Two Different Endothelin B Receptor Subtypes Mediate Contraction of the Rabbit Saphenous Vein

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ABSTRACT—To study endothelin receptor subtypes that mediate venous smooth muscle contraction, effects of some endothelin receptor agonists and antagonists on the rabbit lateral saphenous vein were examined and compared with those on the saphenous artery. In the artery, endothelin (ET)-1 elicited concentration-dependent contractions, while selective ET_B-receptor agonists, IRL1620 (Suc-[Glu^9,Ala^{11,15}]ET-1(8–21)) and sarafotoxin 6c (S6c) had almost no effect. The ET-1-induced responses shifted in parallel to the right by BQ-123 (cyclo (-D-Trp-D-Asp-Pro-D-Val-Leu-)), an ET_A-receptor antagonist, or PD142893 (Ac-D-Dip-Leu-Asp-Ile-Ile-Trp), an ET_A/ET_B-receptor antagonist, indicating the involvement of the ET_A receptor in this response. In the saphenous vein, not only ET-1 and ET-3, but also ET_B-receptor agonists, IRL1620, S6c and [Glu^9]sarafotoxin 6b ([Glu^9]S6b), produced concentration-dependent, BQ-123-insensitive contractions. PD142893 did not affect the ET-1-induced contraction, but it shifted greatly the IRL1620-induced concentration-response curve in parallel to the right. The major components of ET-3-, S6c- and [Glu^9]S6b-induced contractions were resistant to PD142893. These results indicate that two different vasoconstrictive ET_B-receptor subtypes, ET_{B1} (sensitive to IRL1620 and PD142893) and ET_{B2} (insensitive to IRL1620 and PD142893), are located on the smooth muscle of the saphenous vein.

Keywords: Endothelin, Sarafotoxin, Saphenous artery and vein (rabbit), Endothelin receptor, Endothelin receptor antagonist

Since the original discovery of endothelin (ET)-1 in the culture media of porcine aortic endothelial cells (1), its effects on the cardiovascular system have been intensely studied, because this natural peptide of vascular origin has highly potent vasoconstrictor activity. Three endogenous endothelin isopeptides, ET-1, ET-2 and ET-3 (2), and at least two distinct endothelin receptors, ET_A and ET_B (3), are currently known. The ET_A receptor has higher affinities for ET-1 and ET-2 than for ET-3 (4), and the ET_B receptor is non-selective towards all three endothelin isopeptides (5). Until recently, it was generally assumed that in vascular tissues, the vasoconstrictor effects of endothelins are mediated by ET_A receptors on the smooth muscle cells (6), and that ET_B receptors on the endothelial cells mediate the release of prostacyclin and endothelium-derived relaxing factor (EDRF) induced by endothelins (7, 8).

However, considerable accumulated evidence has indicated that a non-ET_A or ET_B-like receptor on vascular smooth muscle is also involved in endothelin-induced vasoconstriction in some blood vessels, especially in veins or some particular vascular beds, e.g., the rabbit saphenous vein, the rabbit jugular vein, the rat renal vascular bed and the porcine pulmonary vein (9–14). Most of these suggestions came from studies with selective ET_A-receptor antagonists, such as BQ-123 (cyclo (-D-Trp-D-Asp-Pro-D-Val-Leu-)), in which these antagonists did not fully antagonize the vascular smooth muscle contractions induced by endothelins. Similar proposals have also been made based on experiments using selective ET_B-receptor agonists such as IRL1620 (Suc-[Glu^9,Ala^{11,15}]ET-1(8–21)) (15) and sarafotoxin 6c (S6c) (16, 17).

For further definitive pharmacological confirmation of the receptor subtypes on the vascular smooth muscle cells, effects of ET_B-receptor antagonists should be examined. IRL1038 is a selective ET_B-receptor antagonist

and is known to be effective in antagonizing the ET_B receptor-mediated vasodilation (14, 18). A study using this antagonist in swine pulmonary blood vessels, however, revealed that the contractile responses induced by some ET_B-receptor agonists cannot be inhibited by IRL1038. This observation suggests that the IRL1038-insensitive ET_B receptor that mediates contraction is different from the IRL1038-sensitive ET_B receptor that mediates endothelium-dependent relaxation (14).

Coexistence of both ET_A and ET_B receptors on the same vascular smooth muscle may complicate pharmacological analysis of receptor subtypes. If only typical ET_A and typical ET_B receptors are colocalized, a nonselective ET_A/ET_B-receptor antagonist is expected to be effective in antagonizing endothelin-induced contraction of such smooth muscles. However, Warner and colleagues, who investigated effects of an ET_A/ET_B-receptor antagonist, PD142893 (Ac-D-Dip-Leu-Asp-Ile-Ile-Trp) (19), on the rabbit pulmonary artery, rat stomach strip and the rat perfused mesentery again revealed receptor heterogeneity among the ET_B receptors (20). Therefore, in the present study, we investigated the effects of PD142893 on the contractions induced by some endothelin receptor agonists in the isolated rabbit lateral saphenous vein and compared them with those in the saphenous artery, in order to characterize the endothelin receptor subtypes involved in endothelin-induced contractions of venous smooth muscle.

## MATERIALS AND METHODS

Tissue preparation

The lateral saphenous veins or the saphenous arteries were removed from male Japan White rabbits (2.4–3.0 kg body weight; Funabashi Farm, Funabashi) anesthetized with pentobarbital sodium (50 mg/kg, i.v.). After careful dissection to remove the connective tissue from the isolated blood vessels in a cold modified Krebs-Ringer bicarbonate solution, they were divided into rings of 2–3 mm in length. The vascular endothelium was removed by gentle mechanical rubbing of the internal surface. Each ring preparation was then mounted horizontally between two stainless steel hooks in an organ chamber filled with 5 ml of the modified Krebs-Ringer bicarbonate solution. One hook was connected to a micrometer for controlling the tissue length and the other, to a force transducer (TB-612T; Nihon Kohden, Tokyo) for isometric force recording. The modified Krebs-Ringer bicarbonate solution was aerated constantly with a 95% O_2 and 5% CO_2 gas mixture and maintained at 37°C. The endothelium removal of each ring was verified by the absence of a relaxant response to acetylcholine (1 μM) in the rings precontracted with phenylephrine (1–3 μM).

Experimental procedures

During an initial equilibration period of 60 min, rings of the saphenous arteries and the lateral saphenous veins were stretched by resting loads of 0.8 g and 0.2 g, respectively. Then the contractility of each preparation was examined by increasing the KCl concentration in the bathing medium (final concentration of 60 mM for the saphenous artery and 100 mM for the saphenous vein). Such K^+ deporalization was repeated at intervals of 30–40 min until the contractile response attained a steady state (usually 3–4 times). Cumulative concentration-response curves for endothelin or sarafotoxin peptides were then constructed by increasing the concentration in the organ chamber in half-log increments. Contractile responses induced by these agonists were expressed as percents of the above-mentioned maximal response to KCl. When the effects of BQ-123 and PD142893 were examined, each compound was added to the organ chamber 15–20 min before addition of the first dose of the agonists, and appropriate parallel control experiments were carried out with the vehicle alone. When ethanol, methanol or DMSO was used as a solvent, their final concentrations did not exceed 0.3%, 1.0% or 0.5%, respectively; and they showed no significant influence on the contractile responses in the present experiments.

Drugs and solutions

The composition of the modified Krebs-Ringer bicarbonate solution used in the present study was as follows: 118.4 mM NaCl, 4.7 mM KCl, 1.2 mM $MgSO_4$, 1.9 mM $CaCl_2$, 1.2 mM KH_2PO_4, 10.1 mM glucose and 25.0 mM $NaHCO_3$. ET-1, ET-2, ET-3 and S6c were purchased from Peptide Institute, Inc. (Osaka). The selective ET_B-receptor agonist [Glu^9]sarafotoxin 6b ([Glu^9]S6b) was synthesized as reported previously (21). These peptides were dissolved in phosphate-buffered saline (pH 7.2) containing 0.05% bovine serum albumin. IRL1620 was synthesized at International Research Laboratories, Ciba-Geigy Japan (Takarazuka), as reported previously (15), and dissolved in 0.01 N NaOH. BQ-123 was purchased from Peninsula Laboratories, Inc. (Belmont, CA, USA) and dissolved in absolute ethanol. PD142893 was synthesized at Research Laboratories, Nippon Chemiphar Co., Ltd. (Misato) and dissolved in absolute methanol. The other drugs used were: phenylephrine hydrochloride, acetylcholine chloride, pyrilamine maleate, indomethacin, nordihydroguaiaretic acid (Sigma, St. Louis, MO, USA), atropine sulfate (Nacalai Tesque, Kyoto), phentolamine mesylate (Ciba-Geigy Japan), guanethidine sulfate (Tokyo Kasei, Tokyo) and methysergide hydrogenmaleinate (Sandoz, Basel, Switzerland). Indomethacin was dissolved in absolute ethanol and nordihydroguaiaretic acid was dissolved in 50% DMSO. The other drugs were dis-
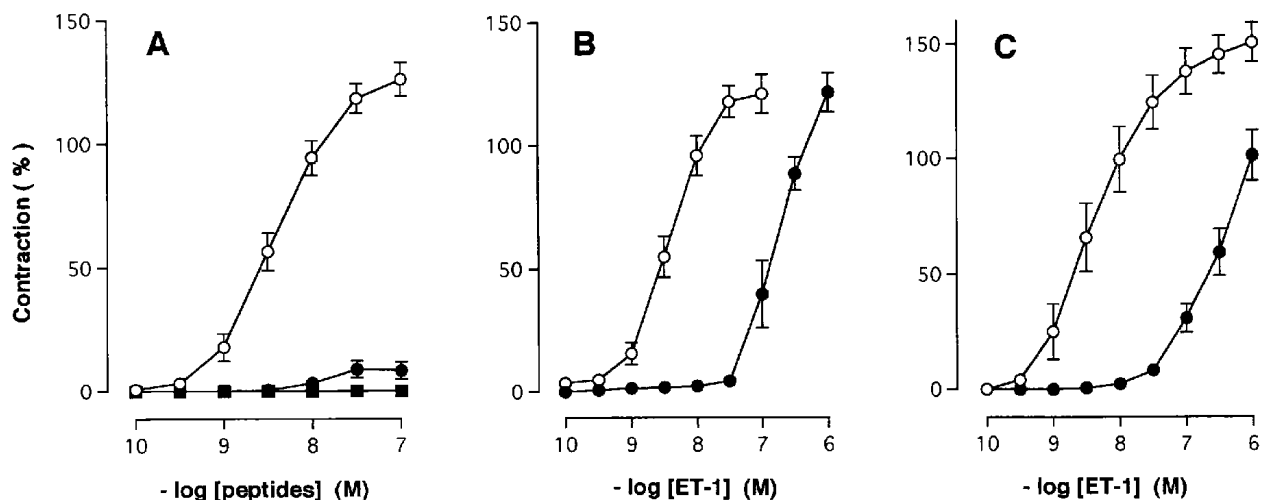
Fig. 1. Contractile responses to endothelin-1 (ET-1), sarafotoxin 6c (S6c) and IRL1620 and effects of BQ-123 and PD142893 on the ET-1-induced contraction in isolated rabbit saphenous artery. A: Concentration-response curves for ET-1 (○), IRL1620 (●) and S6c (■). B: Concentration-response curves for ET-1 in the absence (○) and presence (●) of 3 μM BQ-123. C: Concentration-response curves for ET-1 in the absence (○) and presence (●) of 3 μM PD142893. Contractile responses are expressed as percentages of the maximal tension induced by 60 mM KCl. Bars represent ±S.E.M. (n=5−8).

solved in distilled water.

Analyses of the data

Concentration-response curves for the agonists were analysed by means of a curve-fitting computer program. Maximal responses (E_{max}) and the EC_{50} values are expressed as the means ± S.E.M. The data were statistically evaluated by Student's t-test. Values were considered to differ significantly when the probability values were less than 0.05.

RESULTS

Effects of ET-1, IRL1620 and S6c on the saphenous artery

ET-1 (0.1−100 nM) elicited concentration-dependent contraction of the saphenous artery. In contrast, one of the ET_B-receptor agonist, IRL1620 (up to 1 μM), caused only very weak contraction and the other ET_B-receptor agonist, S6c (up to 1 μM), showed no effect at all (Fig. 1A). The ET-1-induced responses were clearly shifted in parallel to the right by the ET_A-receptor antagonist BQ-123 (3 μM) and the ET_A/ET_B-receptor antagonist PD142893 (3 μM) without any reduction in the maximal responses (Fig. 1 and Table 1). These concentrations of BQ-123 and PD142893 alone produced no mechanical response in the isolated saphenous artery.

Vasoconstrictor responses induced by ET-1 and related peptides in the saphenous vein and effects of BQ-123

ET-1 and IRL1620 produced strong concentration-

dependent contractions of the lateral saphenous vein and were almost equipotent in this tissue (Table 2). These contractile responses were resistant to the following agents: the muscarinic antagonist atropine (5 μM), the α-adrenoceptor antagonist phentolamine (10 μM), the adrenergic neuron blocking agent guanethidine (1 μM), the histamine H_1-receptor antagonist pyrilamine (0.3 μM), the 5-HT-receptor antagonist methysergide (10 μM), the cyclooxygenase inhibitor indomethacin (10 μM) and the lipoxygenase inhibitor nordihydroguaiaretic acid

Table 1. Effects of BQ-123 and PD142893 on the EC_{50} values and maximal responses for endothelin-1 (ET-1) in isolated rabbit saphenous artery

	n	EC_{50} (nM)	E_{max} (%)
Without BQ-123	8	3.95 (2.42−6.47)	124.7±7.3
With BQ-123 (3 μM)	5	163[a] (64.3−327)	123.0±7.9
Without PD142893	5	4.86 (1.38−17.2)	150.6±8.2
With PD142893 (3 μM)	5	508[a,b] (197−1310)	N.E.

Values are expressed as the means ± S.E.M. n: number of experiments. E_{max}: maximal responses, expressed as percentages of the maximal tension induced by 60 mM KCl. The 95% confidence limits are given in parentheses under the EC_{50} values. N.E.: not evaluated, because the responses did not reach a maximum even at the highest concentration tested. [a]$P < 0.05$, compared with the control value without BQ-123 or PD142893. [b]The value was calculated by assuming that the E_{max} in the presence of PD142893 was the same as the control value.

Table 2. Effects of BQ-123 on the EC_{50} values and maximal responses for endothelin-1 (ET-1) and related peptides in rabbit lateral saphenous vein

|		Control			With BQ-123 (3 μM)	
	n	EC_{50} (nM)	E_{max} (%)	n	EC_{50} (nM)	E_{max} (%)
ET-1	5	2.36 (1.38−4.01)	167.9± 8.8	5	2.39 (1.93−2.95)	185.4±15.5
ET-3	6	1.07 (0.80−1.44)	119.0± 5.4	6	1.01 (0.58−1.75)	126.4± 3.2
IRL1620	5	2.72 (1.53−4.84)	192.0±15.7	6	1.81 (1.22−2.68)	188.1± 5.4
S6c	5	0.98 (0.71−1.35)	146.5± 3.6	5	0.93 (0.72−1.20)	144.9± 4.1
[Glu⁹]S6b	5	0.67 (0.38−1.18)	123.9± 6.0	6	0.52 (0.37−0.73)	118.0± 4.0

Values are expressed as the means±S.E.M. n: number of experiments. E_{max}: maximal responses, expressed as percentages of the maximal tension induced by 100 mM KCl. The 95% confidence limits are given in parentheses under the EC_{50} values. S6c: sarafotoxin 6c, [Glu⁹]S6b: [Glu⁹]sarafotoxin 6b.

(10 μM) (data not shown).

ET-3 and selective ET_B-receptor agonists, S6c and [Glu⁹]S6b (22), also elicited concentration-dependent contractions of this venous smooth muscle. The EC_{50} values for these sarafotoxins were slightly lower than that for ET-1 (Table 2). In contrast to the antagonistic effect observed in the saphenous artery, BQ-123 (3 μM) did not affect any of the concentration-response curves of these endothelins and related peptides in the saphenous vein (Fig. 2 and Table 2). The EC_{50} values were almost identical in the absence or presence of BQ-123.

Effects of PD142893 on vasoconstrictor responses induced by ET-1 and related peptides in the saphenous vein

When the venous ring preparations were pretreated with 10 μM of PD142893, the IRL1620-induced responses were greatly shifted in parallel to the right (Fig. 3B and Table 3). This antagonist alone had no mechanical effect. In contrast to the IRL1620-induced responses, the ET-1-induced concentration-response curve was not affected at all by the same concentration of PD142893 (Fig. 3A). The EC_{50} value for ET-1 obtained in the presence of PD142893 was not different from that in the absence of
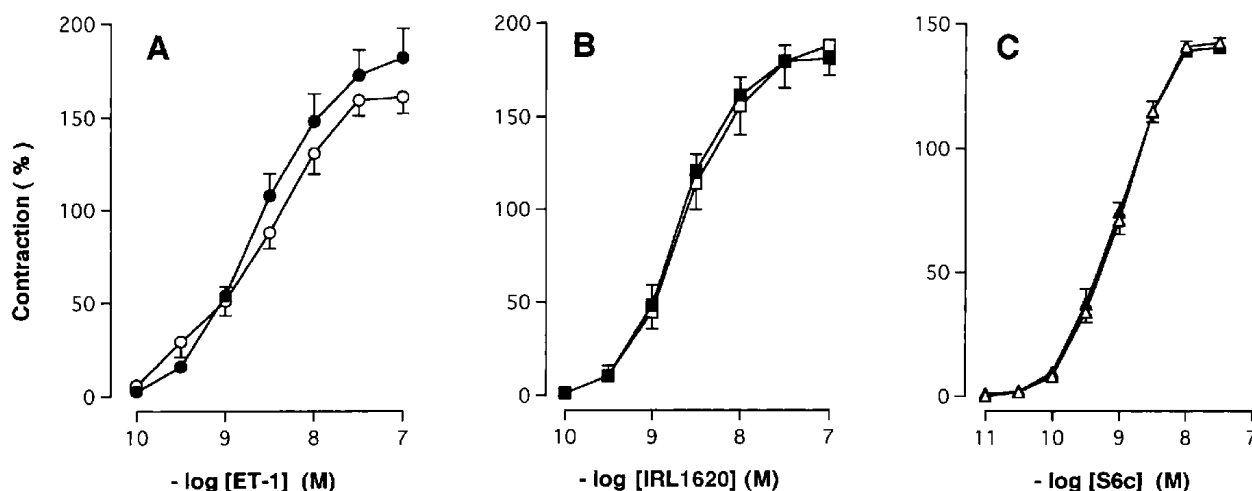
Fig. 2. Effects of 3 μM BQ-123 on the contractile responses induced by endothelin-1 (ET-1), IRL1620 and sarafotoxin 6c (S6c) in isolated rabbit lateral saphenous vein. A: Concentration-response curves for ET-1 in the absence (○) and presence (●) of BQ-123. B: Similar curves for IRL1620 in the absence (□) and presence (■) of BQ-123. C: Similar curves for S6c in the absence (△) and presence (▲) of BQ-123. Contractile responses are expressed as percentages of the maximal tension induced by 100 mM KCl. Bars represent ±S.E.M. (n=5−6).

Table 3. Effects of PD142893 on the EC$_{50}$ values and maximal responses for endothelin-1 (ET-1) and related peptides in isolated rabbit saphenous vein

	Control			With PD142893 (10 μM)		
	n	EC$_{50}$ (nM)	E$_{max}$ (%)	n	EC$_{50}$ (nM)	E$_{max}$ (%)
ET-1	6	1.64 (1.19−2.26)	186.5±10.1	7	1.43 (0.99−2.05)	180.3±6.0
ET-3	6	1.07 (0.80−1.43)	121.6± 5.9	5	2.75[a] (1.25−6.04)	151.3±8.6[a]
IRL1620	5	2.24 (1.75−2.88)	149.3±12.2	6	123[a,b] (96.4−157)	N.E.
S6c	5	0.38 (0.25−0.57)	169.4± 7.3	9	3.02[a] (2.00−4.57)	187.1±9.8
[Glu^9]S6b	6	0.44 (0.29−0.66)	133.9± 5.4	5	1.39[a] (0.95−2.03)	158.4±5.1[a]

Values are expressed as the means±S.E.M. n: number of experiments. E$_{max}$: maximal responses, expressed as percentages of the maximal tension induced by 100 mM KCl. The 95% confidence limits are given in parentheses under the EC$_{50}$ values. N.E.: not evaluated, because the responses did not reach the maximum even at the highest concentration tested. [a]P<0.05, compared with the control value. [b]The value was calculated by taking the value at 1 μM of IRL1620 as the E$_{max}$. S6c: sarafotoxin 6c, [Glu^9]S6b: [Glu^9]sarafotoxin 6b.

this antagonist (Table 3).

In the cases of ET-3, S6c and [Glu^9]S6b, their concentration-response curves shifted to the right by PD142893 (10 μM), but their rightward shifts were much less compared with that in the case of IRL1620, and their maximal responses were increased (Fig. 4 and Table 3). An increased concentration (30 μM) of PD142893 could not cause any further rightward shift of the concentration-response curve for S6c (Fig. 5B). In contrast, 1.0−30 μM of PD142893 showed potent antagonistic effects on

the IRL1620-induced contraction in a dose-dependent manner (Fig. 5A). In the presence of 30 μM of PD142893, 1 μM of IRL1620 could elicit only very weak contraction (less than 20% of KCl contraction), but the same preparations showed full responses, if 100 nM of S6c was added (data not shown).

Furthermore, the effect of combination of BQ-123 (3 μM) and PD142893 (10 μM) on ET-1-induced response in the saphenous vein was not different from that of PD142893 alone (data not shown).
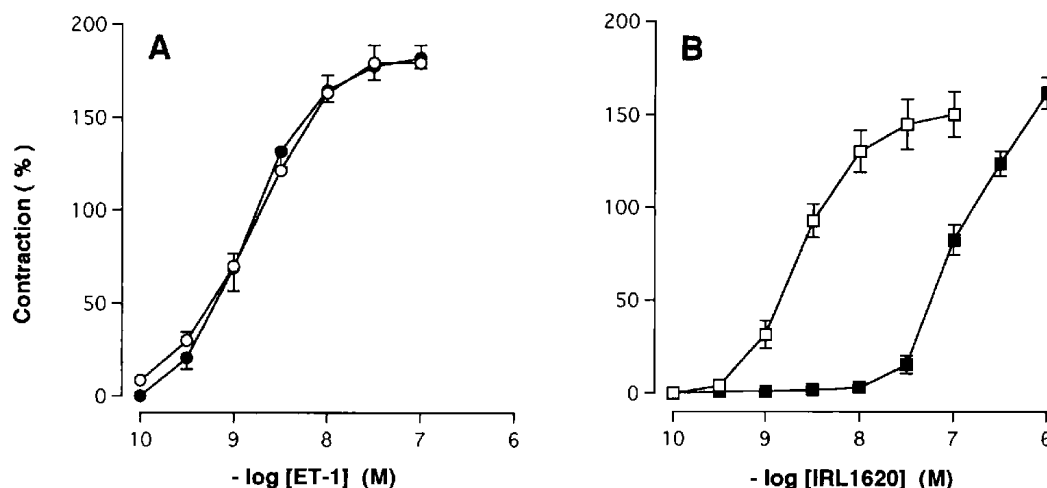
Fig. 3. Effects of 10 μM PD142893 on the contractile responses induced by endothelin-1 (ET-1) or IRL1620 in isolated rabbit saphenous vein. A: Concentration-response curves for ET-1 in the absence (○) and presence (●) of PD142893. B: Similar curves for IRL1620 in the absence (□) and presence (■) of PD142893. Contractile responses are expressed as percentages of the maximal tension induced by 100 mM KCl. Bars represent ±S.E.M. (n=5−7).

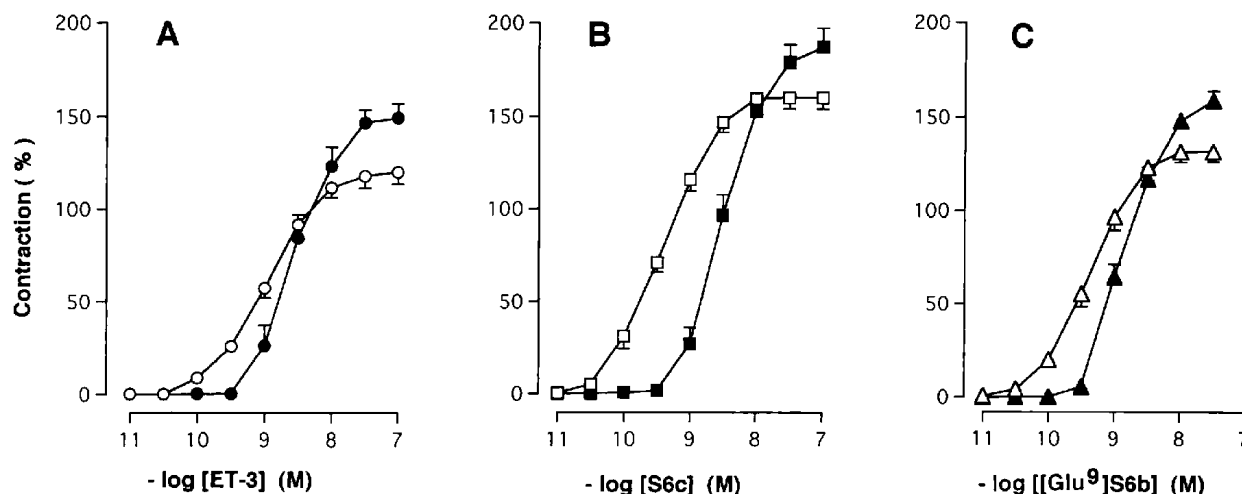**Fig. 4.** Effects of 10 μM PD142893 on the contractile responses induced by endothelin-3 (ET-3), sarafotoxin 6c (S6c) or [Glu⁹]-sarafotoxin 6b ([Glu⁹]S6b) in isolated rabbit saphenous vein. A: Concentration-response curves for ET-3 in the absence (○) and presence (●) of PD142893. B: Similar curves for S6c in the absence (□) and presence (■) of PD142893. C: Similar curves for [Glu⁹]S6b in the absence (△) and presence (▲) of PD142893. Contractile responses are expressed as percentages of the maximal tension induced by 100 mM KCl. Bars represent ±S.E.M. (n = 5 – 6).
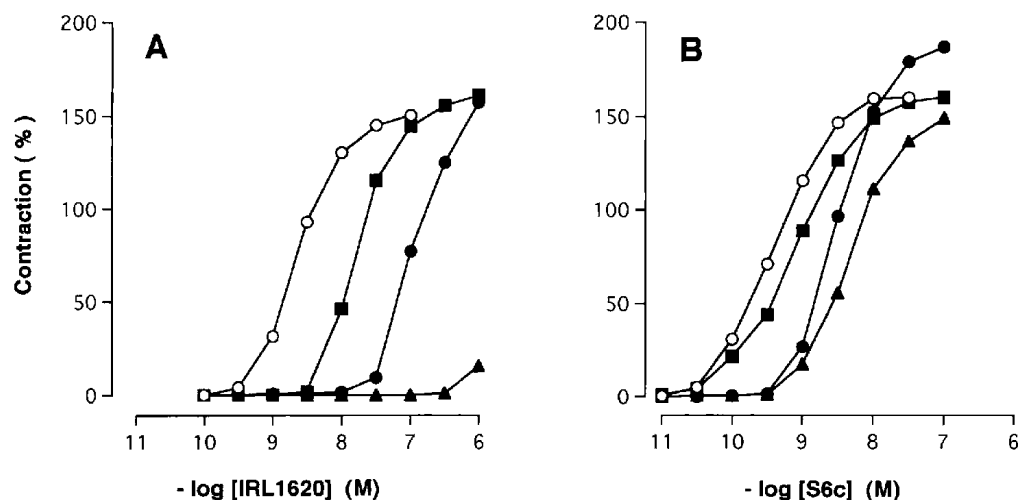

Fig. 5. Effects of various concentrations of PD142893 on the contractile responses induced by IRL1620 and sarafotoxin 6c (S6c) in isolated rabbit saphenous vein. A: Concentration-response curves for IRL1620 in the absence (○) and presence of 3 μM (■), 10 μM (●) or 30 μM (▲) of PD142893. B: Similar curves for S6c in the absence (○) and presence of 3 μM (■), 10 μM (●) or 30 μM (▲) of PD142893. Contractile responses are expressed as percentages of the maximal tension induced by 100 mM KCl. Each value indicates the mean of 3 – 6 experiments.

DISCUSSION

In the present experiments, ET-1 caused strong contractile responses in the isolated saphenous artery in a concentration-dependent manner, while ET_B-receptor agonists, IRL1620 and S6c, had practically no effect in this tissue. Moreover, the ET-1-induced concentration-response curve for the saphenous artery shifted in parallel to the right by the ET_A-receptor antagonist BQ-123 and the ET_A/ET_B antagonist PD142893 (Fig. 1). These results sug-

gest that the rabbit saphenous artery is dominated by ET_A receptors. This is in agreement with a previous general assumption that vascular smooth muscle cells contain predominantly ET_A receptors.

In the saphenous vein, however, BQ-123 could not alter the ET-1-induced contractile responses, and all of the ET_B receptor agonists were shown to be as potent as ET-1 as vasoconstrictor agents (Fig. 2 and Table 2). These observations indicate that the ET_B receptor on the smooth muscle of rabbit saphenous vein mediates vasoconstric-

tion, as reported previously (10).

The antagonistic effect of PD142893 on both ET$_A$ and ET$_B$ receptors was confirmed in the present study, because, as mentioned above, the rightward shift of the ET-1-induced concentration-response curve for the saphenous artery may be explained by its antagonistic effect on ET$_A$ receptors (Fig. 1); and the similar effect on IRL1620-induced contraction of the saphenous vein may be attributable to its effect on ET$_B$ receptors (Fig. 3B).

Nevertheless, PD142893 could not affect the ET-1-induced contractile response of the saphenous vein at all (Fig. 3A). The concentration-response curves for ET-3 and ET$_B$-receptor-selective sarafotoxins, S6c and [Glu^9]-S6b, were slightly shifted to the right by 10 μM of PD142893 (Fig. 4), but further increasing the concentration of PD142893 (30 μM) could not cause any additional shift of the concentration-response curve of S6c, in marked contrast to the very potent antagonistic effect of the same high concentration of PD142893 on the IRL1620-induced contraction in this vein (Fig. 5). The most simple explanation for these apparent contradictory results may be to assume the existence of two distinct ET$_B$-receptor subtypes; namely, PD142893-sensitive and PD142893-insensitive ET$_B$ receptors. If so, IRL1620 can be considered to act mainly on the PD142893-sensitive subtype, whereas the PD142893-insensitive subtype is considered to be responsible for the effects of ET-1, ET-3, S6c and [Glu^9]S6b. The similar results have also been obtained in our laboratory by using another ET$_A$/ET$_B$ antagonist, Ro46-2005, which showed a marked inhibitory effect on IRL1620-induced contraction of the rabbit saphenous vein, in contrast with very weak or no antagonistic effects on ET-1-, ET-3- and S6c-induced responses (unpublished data). These observations also suggest that rabbit saphenous vein contains two different ET$_B$-receptor subtypes.

Some of the recent studies have demonstrated that ET$_B$-receptor antagonists, such as IRL1038 and RES-701-1, as well as an ET$_A$/ET$_B$ antagonist, PD142893, are ineffective in antagonizing ET$_B$-receptor-mediated contractile response of some vascular smooth muscles, in spite of their potent antagonistic effect on ET$_B$-receptor-mediated vasodilation (14, 20, 23). Based on the results about the antagonistic potencies of IRL1038 or RES-701-1, it has been proposed that there are two types of ET$_B$ receptors: an ET$_B$-receptor subtype that is sensitive to these antagonists, termed ET$_{B1}$, and another subtype that is insensitive to them, termed ET$_{B2}$ (23). Applying these considerations to the present results, PD142893-sensitive and insensitive subtypes could be named ET$_{B1}$ and ET$_{B2}$, respectively.

ET-1 and IRL1620 are known to produce vasodilation by the release of EDRF (7, 24), and it has been reported

that the specific binding of [^{125}I]IRL1620 to porcine lung membranes could be displaced by ET-1 (25). Considering that IRL1620 is an ET$_{B1}$ agonist, as mentioned above, these findings suggest that ET-1 may act also on ET$_{B1}$ receptors as an agonist. Therefore, it is likely that ET-1 is a potent agonist for all the types of endothelin receptors, ET$_A$, ET$_{B1}$ and ET$_{B2}$. Although ET-1-induced contraction was not shifted by PD142893 in the rabbit saphenous vein and mediated by the ET$_{B2}$ receptor, it cannot be ruled out that ET$_A$ and ET$_{B1}$ receptors on the saphenous vein also contribute to the contractile response, because it is possible that the ET$_{B2}$-receptor-mediated response may overlap with the responses mediated by ET$_A$- and/or ET$_{B1}$-receptor subtypes.

From a binding study, it has been reported that the endothelin receptor population in the rabbit saphenous vein is made up of ET$_A$ receptors (about 70%) and non-ET$_A$ receptors (about 30%) and that the latter contains two components, one of which displays very high affinity for ET-3 and moderate affinity for S6c (26). These findings seem to support our present study, because there is a possibility that the two components of non-ET$_A$ receptors in this smooth muscle correspond to ET$_{B1}$ and ET$_{B2}$, although the relative functional contribution of ET$_A$ and the two ET$_B$-receptor subtypes in this vasculature cannot be assessed from the present experiment.

In spite of the accumulation of pharmacological evidence for the existence of additional endothelin receptor subtypes, only two endothelin receptors, ET$_A$ and ET$_B$, have been so far cloned (3–5). If there is no other endothelin receptor subtype, an alternative explanation is necessary to account for the results obtained in this study. Since IRL1620 is known to be a highly reversible ligand for the ET$_B$ receptor (25), it is possible that this selective ligand is more easily antagonized than ET-1, a ligand that binds almost irreversibly to the receptor (27). However, PD142893 has been reported to antagonize endothelium-dependent vasodilations induced by ET-1 very effectively (20). Therefore, the difference in binding characteristics (association and dissociation) of agonists can not be the only reason for the different sensitivities to PD142893. It is also possible that pharmacological classification of endothelin receptors is influenced by coexistence of both ET$_A$ and ET$_B$ receptors on the same cell, involvement of multiple intracellular signal transduction systems and/or their possible unknown interactions. To clarify these problems, further studies will be required.

Although the present study indicated that ET$_B$-receptor agonists could elicit full contractile responses in the rabbit saphenous vein, the physiological role(s) of these B-type endothelin receptors on the vascular smooth muscle has not yet been elucidated. It is important to clarify their distribution among various blood vessels of different

animal species. The development of selective ET_{B2}-receptor antagonists and further characterization of endothelin receptor subtypes are essential for elucidating the physiological and pathophysiological roles of endothelins.

In conclusion, our present results suggest that the rabbit saphenous vein contains two different ET_B-receptor subtypes, ET_{B1} and ET_{B2}, both of which can mediate the vasoconstrictive effects of endothelins, and that the saphenous artery contains predominantly ET_A receptors.

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