

Pharmacological Heterogeneity of Both Endothelin ET_A- and ET_B-Receptors in the Human Saphenous Vein

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ABSTRACT—To study endothelin receptor subtypes that mediate the smooth muscle contraction of human saphenous vein, effects of some endothelin-receptor agonists and antagonists were examined. Endothelin (ET)-1 and sarafotoxin 6b (S6b) elicited potent concentration-dependent contractions with similar pD₂ values and similar maximal responses. Selective ET_B-receptor agonists, sarafotoxin 6c (S6c) and IRL1620 (Suc-[Glu⁹, Ala^{11,15}]-endothelin-1(8–21)), also caused contractions, but their maximal responses were about one third of that of ET-1. ET-3 showed a biphasic concentration-response curve. An ET_A-receptor antagonist, BQ-123 (cyclo(-D-Asp-L-Pro-D-Val-L-Leu-D-Trp-)), an ET_A/ET_B-receptor antagonist, PD142893 (Ac-D-Dip-Leu-Asp-Ile-Ile-Trp), or the combination of these two antagonists hardly affected the contractile effect of ET-1, while each of them markedly antagonized the effects of higher concentrations of ET-3 and S6b. Contractions induced by lower concentrations of ET-3 and S6b were resistant to these antagonists. The concentration-response curves for S6c and IRL1620 were not affected by BQ-123. The effect of IRL1620 was markedly inhibited by PD142893, while S6c-induced contractions were much more resistant to PD142893. These different sensitivities to antagonists suggested heterogeneity of both ET_A- and ET_B-receptors [ET_{A1} (sensitive to BQ-123), ET_{A2} (resistant to BQ-123), ET_{B1} (sensitive to PD142893) and ET_{B2} (resistant to PD142893)] in the human saphenous vein, although contractions mediated by ET_B-subtypes have smaller maximal responses than those mediated by the ET_A-subtypes.

Keywords: Endothelin, Sarafotoxin, Saphenous vein (human), Endothelin receptor, Endothelin receptor antagonist

Endothelin (ET)-1 is a highly potent vasoconstrictive peptide, originally isolated from the culture media of porcine aortic endothelial cells (1). Three endogenous endothelin isopeptides, ET-1, ET-2 and ET-3, and at least two distinct endothelin receptors, ET_A and ET_B, are currently known (2). The ET_A-receptor has a higher affinity for ET-1 and ET-2 than for ET-3 (3), and the ET_B-receptor is non-selective to all three endothelin isopeptides (4). It is well-known that ET_A-receptors mediate vascular smooth muscle contractions in many arteries, but considerable evidence is now accumulating that ET_B-receptors on vascular smooth muscle are also involved in endothelin-induced vasoconstriction in some blood vessels, especially in veins or some particular vascular beds; e.g., the rabbit saphenous vein (5–7), the rabbit jugular vein (8), the rat renal vascular bed (9) and the porcine pulmonary vein (10).

Recent development of specific endothelin-receptor

agonists and antagonists has accelerated more precise pharmacological characterization of the endothelin-receptor subtypes, and this has suggested the heterogeneity of endothelin-receptor subtypes in various tissues (11). For example, some ET-1-induced responses, in which the agonist selectivity implied the involvement of ET_A-receptors, have been shown to be substantially insensitive to ET_A-receptor antagonists such as BQ-123 (cyclo(-D-Asp-L-Pro-D-Val-L-Leu-D-Trp-)) and FR139317 ((R)-2-((S)-2-([1-{hexahydro-1*H*-azepynyl}carbonyl]-amino-methyl-pentamoyl))-amino-3-(3-[1-methyl-1*H*-indolyl])propionyl-amino-3-(2-pyridyl)propionic acid). In these cases, some investigators have suggested that two subtypes of ET_A-receptors may exist (6, 12), but others have referred to their observations as “novel”, “atypical” or “non-ET_A, non-ET_B” endothelin receptors (13–15). Similarly, heterogeneity of ET_B-receptors has been pointed out by the use of some ET_A/ET_B-receptor antagonists such as PD142893

(Ac-Dip-Leu-Asp-Ile-Ile-Trp) or selective ET_B-receptor antagonists such as IRL1038 ([Cys¹¹-Cys¹⁵]ET-1(11–21)) or RES-701-1 (cyclic(Gly¹-Asp⁹)(Gly-Asn-Trp-His-Gly-Thr-Ala-Pro-Asp-Trp-Phe-Asn-Tyr-Tyr-Trp) (6, 7, 10, 16–18).

Regarding the endothelin-receptor subtypes of human vascular tissues, some reports have suggested that endothelin-induced contraction is predominantly mediated by ET_A-receptors (19, 20), but ET_B-receptors or atypical endothelin receptors as mentioned above may also be involved, at least in part, in human small arteries or veins (11, 12, 21, 22). Therefore, for further definitive pharmacological characterization of the endothelin-receptor subtypes on the human venous smooth muscle cells, we investigated the effects of BQ-123 and PD142893 on the contractions induced by some endothelin receptor agonists in isolated human saphenous vein.

MATERIALS AND METHODS

Tissue preparation

Sections (1–3 cm) of human saphenous veins were obtained from veins prepared for coronary bypass surgery and used the same day. After careful dissection to remove the connective tissue in a cold modified Krebs-Ringer bicarbonate solution, the veins were cut into rings of 2 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 modified Krebs-Ringer bicarbonate solution. One hook was connected to a micrometer for control of the tissue length, the other to a force transducer (TB-612T; Nihon Kohden, Tokyo) for isometric force recording. The Krebs solution was aerated constantly with a 95% O₂ and 5% CO₂ gas mixture and maintained at 37°C. Endothelium removal from each ring was verified by the absence of a relaxant response to substance P (0.1 μM) in rings precontracted with phenylephrine (3.0 μM).

Experimental procedures

During an initial equilibration period of 60 min, rings of the saphenous veins were stretched by resting loads of 1.0 g. Then the contractility of each preparation was examined by increasing the KCl concentration in the bathing medium to 80 mM. Such K⁺ depolarization was repeated at intervals of 30–40 min until the contractile response attained 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 in terms of percent of the above-men-

tioned maximal response to KCl. When the effects of BQ-123 and PD142893 were to be examined, each compound was added to the organ chamber 15–20 min before addition of the first dose of the agonists. When ethanol or methanol was used as a solvent, the final concentration of the vehicle did not exceed 0.3% or 1.0%, respectively, and they showed no significant influence on the contractile responses in the present experiments.

Drugs and solutions

The composition of modified Krebs-Ringer bicarbonate solution used in the present study was as follows: 118.4 mM NaCl, 4.7 mM KCl, 1.2 mM MgSO₄, 1.9 mM CaCl₂, 1.2 mM KH₂PO₄, 10.1 mM glucose and 25.0 mM NaHCO₃. ET-1, ET-2, ET-3, sarafotoxin 6b (S6b) and sarafotoxin 6c (S6c) were purchased from Peptide Institute, Inc. (Osaka) and were dissolved in phosphate-buffered saline (pH 7.2) containing 0.05% bovine serum albumin. IRL1620 (Suc-[Glu⁹,Ala^{11,15}]-endothelin-1(8–21)) was synthesized at International Research Laboratories, Ciba-Geigy Japan (Takarazuka) 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.

Analyses of the data

Concentration-response curves for the agonists were analyzed by means of a curve-fitting computer program. The pD₂ values and the maximal responses are expressed as means ± S.E.M. The data were evaluated statistically by Student's *t*-test. Values were considered to differ significantly when the probability values were less than 0.05. In experiments with BQ-123, Schild plots were constructed. The dose ratios of concentrations of ET-1 or S6b eliciting 50% of the maximal contraction to each agonist in the presence and absence of BQ-123 were used. The pA₂ values were calculated by linear regression analysis, if the slope of the plot did not differ significantly from unity.

RESULTS

Effects of ET-1 and related peptides on the human saphenous vein

Figure 1 shows contractile effects of 0.1 nM–1.0 μM ET-1 and related peptides on the human saphenous vein. ET-1 and S6b elicited concentration-dependent contractions with similar pD₂ values (8.21 ± 0.10 for ET-1, *n* = 9 and 8.11 ± 0.07 for S6b, *n* = 7) and similar maximal responses (124.2 ± 4.1% for ET-1, *n* = 9 and 127.7 ± 5.3% for S6b, *n* = 7). Selective ET_B-receptor agonists, S6c and

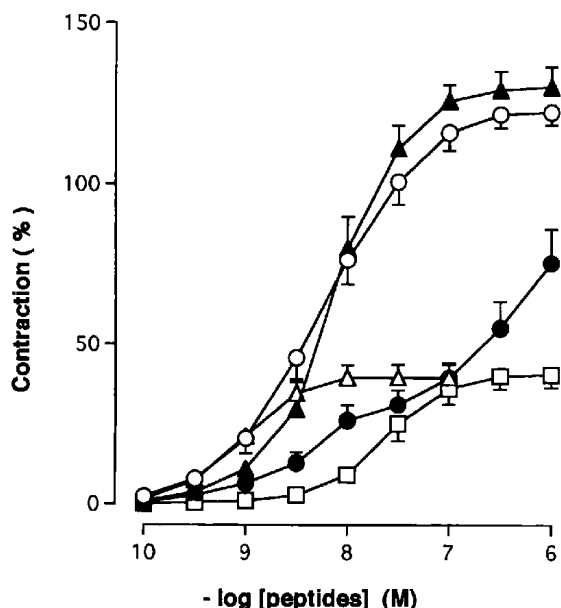


Fig. 1. Concentration-response curves for endothelin-1 (ET-1) (○), endothelin-3 (ET-3) (●), sarafotoxin 6b (S6b) (▲), sarafotoxin 6c (S6c) (△) and IRL1620 (□) in isolated human saphenous vein. Contractile responses are expressed as percentages of the maximal tension induced by 80 mM KCl. Bars represent \pm S.E.M. ($n=6-9$).

IRL1620, also caused contractions, but their maximal responses were about one third of that of ET-1 ($40.3 \pm 4.7\%$ for S6c, $n=7$ and $40.6 \pm 4.4\%$ for IRL1620, $n=7$).

When ET-3 was applied cumulatively to the human saphenous vein, 0.1–10 nM ET-3 caused concentration-

dependent contractions, but the following addition of 10–100 nM of ET-3 showed only minimal additional contractile effect in many preparations; and at concentrations higher than 100 nM, ET-3 again showed apparent concentration-dependent contractions. The amplitude of contractions induced by 10–100 nM ET-3 corresponded approximately to the maximal responses to S6c or IRL1620. The resultant concentration-response curve for ET-3 seemed biphasic and was best fitted to a two site model statistically. The estimated pD_2 values were 8.75 and 6.14.

Effects of BQ-123 on the contractile responses induced by ET-1 and related peptides

In the isolated human saphenous vein, BQ-123 alone caused no mechanical response at concentrations up to 30 μ M. As shown in Fig. 2A, the concentration-response curve for ET-1 shifted in parallel to the right by 1.0–10 μ M BQ-123, an ET_A -receptor antagonist. A higher concentration (30 μ M) of BQ-123 showed no further inhibitory effect. On the other hand, much lower concentrations (30 nM–3.0 μ M) of this antagonist showed marked antagonistic effects on the major part of S6b-induced contractions, although the responses induced by S6b at concentrations lower than 1.0 nM remained unaffected (Fig. 2B). The pA_2 values for BQ-123 against ET-1 and S6b were 6.34 ± 0.10 and 7.65 ± 0.08 , respectively, indicating a 20-fold difference. The slopes of the Schild plots were not significantly different from unity in either case (ET-1: 0.90 ± 0.09 , S6b: 1.15 ± 0.09).

Contractions induced by 0.1–100 nM ET-3 were not

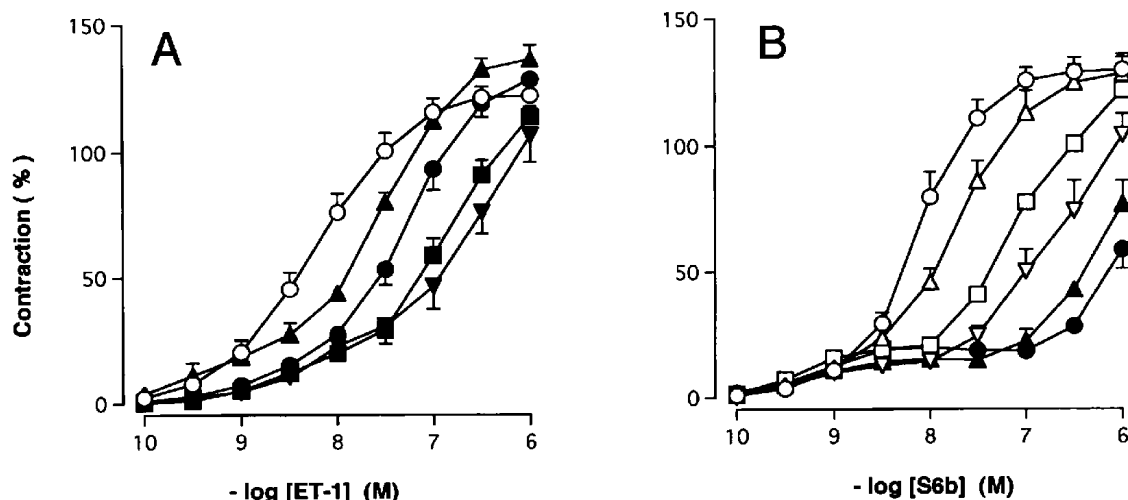


Fig. 2. Concentration-response curves for endothelin-1 (ET-1) (A) and sarafotoxin 6b (S6b) (B) in human saphenous vein in the absence (○) and presence of 30 nM (△), 100 nM (□), 300 nM (▽), 1.0 μ M (▲), 3.0 μ M (●), 10 μ M (■) and 30 μ M (▼) of BQ-123. Contractile responses are expressed as percentages of the maximal tension induced by 80 mM KCl. Bars represent \pm S.E.M. ($n=5-8$).

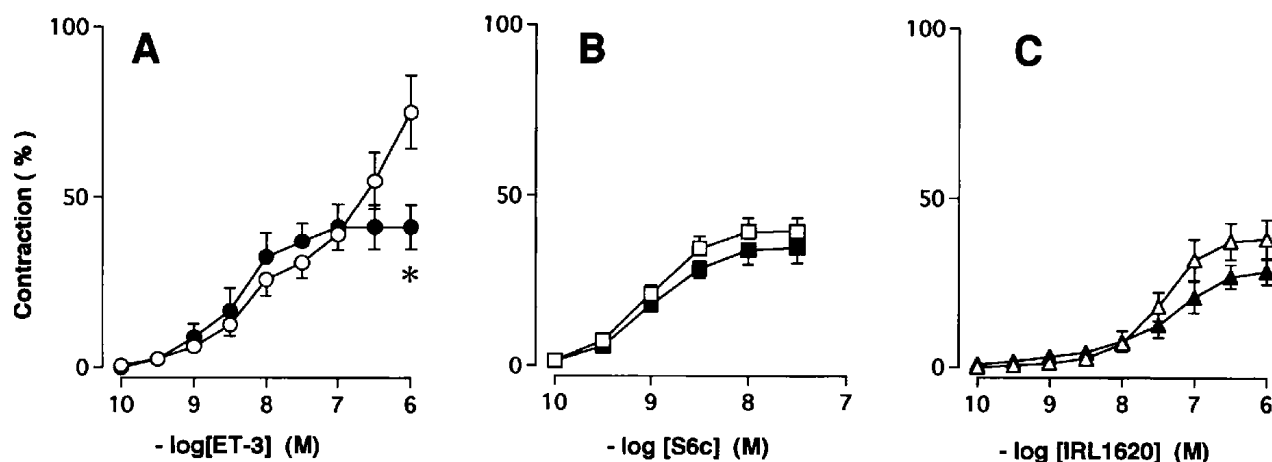


Fig. 3. Effects of BQ-123 ($3.0 \mu\text{M}$) on the endothelin-3 (ET-3)-, sarafotoxin 6c (S6c)- and IRL1620-induced contractions of human saphenous vein. A: concentration-response curves for ET-3 in the absence (○) and presence (●) of BQ-123. B: those for S6c in the absence (□) and presence (■) of BQ-123. C: those for IRL1620 in the absence (△) and presence (▲) of BQ-123. Contractile responses are expressed as percentages of the maximal tension induced by 80 mM KCl. Bars represent \pm S.E.M. ($n=5-7$). * $P<0.05$, compared with the control value.

inhibited at all by $3 \mu\text{M}$ BQ-123, while those induced by concentrations higher than 300 nM were markedly suppressed (Fig. 3A). Consequently, the concentration-response curve for ET-3 in the presence of BQ-123 was monophasic, in contrast to the biphasic curve observed in the absence of this antagonist, as described above. The concentration-response curves for S6c and IRL1620 were not affected significantly by $3 \mu\text{M}$ BQ-123 in the entire concentration range tested (Fig. 3: B and C).

Effects of PD142893 on the contractile responses induced by ET-1 and related peptides

Application of PD142893 up to $30 \mu\text{M}$ alone produced no response in the isolated human saphenous vein. PD142893 ($10 \mu\text{M}$) hardly affected the contractile effect of ET-1 on the human saphenous vein (Fig. 4A). Although contractions induced by 1.0–10 nM ET-1 were slightly suppressed by this concentration of PD142893, the pD_2 values for ET-1 in the absence and presence of this antagonist were 8.20 ± 0.11 ($n=9$) and 7.91 ± 0.05 ($n=6$), respectively, and the difference was not significant

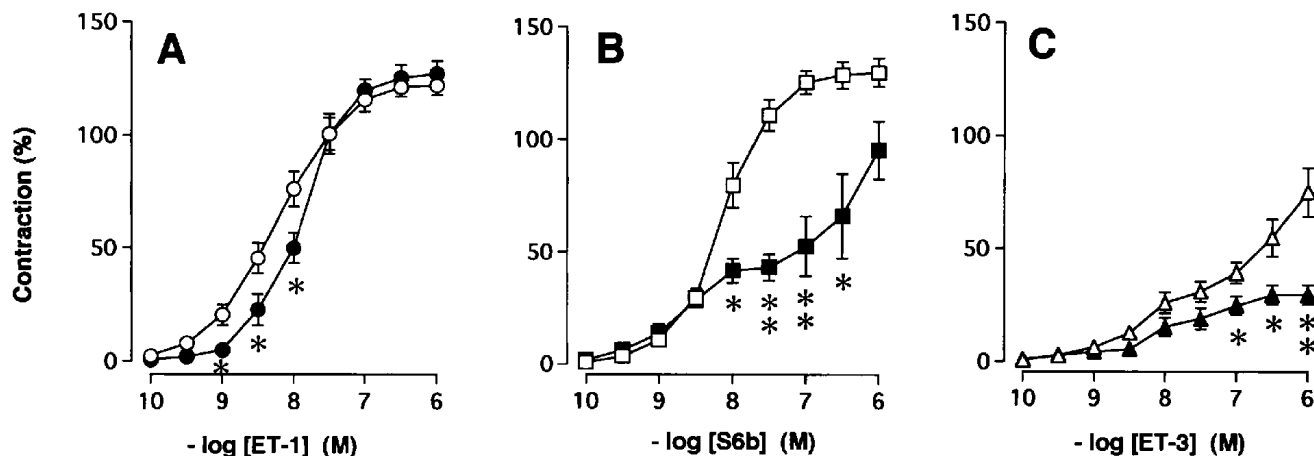


Fig. 4. Effects of PD142893 ($10 \mu\text{M}$) on the endothelin-1 (ET-1)-, sarafotoxin 6b (S6b)- and endothelin-3 (ET-3)-induced contractions of the human saphenous vein. A: concentration-response curves for ET-1 in the absence (○) and presence (●) of PD142893. B: those for S6b in the absence (□) and presence (■) of PD142893. C: those for ET-3 in the absence (△) and presence (▲) of PD142893. Contractile responses are expressed as percentages of the maximal tension induced by 80 mM KCl. Bars represent \pm S.E.M. ($n=5-9$). * $P<0.05$ and ** $P<0.01$, compared with the control value.

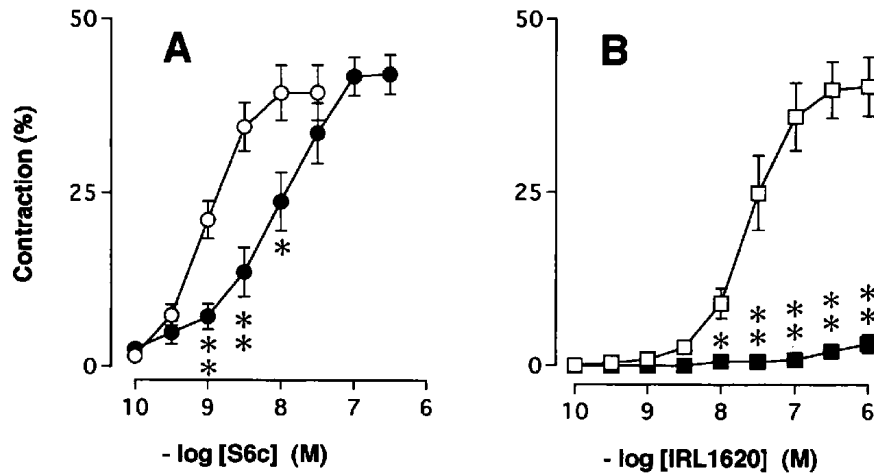


Fig. 5. Effects of PD142893 (10 μ M) on sarafotoxin 6c (S6c)- and IRL1620-induced contraction of human saphenous vein. **A:** concentration-response curves for S6c in the absence (○) and presence (●) of PD142893. **B:** those for IRL1620 in the absence (□) and presence (■) of PD142893. Contractile responses are expressed as percentages of the maximal tension induced by 80 mM KCl. Bars represent \pm S.E.M. ($n=5-7$). * $P<0.05$ and ** $P<0.01$, compared with the control value.

statistically. Furthermore, the combination of BQ-123 (10 μ M) and PD142893 (30 μ M) showed no further inhibitory effect on the ET-1-induced contractions, when compared to the effects of 10 μ M BQ-123 or 30 μ M PD142893 alone (data not shown).

However, PD142893 (10 μ M) markedly inhibited S6b-induced contractions, when concentrations of S6b were higher than 10 nM (Fig. 4B). Similarly, contractions induced by ET-3 in concentrations higher than 100 nM were inhibited by PD142893 (10 μ M), although contractions induced by low concentrations of S6b and ET-3 were resistant to PD142893 (Fig. 4: B and C).

Contractions induced by 1.0–10 nM S6c were suppressed by 10 μ M PD142893, but the maximal responses were unaffected (Fig. 5A). The pD_2 values for S6c in the absence and presence of this antagonist were 9.02 ± 0.05 ($n=7$) and 8.03 ± 0.15 ($n=6$), respectively, indicating a tenfold rightward shift. In contrast, IRL1620-induced responses were almost completely abolished by the same concentration of PD142893 (Fig. 5B).

DISCUSSION

In the human saphenous vein, ET-1 and S6b showed potent contractile effects with similar pD_2 values and maximal responses. The selective ET_B -receptor agonists S6c and IRL1620 also caused contractions, but their maximal responses were about one third of that induced by ET-1. ET-3 showed a biphasic concentration-response curve, with a plateau-like phase at a level similar to the maximal responses to S6c and IRL1620. The ET_A -receptor is known to recognize ET-1, S6b and high concentra-

tions of ET-3 as agonists, but not IRL1620 and S6c. Therefore, the data obtained in this study indicate that the human saphenous vein contains both ET_A - and ET_B -receptors, although ET_B -receptor-mediated responses were much smaller than those mediated by ET_A -receptors in this vein. Since ET-3 is known to act as an ET_B -receptor agonist at low concentrations, the contractions induced by low concentrations of ET-3 in the human saphenous vein are considered to be ET_B -receptor-mediated responses. On the other hand, at concentrations higher than 100 nM, ET-3 is approximately 100-fold less potent than ET-1. Therefore, the contractions induced by high concentrations of ET-3 are considered to be mediated by ET_A -receptors, because ET-3 is known to be less potent as an ET_A -receptor agonist than ET-1.

The concentration-response curves for S6c and IRL1620 were not affected by the ET_A -receptor antagonist BQ-123. These results are consistent with the interpretation that these responses are mediated by ET_B -receptors. Contractions induced by low concentrations of ET-3 and S6b were resistant to BQ-123, whereas contractions induced by high concentrations of these agonists were very sensitive to BQ-123 (Figs. 3A and 2B). These observations imply the involvement of ET_A -receptors in the BQ-123-sensitive components of these contractions and the involvement of ET_B -receptors in the BQ-123-resistant components.

However, ET-1 was much more resistant to BQ-123 than S6b in the entire concentration range tested in this study (Fig. 2). The pA_2 values against ET-1 and S6b indicate an approximately 20-fold difference in the antagonistic potency and may imply the heterogeneity of endothe-

lin receptors. Similarly, the ET_A/ET_B -receptor antagonist PD142893 hardly affected the contractile effect of ET-1, while it markedly antagonized the contractile effects of high concentrations of ET-3 and S6b in the present experiments. Probably, the ET-1- and S6b-mediated responses were mediated by ET_A -receptor subtypes, because S6c- and IRL1620-induced contractions were much weaker than ET-1- and S6b-induced contractions. In support of this view, similar potent contractile responses to ET-1 and S6b with different sensitivity to BQ-123 were observed after desensitization of ET_B -receptors by pretreatment with S6c in the human saphenous vein (M. Nishiyama et al., unpublished data). The simplest explanation for these results may be to assume that ET-1 and S6b exert their effects through two different ET_A -receptor subtypes, one subtype that is highly sensitive to BQ-123 and PD142893 and the other, much less sensitive to these antagonists. S6b and high concentrations of ET-3 may be recognized by the former subtype, whereas the ET-1-induced response may be mediated by the latter subtype.

Endothelin receptors are currently classified primarily according to the rank order of binding affinities or pharmacological potencies of endothelin isopeptides, but also according to those of some antagonists. Pharmacologically, in typical ET_A -receptor-mediated responses, it is expected that ET-1 is more potent than ET-3, that BQ-123 or FR139317 is effective as an antagonist, and that ET_B -receptor-selective compounds (for example, S6c, IRL1620 or [Ala^{1,3,11,15}]ET-1) have no effect. Similarly, in typical ET_B -receptor-mediated responses, it is expected that ET-3 is as potent as ET-1, that BQ-123 or FR139317 is ineffective as an antagonist, and that ET_B -receptor agonists are fully active. At present, however, there is a considerable number of reports indicating that ET-1-induced responses in some cases are curiously much more resistant to BQ-123 than ET-3- or S6b-induced responses. The responsible receptors are sometimes called "atypical", but possibly, they belong to the ET_A -receptors, since ET_A -receptor antagonists are moderately or highly potent and selective ET_B -receptor agonists are usually inactive (11). In such cases, it is conceivable that ET-1 and S6b (or ET-3) may exert their actions through two different types of ET_A -receptors. Sudjarwo et al. have proposed the classification of the ET_A -receptors into a BQ-123-sensitive (tentatively termed ET_{A1}) subtype and a BQ-123-insensitive (tentatively termed ET_{A2}) subtype (6). The present results suggested that both subtypes are located in the human saphenous vein, and indicated that the BQ-123-sensitive response is also sensitive to PD142893, and that the BQ-123-insensitive response is insensitive to PD142893.

On the other hand, IRL1620-induced contractions of the human saphenous vein were resistant to BQ-123, but

markedly inhibited by PD142893, indicating involvement of ET_B receptors. Contractions induced by S6c were also insensitive to BQ-123, but in contrast to IRL1620, they were relatively insensitive to PD142893 (Fig. 5). Contractions induced by low concentrations of ET-3 and S6b were also resistant to PD142893 (Fig. 4). Although both IRL1620 and S6c are known selective ET_B -receptor agonists, their sensitivities to PD142893 were markedly different. It suggests again the involvement of the heterogeneous subtypes of ET_B -receptors in the endothelin-induced contraction of human saphenous vein. Recent studies have demonstrated that some ET_B -receptor antagonists such as IRL1038 or RES-701-1, as well as the ET_A/ET_B antagonist PD142893, are quite ineffective in antagonizing ET_B -receptor-mediated contractile responses of some vascular smooth muscles, in spite of their potent antagonistic effects on the vasodilation mediated by ET_B -receptors on endothelial cells (6, 7, 10, 11, 16–18). One possible way to explain these findings is to assume the existence of two ET_B -receptor subtypes, and it has been proposed that the ET_B -receptor subtype sensitive to these antagonists should be termed ET_{B1} and the other insensitive subtype, ET_{B2} (17, 18). Although the ET_B -receptor subtypes mediating vasorelaxation and vasoconstriction are sometimes designated as ET_{B1} and ET_{B2} , respectively (23), the antagonistic potency may be more adequate as a criterion for pharmacological classification of ET_B -receptors than the types of responses, as it is possible that both subtypes are located on the smooth muscle and mediate vasoconstriction (7). From a binding study, it has been reported that ET_B -receptors in the rabbit saphenous vein is made up of two components with different affinities for ET-3 and S6c (24). This finding seems to support the idea of two different ET_B -receptor subtypes, although it is not known how the two binding components correspond to the two ET_B -receptor subtypes discussed above. Present pharmacological results suggest that the human saphenous vein contains both ET_{B1} (sensitive to PD142893)- and ET_{B2} (resistant to PD142893)-receptors, in addition to the ET_A -subtypes mentioned above.

In addition to ET_A - and ET_B -receptors, a possibility of a third endothelin receptor cannot be denied. The identification of a cDNA encoding a novel ET_C -receptor with relatively high affinity for ET-3 has been reported in *Xenopus* dermal melanophores (25). However, it is not yet clear whether it actually represents a species variant of ET_A - or ET_B -receptors, or whether it is a distinct subtype, ET_C , that is highly selective to ET-3 and has a distinct mammalian homologue. Recently, Douglas et al. have reported that the low affinity site in the rabbit lateral saphenous vein has the characteristics of an ET_C -receptor, since ET-3 and S6c were significantly more potent

than ET-1 (26). Because of the scarcity of evidence for the presence of an ET_C-receptor molecule mediating the ET_C-type responses and the lack of selective ET_C-receptor ligands other than ET-3, it appears too premature to discuss whether the above mentioned BQ-123-sensitive response to ET-3 or S6b are related to the ET_C-receptor.

In spite of the accumulation of pharmacological evidence for the existence of additional endothelin receptor subtypes, its possibility is still controversial. At present, only two endothelin receptors, ET_A and ET_B, have so far been cloned (2). In the rabbit saphenous vein, low stringency Northern analysis with ET_A- and ET_B-receptor cDNA probes failed to detect additional RNA species other than those of the known ET_A- and ET_B-receptors (26). If there is no other endothelin receptor subtype, an alternative explanation is necessary to account for the apparent heterogeneous responses to endothelins, although we cannot rule out the possibility that different post-translational modification results in the receptor heterogeneity. Since pharmacological responses can be influenced by many factors, the observed atypical responses may be mediated by the known ET_A- and ET_B-receptors. For example, it is possible that different association/dissociation rates for receptor-ligand complexes or a difference in their internalization might affect functional observations. Another possibility is that differences in antagonistic potency might reflect differential binding characteristics of agonists and antagonists to distinct subdomains of a single type of receptor. Alternatively, if ET_A- and ET_B-receptors coexist in different ratios in various tissues, their total responses to endothelins might give the impression of different receptor subtypes. In this situation, involvement of multiple intracellular signal transduction systems with the selective trafficking of receptors to different G proteins and/or their possible unknown interactions may be important points for consideration (27). Biochemical analyses and molecular expression studies using a wide spectrum of both agonists and antagonists seem essential to clarify the pharmacological heterogeneity of the responses to endothelins.

Our present results indicated pharmacological heterogeneity of both ET_A- and ET_B-receptor-mediated contractions in human saphenous vein, although the maximal responses mediated by ET_B-receptors were much smaller than those mediated by ET_A-receptors. The currently available data, as well as those obtained in the present study, clearly indicate the pharmacological heterogeneity of responses to endothelins, but they are not yet sufficient to provide conclusive evidence for endothelin receptor nomenclature. Therefore, additional data on the binding characteristics, on the second messenger production and on the DNA nucleotide sequences of the possible novel subtypes are eagerly awaited. Further investigation of

these endothelin receptor subtypes is essential for elucidating the physiological and pathophysiological roles of endothelins, and it will be of particular importance if endothelin receptor antagonists are considered as potentially useful drugs for therapeutic purposes.

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