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Atypical receptors mediate the response to endothelin-1 and sarafotoxin S6b in the human umbilical artery

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Abstract

The receptors mediating smooth muscle response to endothelin-1 and sarafotoxin S6b in the human umbilical artery were investigated in vitro. Both agonists induced contractions that were unaffected by the endothelin ET_B receptor antagonist BQ 788 (10^{-9}, 10^{-8}, 10^{-7} M). The non-selective endothelin $ET_{A/B}$ receptor antagonist PD 142893 (10^{-7} M) decreased the contraction induced by endothelin-1. PD 142893 (10^{-9} M) enhanced the contraction induced by sarafotoxin S6b whereas higher concentrations had no effect. Removing the endothelium did not affect the antagonising action of PD 142893 on endothelin-1-induced contractions while the enhancement of the sarafotoxin S6b-induced contraction was abolished. Sarafotoxin S6b induced relaxation in segments precontracted by 5-hydroxytryptamine and exposed to the endothelin ET_A receptor antagonist BQ 123 (10^{-7} M) and PD 142893 (10^{-9} M) abolished this relaxation. These endothelial receptors seem neither to be classical endothelin ET_A nor endothelin ET_B receptors and they are not activated by endothelin-1.

Keywords: Umbilical artery; (Human); Endothelin-1; Sarafotoxin S6b; PD 142893; BQ 788; Endothelial receptor

1. Introduction

The endothelins constitute a group of endothelium-derived structurally similar peptides, named endothelin-1, endothelin-2 and endothelin-3 (Yanagisawa et al., 1988). These peptides bear a close resemblance to the snake venoms sarafotoxins which also exist in several isoforms (Yanagisawa and Masaki, 1989; Stjernquist, 1998).

The vascular effects of endothelins are mediated via interactions with endothelin receptors. The receptors are typically divided into two major categories, endothelin ET_A and endothelin ET_B. The endothelin ET_A receptor is primarily found in vascular smooth muscle, where it mediates contraction. The endothelin ET_B receptor promotes the release of endothelium derived relaxing factors such as nitric oxide and prostacyclin while situated on the vascular endothelium, or induces contraction while situated on the smooth muscle cells (Stjernquist, 1998). This simple description has been challenged. Alternative main- and subdivisions of endothelin receptors have been proposed (Haynes et al., 1993; Bodelsson and Stjernquist, 1993; Bax and Saxena, 1994). The proportions between the endothelin$_A$ and endothelin$_B$ receptors might also differ in various organs, a condition which also could explain atypical effects (Battistini et al., 1995).

The endothelin receptors can be classified based on the relative potency of various agonists. The currently accepted division is as follows; for the endothelin$_A$ receptor: endothelin-1 = sarafotoxin S6b > endothelin-2 >> endothelin-3 and the endothelin$_B$ receptor:endothelin-1 = sarafotoxin S6b = endothelin-2 = endothelin-3 (Arai et al., 1990; Sakurai et al., 1990).

The human umbilical artery holds several unique characteristics in comparison with other vessels. It lacks tunica adventitia, vasa vasorum and innervation. The artery is thus dependent on humoral and locally released factors for the regulation of the smooth muscle tone.

Endothelin-1 contracts the human umbilical artery smooth muscle and may play a role in the closure of the umbilical vessels postpartum (Haegerstrand et al., 1989; Bodelsson and Stjernquist, 1994). Indeed, the contractile response to endothelin-1 is augmented at high oxygen tension (Bodelsson et al., 1996), a condition the vessel is exposed to immediately after birth. A low oxygen tension will on the other hand reduce the contractile effect of

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endothelin-1 in human umbilical artery suggesting a protective response at hypoxic situations in utero (Bodelsson et al., 1996).

The smooth muscle of the human umbilical artery contracts when exposed to endothelin-1 as well as sarafotoxin S6b in vitro. The selective endothelin$_A$ receptor antagonist, BQ 123 (Ihara et al., 1992), is, however, more potent in inhibiting sarafotoxin S6b, than endothelin-1 induced contraction (Bodelsson and Stjernquist, 1993; Bogoni et al., 1996). Hence, the endothelin-receptors mediating smooth muscular activity in the human artery do not fulfil the criteria for classical endothelin$_A$ receptors.

The aim of the present study was to further investigate the functional receptors mediating and modulating the contractile response to endothelin-1 and sarafotoxin S6b in the human umbilical artery.

2. Material and methods

2.1. Material

Samples of 54 umbilical cords were obtained from normal pregnancies and normal deliveries, immediately after delivery. A 7–8 cm long segment of the umbilical cord within the 15 cm closest to the child was obtained, the arteries atraumatically dissected out and placed in cold (4°C) Krebs–Ringer solution (composition, please see below) until the experiments were carried out, 0.5–24 h after specimen collection.

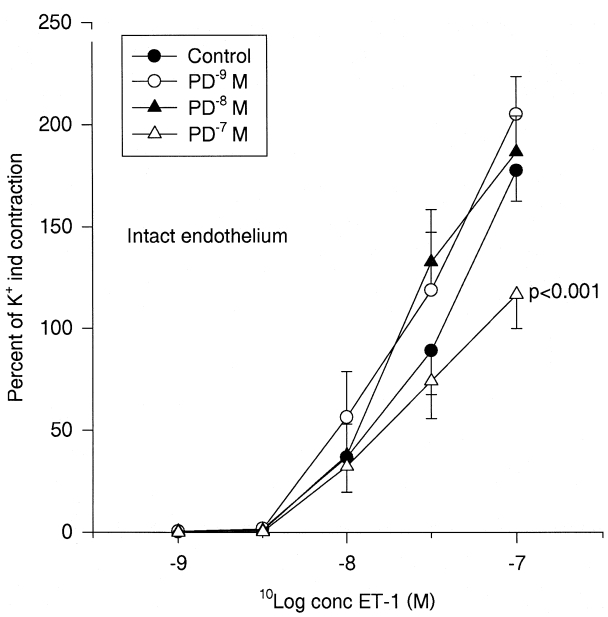

Fig. 1. Contractile response to endothelin-1 in the human umbilical artery in the absence and presence of the nonselective endothelin$_{A/B}$ antagonist PD 142893. Endothelin-1 induced a concentration-dependent contraction which was reduced by PD 142893 at 10^{-7} M. Lower concentrations of PD 143893 (10^{-8} and 10^{-9} M, respectively) did not affect the contraction. Means ± S.E.M. ($n = 18$).

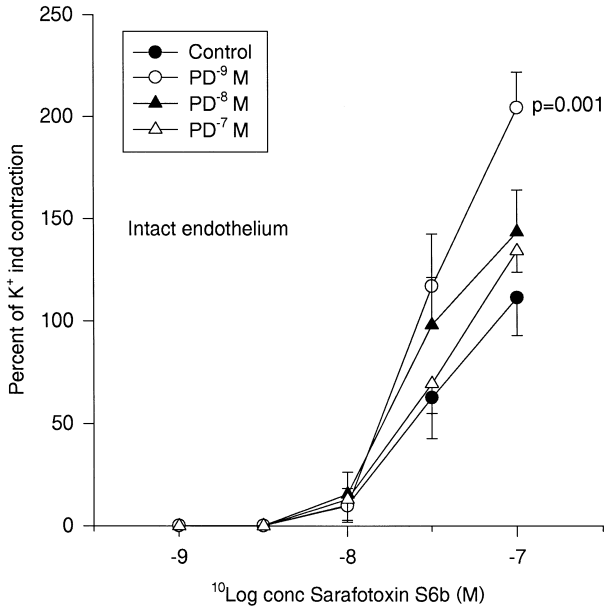

Fig. 2. Contractile response to sarafotoxin S6b in the human umbilical artery in the absence and presence of the nonselective endothelin$_{A/B}$ receptor antagonist PD 142893. Sarafotoxin S6b induced a concentration-dependent contraction which was increased by PD 142893 at 10^{-9} M. Higher concentrations of PD 142893 (10^{-8} and 10^{-7} M respectively) left the contraction unaffected. Means ± S.E.M. ($n = 17$).

2.2. In vitro recording

The arteries were carefully cut into 3–4 mm long ring segments and then slid on to two parallel L-shaped hooks through the lumen of the vessel (Stjernquist and Owman, 1985). The hooks were submersed in an organ bath (volume 4 ml). One of the hooks was connected to a force–displacement transducer (model FT03C, Grass, Quincy, MA, USA), the other one was movable enabling adjustment of smooth muscle tension. The isometric mechanical activity was recorded on a Grass model 7D polygraph.

The organ baths contained a modified Krebs–Ringer solution of the following composition (in mM): NaCl 118, KCl 4.7, $CaCl_2 \times 2H_2O$ 2.0, $MgSO_4 \times 7H_2O$ 1.2, $NaHCO_3$ 24.8, KH_2PO_4 1.2 and glucose 5.6. The organ chambers were continuously aerated with a gas mixture of 88.5% oxygen and 11.5% carbon dioxide, giving a PO_2 of 45 kPa and a PCO_2 of 5.0 kPa, resulting in a pH of 7.35 to 7.45. The temperature of the fluid was thermostatically maintained at 37°C, throughout the experiment. The initial smooth muscle tension was set at 80 mN (Bodelsson and Stjernquist, 1994). After an equilibration period of 1 h, the vascular preparations were contracted by exchanging the 118 mM NaCl in the bathing fluid for 126 mM KCl. The resulting contractile response was registered and used as an internal reference for each vessel segment. After rinsing, concentration–response experiments were carried out as described below.

In a first series of experiments, the selective endothelin$_B$ receptor antagonist BQ 788 (Ishikawa et al., 1994) was added to the organ baths at 0, 10^{-9}, 10^{-8} or 10^{-7} M

and 20 min later either endothelin-1 or sarafotoxin S6b were added in a cumulative manner in 0.5 $_{10}$log units, with registration of the achieved contraction.

In a second series of experiments, the effects of the non-selective endothelin $_{A/B}$ receptor antagonist PD 142-893 (0, 10^{-9}, 10^{-8} or 10^{-7} M; Cody et al., 1992) was investigated in a similar mode as described above for BQ 788.

In a third series of experiments, the function of the endothelium in the contractile response to endothelin-1 or sarafotoxin S6b was examined in the presence or absence of PD 142893 (0, 10^{-9}, 10^{-8} or 10^{-7} M). The first part of the experiment was carried out using the same method as in the aforementioned second series of experiments. In the second part, after washing, the endothelium in each segment was removed by perfusion of the gas mixture through the lumen with a 0.3 mm cannula for 3–5 min at a flow normally used to aerate the organ baths. The absence of a functioning endothelium was verified by elimination of relaxation by substance P (10^{-7} M), of segments precontracted by the buffer solution containing 126 mM KCl. If substance P still elicited a relaxation, the intraluminal gas treatment was reiterated and subsequently tested until no relaxation could be induced (Bodelsson and Stjernquist, 1994). When the endothelium had been successfully removed, the interaction between the agonists and antagonists were investigated again.

In a fourth series of experiments, the possible relaxatory effect of sarafotoxin S6b was investigated in the presence or absence of PD 142893 (10^{-9} M). The endothelin$_{A}$ selective receptor antagonist BQ 123 (10^{-7} M; Ihara et al., 1992) was added to each of the organ baths. After precontraction by 5-hydroxytryptamine (5-HT, 10^{-6} M), a stable contraction was awaited and then sarafotoxin S6b (10^{-10}–10^{-7} M) was added cumulatively. The same protocol could not be used for endothelin-1, as BQ 123 in the concentrations used, does not antagonise endothelin-1 in this particular artery (Bodelsson and Stjernquist, 1993).

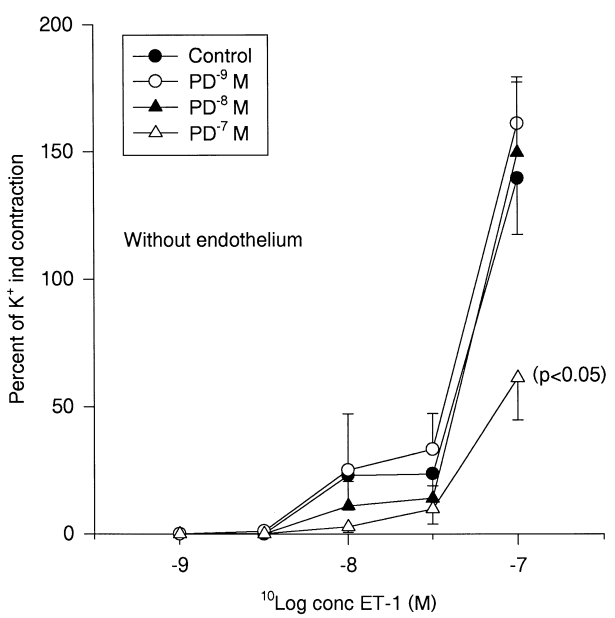

Fig. 3. Contractile response to endothelin-1 in segments of human umbilical artery without endothelium. The non-selective endothelin$_{A/B}$ receptor antagonist PD 142893 (10^{-7} M) still antagonised the contraction induced by endothelin-1. Means ± S.E.M. ($n = 9$).

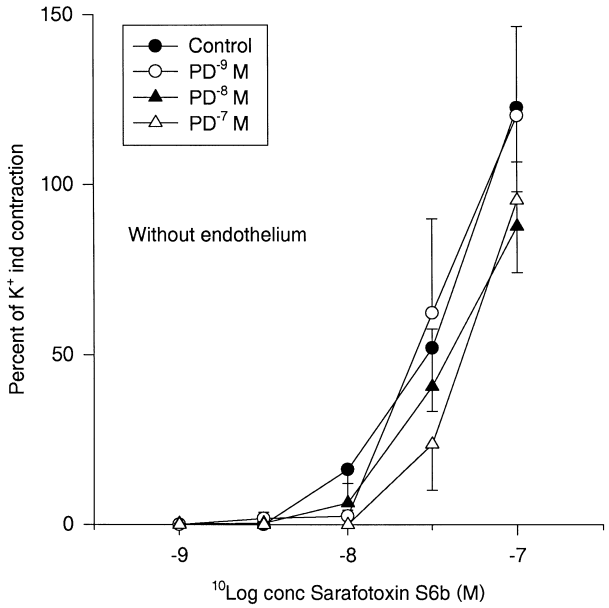

Fig. 4. Contractile response to sarafotoxin S6b in segments of human umbilical artery deprived of their endothelium. The nonselective endothelin$_{A/B}$ receptor antagonist PD 142893 at 10^{-9} M no longer increased the contraction induced by sarafotoxin S6b. Means ± S.E.M. ($n = 8$).

2.3. Drugs

The following compounds were used: human endothelin-1 (Cys-Ser-Cys-Ser-Leu-Met-Asp-Lys-Glu-Cys-Val-Tyr-Phe-Cys-His-Leu-Asp-Ile-Ile-Trp), 5-hydroxytryptamine creatinine sulphate, sarafotoxin S6b (Cys-Ser-Cys-Lys-Asp-Met - Thr-Asp - Cys-Glu -Cys -Leu -Tyr-Phe-Cys-His-Glu-Asp-Val-Ile-Trp), substance P acetate (Sigma, St Louis, MO, USA); BQ 123 (cyclo(D-Asp-Pro-D-Val-Leu-D-Trp) and BQ 788 (*N-cis*-2,6-dimethylpiperidinocarbonyl-L-gamma-methylleucyl-D-1-methoxy-carbonyltryptophanyl-D-norleucine; Calbiochem-Novabiochem. Läufelfingen, Switzerland); PD 142893 (Ac(3,3-D-diphenylalanyl)-L-Leu-L-Asp-L-Ile-L-Ile-L-Trp trifluoroacetate; Neosystem, France). All substances except 5-hydroxytryptamine were dissolved in 0.9% NaCl. 5-hydroxytryptamine was dissolved in 0.9% NaCl containing 0.5 mg EDTA/50 ml NaCl. The concentrations given are final molar concentrations in the organ baths.

2.4. Analysis of data

Values are given as mean ± S.E.M. The number of individuals is indicated by *n*. The results were analysed using two-way repeated measurements analysis of variance

on log transformed data (Ludbrook, 1994). $P < 0.05$ was chosen as level of significance.

3. Results

Both endothelin-1 and sarafotoxin S6b induced comparable concentration-dependent contractions in the human umbilical artery segments. BQ 788 affected neither the contraction in response to endothelin-1 ($n = 5$) nor the one to sarafotoxin S6b ($n = 8$, data not shown). PD 142893 at 10^{-7} M markedly decreased the contractile response to endothelin-1 while lower concentrations (10^{-8} and 10^{-9} M) had no effect (Fig. 1). In the presence of PD 142893 at 10^{-9} M the contractile response to sarafotoxin S6b was increased, whereas higher concentrations of the antagonist did not affect the concentration–response curve (Fig. 2).

Destruction of the endothelium did not affect the contractile response to endothelin-1 ($n = 9$) or sarafotoxin S6b ($n = 8$, data not shown). The effects of PD 142893 on endothelin-1-induced contractions in endothelium deprived segments (Fig. 3) were identical in comparison with segments with an intact endothelium. PD 142893 did not affect the contractile response induced by sarafotoxin S6b in endothelium-deprived segments (Fig. 4).

In vessel segments precontracted by 5-HT and exposed to BQ 123 a small sarafotoxin S6b-induced relaxation was recorded. No relaxation was seen when PD 142893 (10^{-9} M) also was present in the organ chamber (Fig. 5).

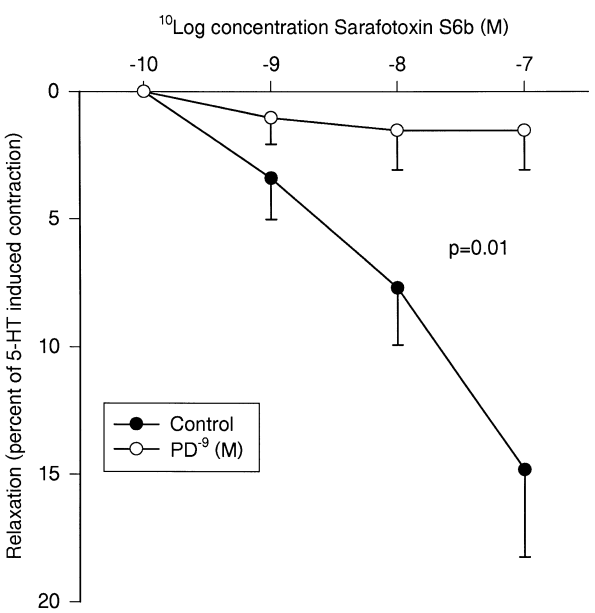

Fig. 5. Relaxatory effect of sarafotoxin S6b in segments of human umbilical artery precontracted by 5-HT (10^{-6} M) and exposed to the selective endothelin$_A$ receptor antagonist BQ 123 (10^{-7} M). Sarafotoxin S6b at 10^{-7} M induced a relaxation. In the presence of the nonselective endothelin$_{A/B}$ receptor antagonist PD 142893 (10^{-9} M) a relaxation could no longer be detected. Means ± S.E.M. ($n = 5$).

4. Discussion

In preparations with multiple receptor subtypes for a common agonist, the effect of the agonist may turn out to be complex. Thus, in vascular preparations with both contraction and relaxation mediating receptors, the net effect of an agonist depends on the balance between simultaneous contracting and relaxing stimulation (see Bodelsson et al., 1989).

The contraction of the human umbilical artery induced by sarafotoxin S6b was enhanced by low concentrations of PD 142893 (10^{-9} M). This could be due to an antagonistic effect by PD 142893 on receptors mediating relaxation, resulting in an enhancement of the contractile effect due to interaction of the agonist with the smooth muscle receptors. Previous studies have revealed that the contractile endothelin receptors in the human umbilical artery do not fulfil the criteria for classical endothelin$_A$ receptors (Bodelsson and Stjernquist, 1993). The present findings indicate that the human umbilical artery also contains relaxation-mediating receptors.

This assumption is supported by the finding that sarafotoxin S6b relaxed vessel segments precontracted by 5-HT in the presence of the endothelin$_A$ receptor antagonist BQ123. PD 142893 at low concentrations corresponding to the concentration at which the contractile response to sarafotoxin S6b was enhanced inhibited the relaxation. Seo (1996) has previously observed an antagonist-induced augmentation of contractile response in response to endothelin-1 in the human gastroepiploic artery. In this preparation, the endothelin$_B$ receptor antagonist Res 701-1 augmented the contractile response induced by endothelin-1. However, another endothelin$_B$ receptor antagonist, BQ 788, was without effect. This is in concordance with our findings: BQ 788 did not alter the contractions induced by sarafotoxin S6b in the human umbilical artery. Nevertheless, BQ 788 has been shown to have considerable affinity for human endothelin$_B$ receptors (Reynolds et al., 1995). The lack of effect of this compound may indicate that the relaxing receptors activated by sarafotoxin S6b are not of the classical endothelin$_B$ subtype. The relaxant response to sarafotoxin S6b in the presence of the endothelin$_A$ receptor antagonist BQ 123 suggests that the receptors involved might not be of the endothelin$_A$ receptor subtype either. They could, therefore, represent non-endothelin$_A$-non-endothelin$_B$ receptors at which sarafotoxin S6b acts as an agonist and PD 142893 as an antagonist. BQ 123 and BQ 788, at the concentrations used, seem not to interact with this receptor.

Removal of the endothelium abolished the PD 142893-induced enhancement of the contraction in response to sarafotoxin S6b. This indicates that the relaxing receptors activated by sarafotoxin S6b are situated on the endothelium. Previously, receptors of the endothelin$_B$ receptor subtype have been shown to mediate endothelium-depen-

dent relaxation (Takayanagi et al., 1991). However, due to lack of effect of BQ 788, the endothelial receptors in the human umbilical artery do not seem to be of the endothelin$_B$ receptor subtype. The presence of an endothelium-dependent relaxation in response to sarafotoxin S6b would probably result in an enhancement of the sarafotoxin S6b induced contraction in endothelium deprived vessel segments. However, this was not the case in the present study and the reason remains to be elucidated. One explanation could be that the endothelium also releases hitherto unknown vasoconstrictors in response to sarafotoxin S6b, and that this mechanism is unaffected by PD 142893.

In the presence of higher concentrations of PD 142893 (10^{-8}–10^{-7} M), the enhancement of the contractile response to sarafotoxin S6b observed at 10^{-9} M PD 142893 was lost. This could be due to an antagonistic effect on contracting receptors at these higher concentrations. Indeed, sarafotoxin S6b contracts the human umbilical artery via receptors highly sensitive to the antagonistic effect of BQ 123 whereas the endothelin-1 mediated contraction is unaffected by BQ 123 at these concentrations (Bodelsson and Stjernquist, 1993; Bogoni et al., 1996). This suggests that the receptors mediating the contractile response to sarafotoxin S6b in the human umbilical artery have some properties in common with endothelin$_A$ receptors. PD 142893 is classically known as a nonselective endothelin$_{A/B}$ receptor antagonist (Cody et al., 1992). It therefore seems reasonable to assume that PD 142893 could antagonise the contractile endothelin$_A$ receptors of the human umbilical artery, at least at higher concentrations.

The contraction induced by endothelin-1 was not augmented by PD 142893 but antagonised at the highest PD 142893 concentration used (10^{-7} M). Removal of the endothelium did not alter this pattern. A possible explanation could be that endothelin-1 does not activate the endothelial non-endothelin$_A$-non-endothelin$_B$ receptor mediating the relaxation induced by sarafotoxin S6b. If so, this is, to our knowledge, the first indication of a human endothelin-like receptor not activated by endothelin-1. This is supported by the observation that endothelium removal did not affect the concentration response curve for endothelin-1, as also previously demonstrated (Bodelsson and Stjernquist, 1993).

Indeed, findings have been presented not being consistent with the classical concept on endothelin$_A$ and endothelin$_B$ receptors. Evidence for endothelin$_B$ receptor mediated contraction, e.g., in coronary arteries (Wang et al., 1995), has been presented, and this subtype of receptor has been designated 'endothelin$_{B2}$' in contrast to the relaxatory 'endothelin$_{B1}$' (Bax and Saxena, 1994). Evidence has also been presented indicating the existence of endothelin receptors that do not fit the current classification of endothelin$_A$ and endothelin$_B$ receptors (Bax and Saxena, 1994; Haynes et al., 1993; Bodelsson and Stjernquist, 1993). These yet unclassified endothelin-receptors have sometimes been designated endothelin$_C$ or non endothelin$_A$–non endothelin$_B$ receptors (Bax and Saxena, 1994).

In conclusion, the endothelium of the human umbilical artery mediates relaxation in response to sarafotoxin S6b. This relaxation is potently antagonised by PD 142893 resulting in an enhancement of the contractile effect of sarafotoxin S6b. The endothelial receptors involved seem neither to be classical endothelin$_A$ nor classical endothelin$_B$ receptors and they are not activated by endothelin-1.

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