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Short communication

Heterogeneity of endothelin ET_A receptor-mediated contractions in the rabbit saphenous vein

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Abstract

The involvement of endothelin ET_A receptors in endothelin-1-induced contractions of the rabbit saphenous vein was studied. After desensitization of endothelin ET_B receptors by pretreatment with sarafotoxin S6c, endothelin-1 and sarafotoxin S6b and a high concentration of endothelin-3 caused dose-dependent contractions. However, endothelin-1-induced contractions were much less sensitive to an endothelin ET_A receptor antagonist, BQ-123 (cyclo (-D-Asp-L-Pro-D-Val-L-Leu-D-Trp-)), than sarafotoxin S6b-induced responses. The pA_2 values of BQ-123 for endothelin-1- and sarafotoxin S6b-induced contractions were 5.69 and 7.65, respectively. These results suggest pharmacological heterogeneity of endothelin ET_A receptors in the rabbit saphenous vein.

Keywords: Endothelin; Sarafotoxin; BQ-123; Endothelin ET_A receptor; Saphenous vein, rabbit

1. Introduction

Endothelin-1 is a highly potent vasoconstrictor peptide, originally isolated from the culture media of porcine aortic endothelial cells (Yanagisawa et al., 1988). Three endogenous endothelin isopeptides, endothelin-1, endothelin-2 and endothelin-3, and two distinct endothelin receptors, ET_A and ET_B, are currently known (Masaki et al., 1994). The endothelin ET_A receptor has a higher affinity for endothelin-1 and the endothelin-2 than for endothelin-3, and the endothelin ET_B receptor is non-selective to all three endothelin isopeptides. It has been assumed that, in vascular tissues, the vasoconstrictor effects of endothelins are mediated by endothelin ET_A receptors on smooth muscle cells, while endothelin ET_B receptors on endothelial cells mediate vasodilation through release of prostacyclin and endothelium-derived relaxing factor. However, recent studies have suggested that the endothelin ET_B receptor is also located on the vascular smooth muscle cells and involved in endothelin-induced vasoconstriction in some blood vessels, especially in veins, e.g., the rabbit jugular vein (Sumner et al., 1992), the rabbit saphenous vein (Moreland et al., 1992; Sudjarwo et al., 1994; Nishiyama et al., 1995) and the porcine pulmonary vein (Sudjarwo et al., 1993).

It has been reported that, in the rabbit saphenous vein, endothelin-3 and endothelin ET_B receptor-selective agonists, such as IRL1620 (Suc-[Glu^9,$Ala^{11,15}$]endothelin-1-(8–21)) and sarafotoxin S6c are as potent as endothelin-1 to induce vasoconstriction and that a selective endothelin ET_A receptor antagonist, BQ-123 (cyclo (-D-Asp-L-Pro-D-Val-L-Leu-D-Trp-)), does not inhibit these contractions at all, suggesting involvement of endothelin ET_B receptors (Moreland et al., 1992; Sudjarwo et al., 1993, 1994; Nishiyama et al., 1995). A binding study has indicated that the endothelin receptor population in this vein is made up of endothelin ET_A (about 70%) and ET_B (about 30%) receptors (Webb et al., 1993). However, it is difficult to estimate the physiologically relevant contribution of the endothelin ET_A receptor in the endothelin-1-induced contraction of the rabbit saphenous vein, firstly because endothelin ET_A and ET_B receptor-mediated contractions overlap in this blood vessel, and secondly because no suitable selective endothelin ET_A receptor agonist is available at present.

Recently, it has been demonstrated that the endothelin ET_A receptor in this vein is also involved in

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endothelin-1-induced contractile responses, since endothelin-1 and high concentrations of endothelin-3 showed contractile effects after desensitization of endothelin ET_B receptors (Sudjarwo et al., 1994). In this case, it was noted that BQ-123 was much more effective to antagonize the contractile effect of endothelin-3 than that of endothelin-1, suggesting the need for subclassification of endothelin ET_A receptors. Similarly, for some human blood vessels, it has also been reported that the contractile effects of sarafotoxin S6b and a high concentration of endothelin-3 are much more easily antagonized by BQ-123 than that of endothelin-1 (Bax et al., 1993, 1994; Riezebos et al., 1994; White et al., 1994). Therefore, in the present study, we investigated the antagonistic potency of BQ-123 on endothelin-1-, endothelin-3- and sarafotoxin S6b-induced contractions in the rabbit saphenous vein after pretreatment with sarafotoxin S6b, for further characterization of the endothelin ET_A receptor subtypes in this vein.

2. Materials and methods

The lateral saphenous veins were isolated from male Japan White rabbits (2.4–3.0 kg body weight) anesthetized with pentobarbital (50 mg/kg i.v.). The blood vessels were divided into rings of 3 mm in length and the endothelium was removed by gentle mechanical rubbing. Each preparation was mounted horizontally in an organ chamber filled with 5 ml of modified Krebs solution (mM: NaCl 118.4, KCl 4.7, $MgSO_4$ 1.2, $CaCl_2$ 1.9, KH_2PO_4 1.2, glucose 10.1 and $NaHCO_3$ 25), aerated with 95% O_2 and 5% CO_2, and maintained at 37°C. Mechanical responses were recorded isometrically under resting loads of 0.2 g. Endothelium removal from each ring was verified by the absence of a relaxant response to acetylcholine (1 μM) in rings precontracted with phenylephrine (1.0–3.0 μM). After washout, 100 mM KCl was applied repeatedly at intervals of 30–40 min until the contractile response attained steady state (usually 3–4 times). A single cumulative concentration-response curve for endothelin or sarafotoxin peptides was then obtained on each preparation. In some experiments, for desensitization of endothelin ET_B receptors, the preparations were pretreated with 300 nM of sarafotoxin S6c. This concentration of sarafotoxin S6c elicited a transient strong contraction but the tension returned to its resting level in about 1 h. Concentration-response curves for endothelin-1, endothelin-3 and sarafotoxin S6b were then obtained in the new bath solution containing the same concentration of sarafotoxin S6c. Contractile responses were expressed in terms of percent of the maximal response to 100 mM KCl. When the effects of BQ-123 were to be examined, it was added to the organ chamber 15 min before application of the first dose of the agonists. Experiments with or without the antagonist were conducted in a parallel paired manner.

Endothelin-1, endothelin-3, sarafotoxin S6b and sarafotoxin S6c were purchased from Peptide Institute (Osaka, Japan). BQ-123 (sodium salt) was supplied by Nippon Chemiphar, Co. These peptides were dissolved in phosphate-buffered saline (pH 7.2) containing 0.05% bovine serum albumin. IRL1620 was a gift from Ciba-Geigy Japan and dissolved in 0.01 N NaOH.

Concentration-response curves were analyzed by a curve-fitting computer program. Maximal responses (E_{max}) and pD_2 values were expressed as the means ± S.E.M. The data were evaluated statistically using Student's t-test and $P < 0.05$ was taken as significant. In experiments with BQ-123, Schild plots were constructed and the pA_2 values were calculated by linear regression analysis if the slope did not differ significantly from unity.

3. Results

All the agonists, endothelin-1, endothelin-3, sarafotoxin S6b, sarafotoxin S6c and IRL1620 induced concentration-dependent contractions with similar pD_2 values in the preparations untreated with sarafotoxin S6c (Table 1). After desensitization of endothelin ET_B receptors by pretreatment with sarafotoxin S6c, the pD_2 value for endothelin-1 was scarcely affected, while the contractile potency of sarafotoxin S6b and endothelin-3 decreased significantly. On the other hand, neither IRL1620 nor the second application of sarafotoxin

Table 1
Effects of S6c pretreatment on the pD_2 values and maximal responses for endothelin-1 and related peptides in the rabbit saphenous vein

	Control			After S6c pretreatment		
	n	pD_2	E_{max} (%)	n	pD_2	E_{max} (%)
Endothelin-1	7	8.65 ± 0.07	164.1 ± 6.6	5	8.64 ± 0.23	140.9 ± 8.4
Endothelin-3	8	8.95 ± 0.04	119.9 ± 3.4	5	7.39 ± 0.06 [a]	122.9 ± 5.3
S6b	5	9.60 ± 0.18	161.3 ± 7.2	6	8.77 ± 0.06 [a]	166.5 ± 9.0
S6c	7	9.23 ± 0.07	165.3 ± 4.0	4	No response	
IRL1620	7	8.65 ± 0.04	163.2 ± 8.9	4	No response	

Each value represents the mean ± S.E.M. of n experiments. [a] $P < 0.05$ compared with the control value.

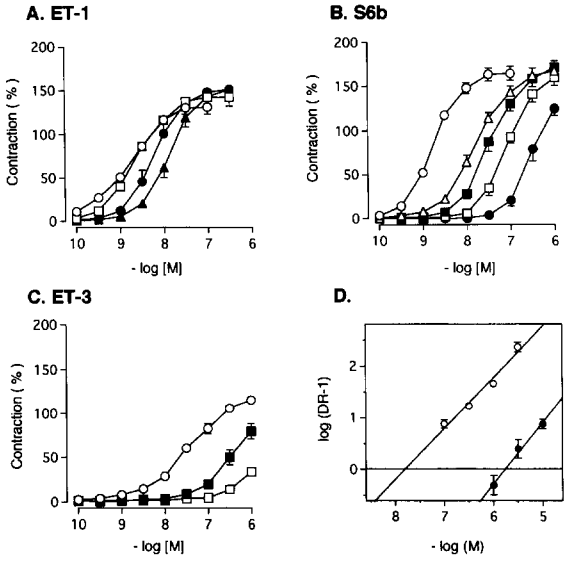

Fig. 1. Concentration-effect curves for ET-1 (A), S6b (B) and ET-3 (C) in the isolated rabbit saphenous vein preparations pretreated with 300 nM of S6c in the absence (○) and presence of 0.1 mM (△), 0.3 mM (■), 1.0 mM (□), 3.0 mM (●) and 10 mM (▲) of BQ-123. Contractile responses are expressed as percentages of the maximal tension induced by 100 mM KCl. Vertical bars represent S.E.M. ($n = 5$–8). (D) Schild plot for BQ-123 on contractions evoked by ET-1 (●) and S6b (○). The calculated pA_2 values for BQ-123 on ET-1 and S6b-induced contractions were 5.69 and 7.65, respectively.

S6c could elicit any contraction after desensitization of the endothelin ET_B receptor, in marked contrast with their very potent vasoconstrictive effects observed in the untreated preparation (Table 1).

In the preparation untreated with sarafotoxin S6c, 3 μM of BQ-123 did not affect the contractile responses induced by any of these peptides. The pD_2 values for endothelin-1, endothelin-3, sarafotoxin S6b, sarafotoxin S6c and IRL1620 in the presence of BQ-123 were 8.62 ± 0.03, 9.00 ± 0.09, 9.49 ± 0.13, 9.03 ± 0.04 and 8.74 ± 0.07, respectively ($n = 5$–6). The maximal response to each agonist was also unchanged (data not shown).

As shown in Fig. 1, after desensitization of endothelin ET_B receptors with sarafotoxin S6c, sarafotoxin S6b- and endothelin-3-induced contractions were very effectively antagonized by 0.1–3.0 μM of BQ-123. In contrast, endothelin-1-induced responses were less sensitive to this antagonist. Schild plots for BQ-123 against endothelin-1- and sarafotoxin S6b-induced contractions are shown in Fig. 1D. The calculated pA_2 values for BQ-123 against endothelin-1 and sarafotoxin S6b were 5.69 ± 0.07 and 7.65 ± 0.11, respectively, indicating a 100-fold difference. The slopes of these Schild plots were not significantly different from unity in either case (endothelin-1: 1.18 ± 0.22, sarafotoxin S6b: 0.99 ± 0.08).

4. Discussion

Endothelin-1 and all agonists used in this study elicited strong contractile responses in the normal saphenous vein and BQ-123 could not alter the responses, suggesting the involvement of endothelin ET_B receptors. After pretreatment with sarafotoxin S6c, IRL1620 and sarafotoxin S6c caused no response and the pD_2 value for endothelin-3 decreased from 8.95 to 7.39, corresponding to a 40-fold difference of the EC_{50} value. Therefore this treatment may be effective enough to desensitize endothelin ET_B receptors. Under these conditions, endothelin-1, sarafotoxin S6b and high concentrations of endothelin-3 caused concentration-dependent contractions, and the maximal responses to endothelin-1 and sarafotoxin S6b were similar to those of untreated preparations. These results suggest that desensitization of the endothelin ET_B receptor unmasked the endothelin ET_A receptor-mediated responses. Such results are consistent with previous findings that the endothelin ET_A receptor recognizes endothelin-1, sarafotoxin S6b and high concentrations of endothelin-3 as agonists, but not IRL1620 and sarafotoxin S6c.

However, the effect of BQ-123 on sarafotoxin S6b-induced contraction was greatly different from that on the endothelin-1-induced contraction. Sarafotoxin S6b-induced contraction was antagonized by a very low dose of this antagonist, but endothelin-1-induced responses were much less sensitive, as seen from the difference in their pA_2 values. The simplest explanation for these results is to assume two distinct endothelin receptor subtypes with different sensitivity to BQ-123. Sarafotoxin S6b and high concentrations of endothelin-3 may be considered to act only on a subtype which is highly sensitive to BQ-123, whereas endothelin-1 may act at least on another subtype which is much less sensitive to BQ-123. Since these two hypothetical subtypes are active after thorough desensitization of known endothelin ET_B receptors, they are probably subtypes of the endothelin ET_A receptor. Similar results have been reported by Sudjarwo et al. (1994), who also considered that these two subtypes belong with the endothelin ET_A receptors. Other studies using sarafotoxin S6b have also suggested that endothelin ET_A receptors in human small arteries are different from endothelin ET_A receptors in human small veins, or that endothelin-1 and sarafotoxin S6b contract the human coronary artery via different receptors, which are probably subtypes of the endothelin ET_A receptor (Bax et al., 1993, 1994; Riezebos et al., 1994; White et al., 1994).

If two endothelin receptor subtypes other than the endothelin ET_B receptor are assumed, the present results suggest that endothelin-1 acts at least on the subtype which is less sensitive to BQ-123. On the other

hand, since previously reported pA_2 values of BQ-123 against endothelin-1 in many tissues are 6.8–7.5 (Bax and Saxena, 1994), it is possible that endothelin-1 can act as agonist on the BQ-123-sensitive subtype also. If endothelin-1 acts as agonist on both subtypes, they may be subtypes of endothelin ET_A receptors.

In spite of accumulating pharmacological evidence for the existence of additional endothelin receptor subtypes, only two receptors, endothelin ET_A and ET_B, have so far been cloned. In the rabbit saphenous vein, low stringency Northern analysis failed to detect additional RNA species other than those of known endothelin ET_A and ET_B receptors (Webb et al., 1993). If there is no other endothelin receptor subtype, an alternative explanation is necessary to account for the present results. Pharmacological classification of receptors can be influenced by a number of factors. For example, possible different association/dissociation rates for receptor-ligand complexes or a difference in their internalization might affect functional observations. Another possibility is that different sensitivities to BQ-123 actually reflect differential bindings of agonists to different subdomains of a single type of endothelin ET_A receptor, since similar differential binding of agonists and antagonists to different portions of the tachykinin NK_1 receptor has been reported (Gether et al., 1993). Involvement of multiple intracellular signal transduction systems with the selective trafficking of receptors to different G proteins and/or their possible unknown interactions may also be important points for consideration (Kenakin, 1995). Biochemical analyses and molecular expression studies using extensive numbers of agonists and antagonists seem essential to clarify the pharmacological heterogeneity of endothelin-1-induced responses.

Our present results indicate pharmacological heterogeneity of endothelin-1, endothelin-3 and sarafotoxin S6b-induced contractions in the rabbit saphenous vein, presumably mediated by two distinct endothelin ET_A receptor subtypes. It is possible that both endothelin receptor subtype(s) described in this paper and the known endothelin ET_B receptor are involved in the endothelin-induced vasoconstriction of this vein, probably in a non-additive manner, although the true contribution of each subtype is unknown. Further investigation of these receptor subtypes is essential to elucidate the physiological and pathophysiological roles of endothelins.

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