabbit mesenteric arteries with a pA_2 value of 6.2. The pA_2 values of Bosentan for endothelin ET_{A1}, endothelin ET_{B2}, and endothelin ET_{B1} receptors were 7.4, 6.1, and 6.7, respectively. These results strongly suggest that S-0139 is a potent and selective non-peptide endothelin ET_{A1} receptor antagonist, whose potency is 11- and 15-fold greater than that of BQ-123 and Bosentan, respectively. In a binding experiment, Mihara et al. (1998) reported that the binding affinity of S-0139 for the endothelin ET_A receptor was 100-fold greater than that for the endothelin ET_B receptor.

The contraction was augmented by a high concentration of sarafotoxin S6c in the presence of S-0139 (Fig. 2). A similar effect has also been reported in studies using SB209670 ((±)-(1*S*,2*R*,3*S*)-3-(2-carboxymethoxy-4-methoxyphenyl)-1-(3,4-methylenedioxy-phenyl)-5-(prop-1-yloxy)indane-2-carboxylic acid) and L-754142 ((-)-*N*-(4-*iso*-propylbenzenesulfonyl)-α-(4-carboxy-2-*n*-propylphenoxy)-3,4-methylenedioxyphenylacetamide) against anotherendothelin ET_B-selective receptor agonist, IRL1620 (Ohlstein et al., 1994; Williams et al., 1995). However, the underlying mechanism of this effect has not yet been elucidated. Several investigators have reported an augmented contractile response induced by angiotensin II in the presence of an angiotensin II receptor antagonist, losartan (2-*n*-butyl-4-chloro-5-(hydroxymethyl)-1-[[2′-(1*H*-tetrazol-5-yl)biphenyl-4-yl]methyl]imidazole), in rabbit and canine aorta (Rhaleb et al., 1991; Burns et al., 1994). Robertson et al. (1994) have suggested that this effect could arise from two interconvertible states (active and inactive) of the angiotensin II receptor. The angiotensin II receptor antagonist appears to have a greater affinity for the active state of the receptor and the agonist causes the augmentation. Therefore, the endothelin ET_B receptor mediating vasocontraction may also have two interconvertible states of the receptor.

In canine basilar, coronary, mesenteric and renal arteries, endothelin-1 caused concentration-dependent contractions. Endothelin-1-induced contraction was observed at concentrations above 0.003 nM in basilar and coronary arteries, but only at concentrations above 0.03 nM in mesenteric and renal arteries. Similar sensitive responses to endothelin-1 in canine basilar and coronary arteries have been reported (Tanoi et al., 1992). The EC_{50} value of endothelin-1 in basilar arteries tended to be smaller than that in mesenteric arteries. Sarafotoxin S6c up to 10 μM caused no contraction in either type of artery used in this experiment. As shown in Fig. 4 and Table 2, treatment with S-0139 antagonized the endothelin-1-induced contractions in either types of arteries with similar antagonist activity. Since S-0139 has potent and selective endothelin ET_{A1} receptor antagonist activity, these results suggest that endothelin-1-induced contractions in canine basilar, coronary, mesenteric and renal arteries are mainly mediated via the endothelin ET_{A1} receptor subtype. Teerlink et al. (1994) reported that endothelin ET_B receptor activation induces vasocontraction in the canine coronary circulation. Furthermore, Rigel and Lappe (1993) reported that the contraction elicited by endothelin-1 in canine conduit coronary artery

is mediated predominantly by endothelin ET_A receptors and that the contraction in resistance coronary arteries may at least in part be mediated by endothelin ET_B receptors. This evidence from in vivo experiments suggests that the endothelin-1-induced contraction in canine coronary arteries is at least in part mediated by the endothelin ET_B receptor. In our experiments, however, endothelin ET_B receptor-mediated contraction was not observed in isolated large coronary arteries. Contractions caused by endothelin-1 in proximal portions of the coronary artery may be associated with predominant activation of endothelin ET_A receptor subtypes over endothelin ET_B receptors, whereas those in distal portions of the coronary artery are due to endothelin ET_B receptor activation. As shown in Fig. 4A, treatment with 0.01 μM S-0139 strongly suppressed the contractions induced by a low concentration of endothelin-1 (0.01 to 0.3 nM) in canine basilar arteries. Therefore, it is reasonable to speculate that the antagonistic effect of S-0139 is more sensitive in basilar arteries than in peripheral arteries, such as mesenteric arteries. Suzuki et al. (1992) have suggested that endothelin-1 plays an important role in the pathogenesis of vasospastic events such as cerebral vasospasm after subarachnoid hemorrhage. In fact, it has been reported that the endothelin ET_A receptor antagonist can attenuate cerebral vasospasm in experimental subarachnoid hemorrhage in dogs (Hirose et al., 1995; Sato et al., 1998; Kita et al., 1998). Therefore, S-0139 may be useful as a therapeutic agent for vasospastic diseases.

In the present study, we demonstrated that S-0139 is a potent and selective endothelin ET_{A1} receptor antagonist, and that endothelin-1-induced vasocontractions in canine basilar, coronary, mesenteric and renal arteries are caused mainly by the endothelin ET_{A1} receptor subtype.

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