British Journal of Pharmacology (1997) 120, 25–30

Endothelium-dependent relaxation by substance P in human isolated omental arteries and veins: relative contribution of prostanoids, nitric oxide and hyperpolarization

[1]Susanna M. Wallerstedt & Mikael Bodelsson

Department of Anaesthesiology, University Hospital, S-221 85, Lund, Sweden

1 The objective of the present study was to investigate human omental arteries and veins with respect to: (i) the contractile effect of the thromboxane A_2 analogue U46619, (ii) endothelium-dependency and mediators of the relaxing effect of substance P (SP) and acetylcholine (ACh).

2 Changes in isometric tension in response to administration of U46619, SP and ACh were measured in human isolated omental arteries and veins with and without endothelium. To investigate the mechanism of action of SP, the SP-induced relaxation was measured in the presence of indomethacin (cyclo-oxygenase inhibitor), N^G-monomethyl-L-arginine (L-NMMA, nitric oxide-synthase inhibitor), KCl (inhibitor of endothelium-dependent hyperpolarization), tetraethylammonium (TEA; non-selective inhibitor of K^+-channels, with some preference for the high conductance Ca^{2+}-activated K^+-channel, BK_{Ca}), glibenclamide (inhibitor of the ATP-sensitive K^+-channel) and/or clotrimazole (inhibitor of the cytochrome P450-system and the intermediate conductance Ca^{2+}-activated K^+-channel, IK_{Ca}).

3 U46619 contracted both the artery and the vein segments. Endothelium removal did not alter the contraction.

4 ACh caused neither contraction nor relaxation in artery and vein segments precontracted with U46619.

5 In both artery and vein segments precontracted with U46619, SP produced endothelium-dependent relaxation. The relaxation was unaffected by indomethacin, but was incompletely reduced by L-NMMA and KCl respectively. The L-NMMA-resistent relaxation was abolished in the presence of KCl.

6 TEA inhibited the SP-induced relaxation in artery and vein segments both in the presence and absence of L-NMMA and indomethacin, while glibenclamide and clotrimazole had no effect.

7 In conclusion, the SP-induced relaxation in human omental arteries and veins seems to be mediated via NO and endothelium-dependent hyperpolarization. K_{ATP} and I_{KCa} are probably not involved in the hyperpolarization, but activation of BK_{Ca} may contribute to the hyperpolarization. Prostanoid synthesis and the cytochrome P450-system are probably not involved in the SP-induced relaxation in this area.

Keywords: Human blood vessels; substance P; nitric oxide; hyperpolarization; K^+ channels

Introduction

The regulation of human splanchnic circulation is intricate. The splanchnic vascular bed receives 25% of cardiac output (Folkow & Neil, 1971) and it therefore plays an important role in the blood pressure regulation. Prostanoids and various neuropeptides may modulate the gastrointestinal vascular smooth muscle tone.

The stable thromboxane A_2 analogue U46619, a prostanoid, is a potent vasoconstrictor (Coleman *et al*., 1984), which has been shown to contract various types of human vessels: superficial hand and wrist veins (Arner *et al*., 1991), oesophageal submucosal veins (Madsen *et al*., 1992), inferior epigastric artery and internal mammary artery (Tadjkarimi *et al*., 1993), bronchial and pulmonary artery (Norel *et al*., 1991), small placental arteries (Andrew *et al*., 1994), umbilical artery (Bodelsson *et al*., 1995) and pial arteries (Petersson *et al*., 1995). In this study the effects of U46619 on human omental arteries and veins with and without endothelium were investigated.

Substance P (SP) is a vasoactive neuropeptide. SP has been found to relax vascular smooth muscle of various types of human vessels: pial arteries (Petersson *et al*., 1995), umbilical artery (Bodelsson & Stjernquist, 1994), skeletal muscle arteries (Pernow, 1989) mesenteric arteries and veins (Törnebrandt *et al*., 1987) and superficial hand veins (Bodelsson *et al*., 1990). Whenever investigated, the SP-induced relaxation has been found to be endothelium-dependent in human blood vessels. However, recent studies have shown that endothelium-independent mechanisms can be involved as well, at least in the dog (Enokibori *et al*., 1994). Three mechanisms have been proposed to be involved in the SP-induced relaxation: (i) NO formation, (ii) prostanoid synthesis and (iii) endothelium-dependent hyperpolarization. Depending on species and location of the vessel the mechanism of action seems to be via synthesis of NO (Rosenblum *et al*., 1993), prostanoids (Bodelssoon & Stjernquist, 1994), both NO synthesis and prostanoids (Enokibori *et al*., 1994) or both NO and hyperpolarization (Petersson *et al*., 1995).

The effects of SP on human splanchnic circulation are not yet fully understood. The present study was designed to clarify the endothelium-dependency and the mediators involved in SP-induced relaxation in human omental arteries and veins. As a reference for endothelium-dependent relaxation, experiments with acetylcholine (ACh) were also performed.

Methods

In vitro *experiments*

The study was approved by the Ethical Committee of the University of Lund.

Macroscopically normal segments of human omental arteries and veins were obtained from 42 patients aged 26–93 years (median 70.5) undergoing abdominal surgery. Patients

[1] Author for correspondence

with endocrine tumours, abdominal infections and previous radiotherapy were excluded. The male/female ratio was 1:3. The experiments were carried out within 24 h, meanwhile the vessels, after being dissected free from fat and connective tissue, were stored in aerated Krebs-Ringer solution (composition, see below) at +4°C. The vessels were cut into 2–4 mm long ring segments and were placed in 2 ml tissue baths on two L-shaped hooks, one of which was attached to a Grass FTO3C force-displacement transducer for isometric measurement of tension. The vessel tension was recorded on a Grass polygraph model 7b. The baths were thermostatically kept at 37°C and contained Krebs-Ringer solution (composition, see below), which was continuously aerated with 88.5% O_2 and 11.5% CO_2, to give pH 7.4, P_{CO_2} 5.0 kPa and P_{O_2} approximately 40 kPa.

In the first series of experiments, the optimal resting tension of the vessel segments was determined by stretching the vessels to an equilibrated tension level of 2, 4, 6, 8, 10 and 12 mN and the contraction induced by 90 mM KCl was obtained. The optimal tension for both the artery and vein segments was found to be 6 mN, but did not vary between 4 and 8 mN. In all subsequent experiments the vessel segments were gradually stretched to a resting tension of 4–8 mN, during an equilibration period of 60–90 min.

In a second series of experiments, the contractile effect of U46619 and the effect and endothelium-dependency of SP and ACh were investigated. The endothelium of one artery and one vein segment from 9 patients was removed by gentle injection of the O_2/CO_2 gas mixture through the vessel lumen for five minutes (Bodelsson *et al.*, 1989). The other 1-2 artery and 1-2 vein segments run in parallel served as controls. After the equilibration period, KCl (90 mM) was added to the baths repeatedly, each time followed by wash-out, until consistent contractions were elicited (two to three times). For each vessel segment, the greatest contraction elicited was used as a contraction reference. After thorough wash-out, the stable thromboxane A_2 analogue U46619 was added cumulatively in $\log_{10}$ units (10^{-11}–10^{-5} M) and the resulting contraction was registered. The concentration required to achieve half maximum contraction (EC_{50}) was calculated. After wash-out, U46619, at a concentration corresponding to the calculated EC_{50} value, was added to all baths and the vessel segments were left to equilibrate for 10–15 min, until the level of contraction was stable. Then SP or ACh was added cumulatively in $\log_{10}$ units (10^{-11}–10^{-5} M for SP and 10^{-9}–10^{-4} M for ACh) and the relaxation or contraction was registered. When control vessel segments did not respond to SP all segments from that patient were considered injured and were consequently discarded.

In a third series of experiments, the aim of which was to determine whether the relaxation induced by SP was mediated via prostanoid synthesis or formation of NO, the relaxing effect of SP on intact segments precontracted with U46619 (*vide supra*) was investigated in the presence and absence of the cyclo-oxygenase inhibitor indomethacin (10^{-5} M) or the nitric oxide-synthase inhibitor L-N^G-monomethyl-arginine (L-NMMA, 3×10^{-4} M).

In a fourth series of experiments, the role of hyperpolarization in the SP-induced relaxation was investigated. The relaxing effect of SP on intact vessel segments (precontracted with U46619) treated with L-NMMA (3×10^{-4} M) and indomethacin (10^{-5} M) was compared to that in segments which, in addition to L-NMMA and indomethacin, were also exposed to 30 mM KCl. As a reference, relaxation experiments were also performed on one artery and one vein segment exposed to 30 mM KCl alone.

In a fifth series of experiments the mechanism of the SP-induced hyperpolarization was investigated. Experiments were performed as above with U46619 as a precontractor both in the presence and the absence of L-NMMA and indomethacin and with tetraethylammonium (TEA; non-selective inhibitor of K^+-channels, 10^{-2} M), glibenclamide (inhibitor of the ATP-sensitive K^+-channel, K_{ATP}, 10^{-5} M) or clotrimazole (inhibitor of the cytochrome P450-system and the intermediate conductance Ca^{2+}-activated K^+-channel, I_{KCa}, 10^{-5} M) present in the baths. The solvent dimethyl sulphoxide (DMSO) in equal amount as in the glibenclamide and clotrimazole baths was added to the TEA and control baths. SP (10^{-11}–10^{-6} M) was added and the resulting relaxation was recorded.

In the experiment series 2 to 5, 1–2 concentration-response experiments with SP were performed on the same segment. Initial experiments had shown that the results from two consecutive concentration-response experiments with SP separated with rinsing did not differ. Control experiments were performed on separate segments from the same patient and always run in parallel.

Analysis of data

The maximum response elicited by the agonist (E_{Am}) and the EC_{50} value were calculated. EC_{50} values are expressed as pD_2 values, which are defined as $-\log_{10}(EC_{50})$. When similar experiments were performed on more than one segment from the same patient, the mean for each patient was calculated before presentation and statistical analysis. The number of patients is indicated with *n*. Values are expressed as mean ± s.e.mean. Wilcoxon's signed rank test was used for statistical evaluation. A *P* value less than 0.05 was considered statistically significant and only significant differences are mentioned in the text.

Drugs

The following compounds were used: 9,11-dideoxy-11α,9α-epoxy-methanoprostaglandin $F_{2\alpha}$ (U46619, Sigma); substance P acetate (Sigma); acetylcholine chloride (Sigma); sodium indomethacin (Confortid, Dumex); N^G-monomethyl-L-arginine acetate (L-NMMA, Sigma); dimethyl sulphoxide (DMSO, Sigma); tetraethylammoniumchloride (TEA, Sigma); glibenclamide (Sigma); clotrimazole (Sigma); disodium-calcium EDTA (Sigma). Sodium indomethacin was dissolved in a phosphate buffer and glibenclamide and clotrimazole were dissolved in DMSO. All other compounds were dissolved in distilled water. Indomethacin, L-NMMA, KCl (30 mM), TEA, glibenclamide and clotrimazole were added to the organ baths 15 min before the relaxation experiments. The Krebs-Ringer solution contained (mM): Na^+ 143, K^+ 4.6, Cl^- 126.4, Ca^{2+} 2.5, HCO_3^- 25.0, Mg^{2+} 0.79, SO_4^{2-} 0.79, $H_2PO_4^-$ 1.2, glucose 5.5 and EDTA 0.024.

Results

U46619 induced a concentration-dependent sustained contraction in the human omental artery and vein segments, the E_{Am} and the EC_{50} being greater in the vein segments. Removal of the endothelium did not affect the concentration-response curve for U46619 (Figure 1). During repeated wash-out the U46619-induced active tension returned to baseline level of tension within 45 min.

In both artery and vein segments precontracted with U46619 and with intact endothelium, SP (10^{-11}–10^{-5} M) induced concentration-dependent relaxation (Figure 2). In most vessel segments, the SP-induced relaxation was transient and, unless a consecutive incremental dose of SP was given, the vessel tension returned to the level of precontraction. Neither in artery nor in vein segments denuded of their endothelium did SP induce relaxation. In the presence of L-NMMA, the relaxation induced by SP in vessel segments with intact endothelium was diminished, whereas indomethacin did not alter the SP-induced response (Figure 2). E_{Am} and pD_2 values are shown in Table 1.

Since all vessel segments showed L-NMMA-resistant relaxation, the mechanism for this relaxation was investigated in the presence of both L-NMMA and indomethacin with or without KCl (30 mM). In both artery and vein segments, the SP-induced relaxation was almost completely abolished in the presence of KCl, L-NMMA and indomethacin. The relaxation

in the presence of indomethacin and L-NMMA was similar to the relaxation seen in the experiments with segments exposed to L-NMMA alone (Figures 2 and 3, Table 1). Segments exposed to KCl in the absence of L-NMMA and indomethacin responded to SP concentration-dependently, although the response was weaker than the response seen in the experiments with untreated segments (Figures 2 and 3). E_{Am} and pD_2 values are shown in Table 1.

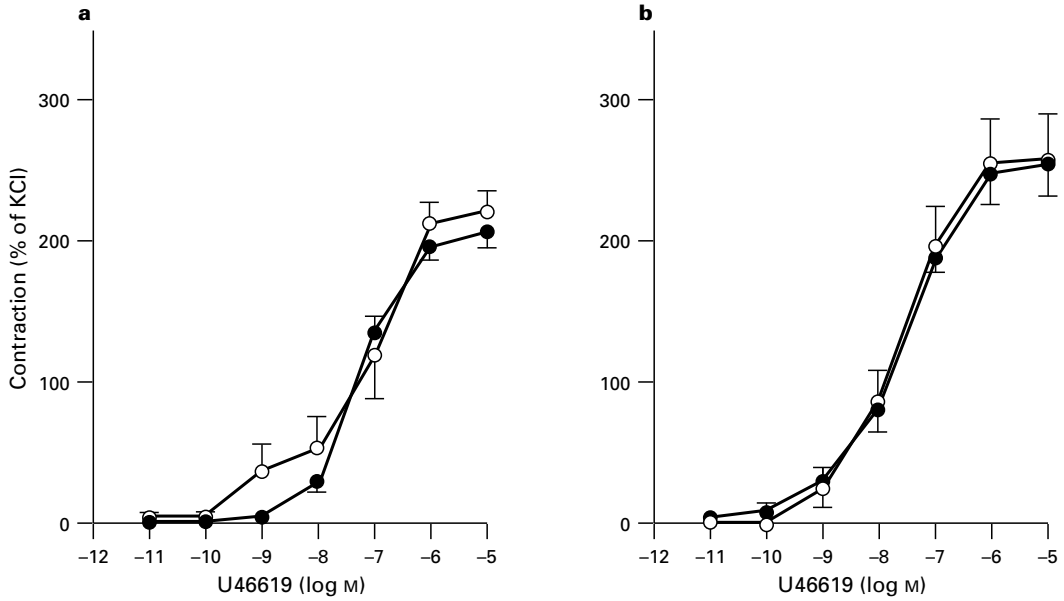

Figure 1 (a) Concentration-response curves for the thromboxane analogue U46619 in human omental artery segments with intact (●, $E_{Am}=206\pm10\%$, $pD_2=7.30\pm0.09$, $n=23$) and denuded (○, $E_{Am}=221\pm16\%$, $pD_2=7.42\pm0.38$, $n=9$) endothelium. The contraction is expressed as % of the contraction induced by KCl (90 mM), which was (mN) 10.8 ± 1.5 (intact endothelium) and 5.7 ± 1.2 (endothelium removed). (b) Concentration-response curves for the thromboxane analogue U46619 in human omental vein segments with intact (●, $E_{Am}=255\pm25\%$, $pD_2=8.10\pm0.23$, $n=16$) and denuded (○, $E_{Am}=258\pm33\%$, $pD_2=7.98\pm0.28$, $n=8$) endothelium. The contraction is expressed as % of the contraction induced by KCl (90 mM), which was (mN) 6.7 ± 1.1 (intact endothelium) and 5.6 ± 1.9 (endothelium removed). The contractile responses to U46619 was not affected by removal of the endothelium in either artery or vein segments. Values are expressed as mean and vertical lines show s.e.mean.

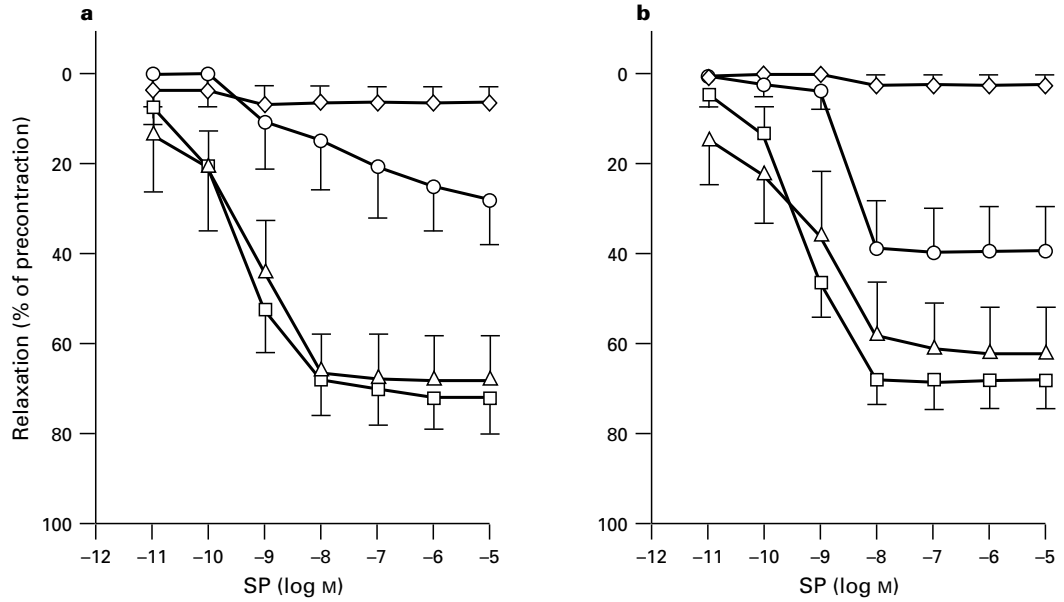

Figure 2 (a) Concentration-response curves for substance P (SP) in human omental artery segments precontracted with U46619; control (□, $n=15$), in the presence of indomethacin (△, 10^{-5} M, $n=5$), in the presence of L-NMMA (○, 3×10^{-4} M, $n=5$) and after removal of the endothelium (◇, $n=6$). The relaxation is expressed as % of the precontraction induced by U46619 before addition of SP, which was (mN) 13.4 ± 1.2 (control), 15.3 ± 2.8 (indomethacin), 15.2 ± 2.1 (L-NMMA) and 8.2 ± 2.3 (endothelium denuded). SP induced relaxation in all artery segments with intact endothelium. (b) Concentration-response curves for substance P (SP) in human omental vein segments precontracted with U46619; control (□, $n=17$), in the presence of indomethacin (△, 10^{-5} M, $n=7$), in the presence of L-NMMA (○, 3×10^{-4} M, $n=9$) and after removal of the endothelium (◇, $n=6$). The relaxation is expressed as % of the precontraction induced by U46619 before addition of SP, which was (mN) 6.7 ± 1.1 (control), 11.5 ± 2.4 (indomethacin), 11.8 ± 2.1 (L-NMMA) and 5.6 ± 1.9 (endothelium-denuded). SP induced relaxation in all vein segments with intact endothelium. In (a) and (b) L-NMMA diminished the relaxation induced by SP, while indomethacin had no effect. Endothelium removal abolished the relaxation induced by SP. Values are expressed as mean and vertical lines show s.e.mean.

Table 1 E_{Am} and pD_2 values for the relaxing response of substance P (SP) in human endothelium intact omental arteries and veins precontracted with U46619

	Artery			*Vein*		
	E_{Am} (%)	pD_2	n	E_{Am} (%)	pD_2	n
Control	72±8	9.38±0.28	15	68±6	9.41±0.18	17
L-NMMA	35±3*	7.92±0.71*	5	39±10*	8.57±0.18*	9
Indomethacin	68±10	10.00±0.50	5	62±11	9.42±0.58	7
KCl	17±6*	9.00±0.29	5	32±4*	9.01±0.15	5
L-NMMA + indomethacin	20±8*	8.21±0.12*	5	36±5*	8.50±0*	5
L-NMMA + indomethacin + KCl	0	–	5	7.8±4.9*	8.50±0*	5

E_{Am} is expressed as % of precontraction. Data shown are means±s.e.mean. *Significantly different from control. Wilcoxon's signed rank test. E_{Am}, maximum response; pD_2, negative $\log_{10}$ of the concentration required to acheive half maximum contraction; L-NMMA, N^G-monomethyl-L-arginine acetate.

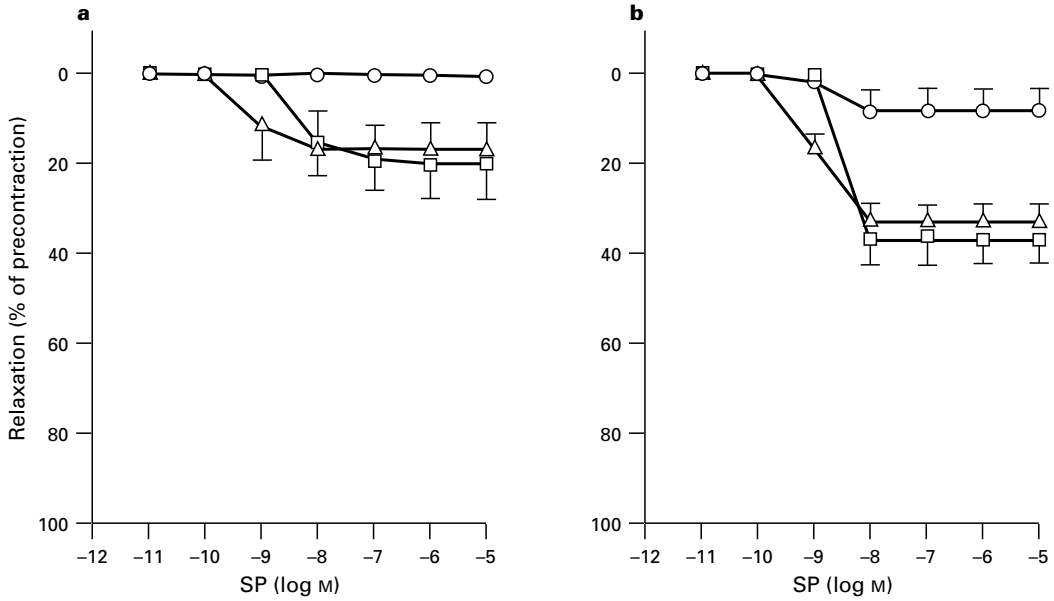

Figure 3 (a) Concentration-response curves for substance P (SP) in human omental artery segments precontracted with U46619 in the presence of L-NMMA and indomethacin (□, $n=5$), 30 mM KCl (△, $n=5$) and L-NMMA, indomethacin and 30 mM KCl (○, $n=5$). The relaxation is expressed as % of the precontraction induced by U46619 before addition of SP, which was (mN) 18.0±2.0 (L-NMMA and indomethacin), 18.6±1.2 (KCl) and 20.2±1.7 (L-NMMA, indomethacin and KCl). (b) Concentration-response curves for substance P (SP) in human omental vein segments precontracted with U46619 in the presence of L-NMMA and indomethacin (□, $n=5$) 30 mM KCl (△, $n=5$) and L-NMMA, indomethacin and 30 mM KCl (○, $n=5$). The relaxation is expressed as % of the precontraction induced by U46619 before addition of SP, which was (mN) 18.7±6.1 (L-NMMA and indomethacin), 15.4±2.4 (KCl) and 14.2±2.0 (L-NMMA, indomethacin and KCl). In (a) and (b) addition of 30 mM KCl abolished the L-NMMA- and indomethacin-resistant relaxation. Values are expressed as mean and vertical lines show s.e.mean.

The L-NMMA-resistant SP-induced relaxation was further investigated in the presence of TEA, glibenclamide or clotrimazole. TEA shifted the SP-induced concentration-response curve to the right and attenuated the maximum response in both artery and vein segments, though the artery segments were more affected than the vein segments. (Figure 4). TEA also inhibited the SP-induced relaxation in the absence of L-NMMA and indomethacin (artery: $n=6$; vein: $n=6$, data not shown). Glibenclamide and clotrimazole had no effect on the SP-induced relaxation, either in the presence or absence of L-NMMA and indomethacin (artery: glibenclamide $n=6$, clotrimazole $n=6$, vein: glibenclamide $n=8$, clotrimazole $n=7$, data not shown).

ACh induced neither relaxation nor contraction in intact or denuded segments (artery: $n=7$, vein: $n=5$).

Discussion

U46619 has been shown to contract human isolated vessels obtained from various locations (Arner *et al*., 1991; Norel *et al*., 1991; Madsen *et al*., 1992; Tadjkarimi 1993; Andrew *et al*., 1994; Bodelsson *et al*., 1995). The results obtained in the present study show that U46619 induces concentration-dependent contraction not affected by endothelium removal in human omental arteries and veins. This indicates that functional receptors for U46619 are present in both human omental arteries and veins, and that these receptors are not located on the endothelium. Tachyphylaxis was not seen and the time for the vessels to return to baseline was long and required repeated wash-outs. A long-lasting, stable contraction not affected by endothelial removal suggests that U46619 is a suitable precontractor in relaxation experiments on human omental vessels.

A previous study on human isolated splanchnic blood vessels has shown that SP dilates both mesenteric arteries and veins in a similar manner (Törnebrandt *et al*., 1987). However, in this study the role of endothelium was not determined. In the present study, SP was found to relax potently human omental arteries and veins with intact endothelium similarly. In endothelium-denuded vessels no significant relaxation was obtained. This indicates an endothelium-dependent relaxing

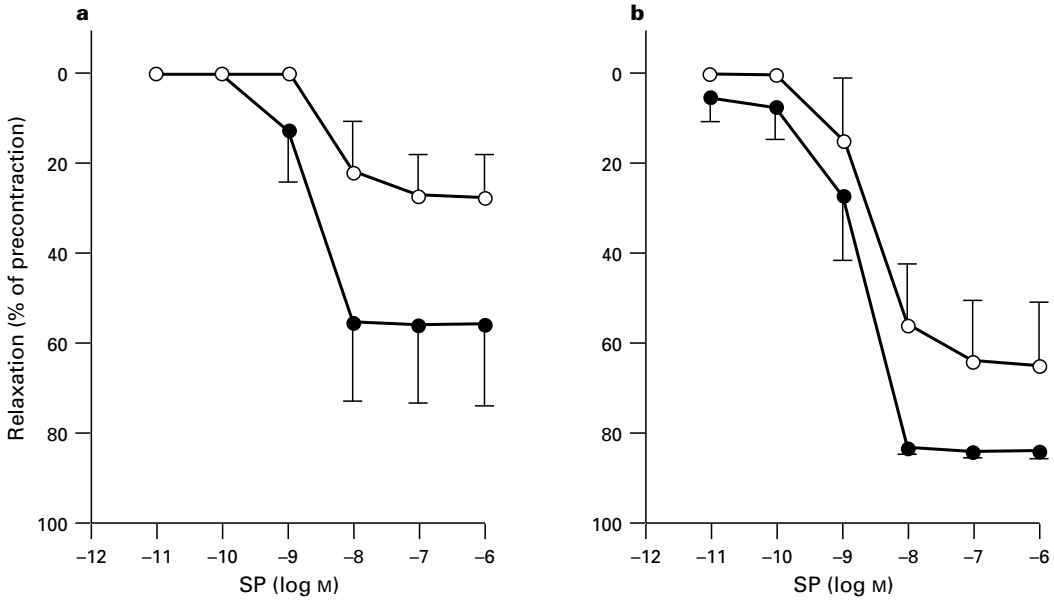

Figure 4 Concentration-response curves for substance P (SP) in human omental artery (a) and vein (b) segments exposed to L-NMMA and indomethacin and precontracted with U46619 in the absence (●) and presence (○) of TEA (10^{-2} M). The relaxation is expressed as % of the precontraction induced by U46619 before addition of SP, which was (mN) 21.1±3.7 (artery, absence of TEA, $n=6$), 28.3±6.6 (artery, presence of TEA, $n=6$), 13.4±4.7 (vein, absence of TEA, $n=6$) and 18.6±5.1 (vein, presence of TEA, $n=6$). TEA inhibited the L-NMMA- and indomethacin-resistent relaxation in both artery and vein segments. Values are expressed as mean and vertical lines show s.e.mean.

action of SP, as previously shown for blood vessels from both experimental animals (Onoue *et al*., 1988; Pernow, 1989) and man (Bodelsson *et al*., 1990; Bodelsson & Stjernquist, 1994; Petersson *et al*., 1995).

NO seems to be an important mediator of the SP-induced relaxation in human omental vessels since the nitric oxide synthase inhibitor L-NMMA diminished the relaxation. This is consistent with findings in some other vessels such as mouse pial arterioles (Rosenblum *et al*., 1993), dog superficial temporal arteries (Enokibori *et al*., 1994), pig and rabbit carotid arteries (Fiscus *et al*., 1992; Gross *et al*., 1994), dog cerebral arteries (Onoue *et al*., 1988) and human pial arteries (Petersson *et al*., 1995). In the present study the cyclo-oxygenase inhibitor indomethacin did not affect the relaxation induced by SP, indicating that prostanoid synthesis is not involved in the relaxing mechanism triggered by SP, which contrasts to the findings in the human umbilical artery, where prostanoids, but not NO, mediate the SP-induced relaxation (Bodelsson & Stjernquist, 1994).

Solutions containing high K^+ concentration can be used to prevent endothelium-dependent hyperpolarization (Nagao & Vanhoutte, 1992; Zygmunt *et al*., 1994a,b). In the present study, addition of KCl abolished the SP-induced relaxation remaining in the presence of L-NMMA and indomethacin. This finding indicates that hyperpolarization probably is involved in the SP-induced relaxation in human omental arteries and veins. This is consistent with findings in guinea-pig carotid artery (Zhang *et al*., 1994), porcine coronary artery (Sharma & Davis, 1994) and human cerebral arteries (Petersson *et al*., 1995). Since no relaxation was seen in endothelium-denuded vessels, the hyperpolarization seems to be endothelium-dependent. Endothelium-dependent hyperpolarization has been suggested to result from formation of endothelium-derived hyperpolarizing factor/factors (EDHF; Taylor & Weston, 1988). Recent evidence suggests that EDHF could be a cytochrome P450-derived arachidonic acid metabolite (Hu & Kim, 1993; Bauersachs *et al*., 1994). Clotrimazole is an inhibitor of the cytochrome P450-system previously shown to inhibit NO/prostanoid-independent vascular smooth muscle relaxation (Lischke *et al*., 1995). It must be noted that clotrimazole also is an inhibitor of the intermediate conductance Ca^{2+}-activated K^+-channel (I_{KCa}; Watson & Girdlestone, 1996). Thus, the inhibitory effect of this compound on NO/prostanoid-independent vascular smooth muscle relaxation could, at least in part, also be an effect on smooth muscle K^+-channels. When used in the present study, clotrimazole had no effect on the SP-induced relaxation either in the presence or absence of L-NMMA and indomethacin. This indicates that SP-induced hyperpolarization in human omental arteries and veins is not due to formation of a cytochrome P450-derived EDHF.

To judge from the relaxation remaining after treatment with L-NMMA and KCl, NO and EDHF synthesis each contributes to about half of the endothelium-dependent relaxation in the vein. In the artery, the effects of L-NMMA and KCl are more pronounced than would be expected by a simple additive effect of NO and EDHF. These findings suggest that in the artery, NO and EDHF act in synergy, potentiating the effect of each other.

L-NMMA shifted the concentration-response curve for SP to the right, while in the presence of 30 mM KCl the E_{Am} value was more affected than the EC_{50} value. This might indicate that the relaxation induced by low concentrations of SP is mainly mediated via the NO pathway. At higher concentrations of SP, hyperpolarization becomes more important. This differentiation of the mechanism mediating relaxation was more evident in the arteries than in the veins. A more prominent role of NO than hyperpolarization in the SP-induced relaxation has also been shown in the pig coronary artery (Kilpatrick & Cocks, 1994).

K^+-channels modify the membrane potential, thus affecting the tone of the smooth muscle cell. An opening of the K^+-channels results in K^+ efflux, hyperpolarization and relaxation (Edwards & Weston, 1994). TEA, a non-selective inhibitor of K^+-channels, with some preference for the high conductance Ca^{2+}-sensitive K^+-channel, BK_{Ca}, inhibited the SP-induced relaxation in the artery and vein segments both in the presence and absence of L-NMMA and indomethacin. This indicates that SP may activate BK_{Ca} in human omental arteries and veins, which results in hyperpolarization and relaxation. The hyperpolarization

in human omental vessels can probably not be solely explained by activation of BK_{Ca}, since the L-NMMA-resistent relaxation was not completely inhibited by TEA. Thus another mechanism for SP-induced hyperpolarization, not identified in the present study, may exist. The more conspicuous inhibition by TEA of the L-NMMA-resistent relaxation in the artery than in the vein is consistent with the more pronounced effect of KCl in this vessel compared to the vein. K_{ATP} and I_{KCa} seem not to be involved in the SP-induced relaxation, since neither glibenclamide nor clotrimazole had any effect on the SP-induced relaxation in the presence or absence of L-NMMA. The assumption that SP acts independently of K_{ATP} agrees with findings in the rat aorta and porcine coronary artery (Bray & Quast, 1991).

In contrast to many other vascular preparations investigated, ACh had no effect on human omental vessels. This indicates that the role of ACh in splanchnic blood flow regulation may be less important.

In conclusion, the relaxing action of SP in human omental arteries and veins seems to be mediated via two different endothelium-dependent pathways: NO-synthesis and smooth muscle hyperpolarization. The hyperpolarization could partly be due to activation of BK_{Ca}.

This study was supported by the Lund University Hospital Research Funds, the Anna Lisa & Sven-Eric Lundgren foundation and the Swedish Society of Medicine.

References

ANDREW, L., MCCARTHY, M.B., WOOLFSON, R.G., EVANS, B.J., DAVIES, D.R., RAJU, S.K. & POSTON, L. (1994). Functional characteristics of small placental arteries. *Am. J. Obstet. Gynecol.*, **170,** 945–951.

ARNER, M., HÖGESTÄTT, E.D. & USKI, T.K. (1991). Characterization of contraction-mediating prostanoid receptors in human hand veins: effects of the thromboxane receptor antagonists BM13,505 and AH23848. *Acta Physiol. Scand.*, **141,** 79–86.

BAUERSACHS, J., HECKER, M. & BUSSE, R. (1994). Display of the characteristics of endothelium-derived hyperpolarizing factor by a cytochrome P450-derived arachidonic metabolite in the coronary microcirculation. *Br. J. Pharmacol.*, **113,** 1548–1553.

BODELSSON, G., MARSAL, K. & STJERNQUIST, M. (1995). Reduced contractile effect of endothelin-1 and noradrenalin in human umbilical artery from pregnancies with abnormal umbilical artery flow velocity waveforms. *Early Hum. Dev.*, **42,** 15–28.

BODELSSON, G. & STJERNQUIST, M. (1994). Endothelium-dependent relaxation to substance P in human umbilical artery is mediated via prostanoid synthesis. *Hum. Reprod.*, **9,** 733–737.

BODELSSON, M., ARNEKLO-NOBIN, B. & TÖRNEBRANDT, K. (1989). Cooling augments contractile response to 5-hydroxytryptamine via an endothelium-dependent mechanism. *Blood Vessels*, **26,** 347–359.

BODELSSON, M., ARNEKLO-NOBIN, B. & TÖRNEBRANDT, K. (1990). Effect of cooling on smooth muscle response to 5-hydroxytryptamine in human hand veins. *Acta Physiol. Scand.*, **140,** 331–339.

BRAY, K. & QUAST, U. (1991). Differences in the K^+ channel opened by cromakalim, acetylcholine and substance P in rat aorta and porcine coronary artery. *Br. J. Pharmacol.*, **102,** 585–594.

COLEMAN, R.A., HUMPHREY, P.P.A., KENNEDY, I. & LUMLEY, P. (1984). Prostanoid receptors - the development of a working classification. *Trends Pharmacol. Sci.*, **5,** 303–306.

EDWARDS, G. & WESTON, A.H. (1994). Effect of potassium channel modulating drugs on isolated smooth muscle. In *Handbook of Experimental Pharmacology*. ed. Szekeres, L. & Papp, J.G. III, pp. 469–531. Heidelberg: Springer.

ENOKIBORI, M., OKAMURA, T. & TODA, N. (1994). Mechanism underlying substance P-induced relaxation in dog isolated superficial temporal arteries. *Br. J. Pharmacol.*, **111,** 77–82.

FISCUS, R.R., GROSS, D.R. & HAO, H. (1992). N-omega-nitro-L-arginine blocks the second phase but not the first phase of the endothelium-dependent relaxations induced by substance P in isolated rings of pig carotid artery. *J. Cardiovasc. Pharmacol.*, **20** (suppl.12), 105–108.

FOLKOW, B. & NEIL, E. (1971). Gastrointestinal and liver circulations. In *Circulation*. pp. 466–467, New York: Oxford University Press.

GROSS, D.R., FISCUS, R.R., AREDEN, W.A., MALEY, R.H., LANZO, S. & SALLEY, R.K. (1994). Substance P induces biphasic endothelium-dependent relaxations in pig and rabbit carotid arteries. *Neuropeptides*, **26,** 329–341.

HU, S. & KIM, H.S. (1993). Activation of K^+ channel in vascular smooth muscle by cytochrome P450 metabolites of arachidonic acid. *Eur. J. Pharmacol.*, **230,** 215–221.

LISCHKE, V., BUSSE, R. & HECKER, M. (1995). Selective inhibition by barbiturates of the synthesis of endothelium-derived hyperpolarizing factor in the rabbit carotid artery. *Br. J. Pharmacol.*, **115,** 969–974.

KILPATRICK, E.V. & COCKS, T.M. (1994). Evidence for differential roles of nitric oxide (NO) and hyperpolarization in endothelium-dependent relaxation of pig isolated coronary artery. *Br. J. Pharmacol.*, **112,** 557–565.

MADSEN, G., TOTTRUP, A., ANDERSSON, K-E. & FORMAN, A. (1992). Mechanical effects of some prostanoids on isolated human oesophageal submucosal veins. *Pharmacol. Toxicol.*, **71,** 330–334.

NAGAO, T. & VANHOUTTE, P.M. (1992). Hyperpolarization as a mechanism for endothelium-dependent relaxations in the porcine coronary artery. *J. Physiol.*, **445,** 355–367.

NOREL, X., LABAT, C., GARDINER, P.J. & BRINK, C. (1991). Inhibitory effects of BAY u3405 on prostanoid-induced contractions in human isolated bronchial and pulmonary arterial muscle preparations. *Br. J. Pharmacol.*, **104,** 591–595.

ONOUE, H., NAKAMURA, N. & TODA, N. (1988). Endothelium-dependent and -independent responses to vasodilators of isolated dog cerebral arteries. *Stroke*, **19,** 1388–1393.

PERNOW, J. (1989). Actions of constrictor (NPY and endothelin) and dilator (substance P, CGRP and VIP) peptides on pig splenic and human skeletal muscle arteries: involvement of the endothelium. *Br. J. Pharmacol.*, **97,** 983–989.

PETERSSON, J., ZYGMUNT, P.M., BRANDT, L. & HÖGESTÄTT, E.D. (1995). Substance P-induced relaxation and hyperpolarization in human cerebral arteries. *Br. J. Pharmacol.*, **115,** 889–894.

ROSENBLUM, W.I., SHIMIZU, T. & NELSON, G.H. (1993). Endothelium-dependent effects of substance P and calcitonin gene-related peptide on mouse pial arterioles. *Stroke*, **24,** 1043–1048.

SHARMA, N.R. & DAVIS, M.J. (1994). Mechanism of substance P-induced hyperpolarization of porcine coronary artery endothelial cells. *Am. J. Physiol.*, **266,** H156–H164.

TADJKARIMI, S., O'NEIL, G.S., SCHYNS, C.H., BORLAND, J.A., CHESTER, A.H. & YACOUB, M.H. (1993). Vasoconstrictor profile of the inferior epigastric artery. *Ann. Thorac. Surg.*, **56,** 1090–1095.

TAYLOR, S.G. & WESTON, A.H. (1988). Endothelium-derived hyperpolarization factor: a new endogenous inhibitor from the vascular endothelium. *Trends Pharmacol. Sci.*, **9,** 272–274.

TÖRNEBRANDT, K., NOBIN, A. & OWMAN, C. (1987). Vasomotor effects of nine neuropeptides on isolated human mesenteric blood vessels. *Peptides*, **8,** 251–256.

WATSON, S. & GIRDLESTONE, D. (1996). 1996 Receptor and ion channel nomenclature supplement. *Trends Pharmacol. Sci.*, **17** (suppl.), 72–77.

ZHANG, G., YAMAMOTO, Y., MIWA, K. & SUZUKI, H. (1994). Vasodilatation induced by substance P in guinea pig carotid arteries. *Am. J. Physiol.*, **266,** H1132–H1137.

ZYGMUNT, P.M., GRUNDEMAR, L. & HÖGESTÄTT, E.D. (1994a). Endothelium-dependent relaxation resistant to N-omega-nitro-L-arginine in the rat hepatic artery and aorta. *Acta Physiol. Scand.*, **152,** 107–114.

ZYGMUNT, P.M., WALDECK, K. & HÖGESTÄTT, E.D. (1994b). The endothelium mediates a nitric oxide-independent hyperpolarization and relaxation in the rat hepatic artery. *Acta Physiol. Scand.*, **152,** 375–384.

(Received June 14, 1996
Accepted September 27, 1996)