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RESEARCH PAPER

Endothelium modulates vasoconstrictor response to prostaglandin I_2 in rat mesenteric resistance arteries: interaction between EP_1 and TP receptors

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Background and purpose: Prostacyclin (PGI_2) is usually described as an endothelium-derived vasodilator, but it can also induce vasoconstriction. We studied the vasomotor responses to PGI_2 in resistance arteries and the role of thromboxane (TP) and prostaglandin E_2 (EP) receptors in this effect.
Experimental approach: Mesenteric resistance arteries were obtained from Sprague-Dawley rats. Vasomotion to PGI_2 was studied in segments of these arteries with and without endothelium and in presence of the nitric oxide (NO) synthase inhibitor N^G-nitro-L-arginine methyl ester (L-NAME), the potassium channel blockers apamin plus charybdotoxin, the non-selective EP receptor antagonist AH6809, the selective TP receptor antagonist SQ29548 or the EP_1 receptor antagonist SC19220. PGI_2-induced NO release was analysed in the absence or presence of SQ29548, AH6809 or SC19220.
Key results: PGI_2 caused contractions in arterial segments that were increased by endothelium removal, L-NAME or L-NAME plus apamin plus charybdotoxin and abolished by SQ29548. In segments with endothelium, AH6809 or SC19220 almost abolished the contractions to PGI_2; this effect was prevented by L-NAME, L-NAME plus apamin plus charybdotoxin or by endothelium removal. PGI_2 induced NO release that was inhibited by the prostacyclin receptor (IP receptor) antagonist, RO1138452, and increased by SQ29548, SC19220 and AH6809. The increase in NO release induced by these separate drugs was inhibited by RO1138452.
Conclusions and implications: PGI_2 activated the TP receptor in mesenteric resistance arteries and produced vasoconstriction, which the endothelium modulated through TP and EP_1 receptors. PGI_2 also released endothelium-derived hyperpolarizing factor and, through IP receptor activation, induced NO release, which in turn, was antagonized by TP and EP_1 receptor activation.
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Keywords: prostacyclin; endothelium; nitric oxide; resistance arteries; prostanoids

Abbreviations: DMSO, dimethyl sulphoxide; EP receptor, prostaglandin E_2 receptor; IP receptor, prostacyclin receptor; KHS, Krebs-Henseleit solution; TP receptor, thromboxane receptor

Introduction

Prostacyclin (PGI_2) is the most abundant prostanoid generated by vascular walls (Bunting *et al.*, 1977; Moncada *et al.*, 1977; Blanco-Rivero *et al.*, 2005). It is produced in endothelial cells from arachidonic acid-derived prostaglandin H_2 through the action of the enzyme PGI_2 synthase. It is generally described as an endothelium-derived vasodilator that, by stimulating the PGI_2 receptors (IP receptors; nomencalture follows Alexander *et al.*, 2008) and activating adenylate cyclase, induces an increase in intracellular cyclic-AMP concentration thereby producing smooth muscle relaxation (Wise and Jones, 1996). However, it is often forgotten that PGI_2 can also promote a vasoconstriction that is mediated by activation of thromboxane A_2 receptors (TP receptors) (Levy, 1978; 1980; Davis *et al.*, 1980; Williams *et al.*, 1994; Zhao *et al.*, 1996; Gluais *et al.*, 2005; Xavier *et al.*, 2008).

We have recently demonstrated in conductance and resistance vessels from aldosterone-treated rats that endogenous PGI_2, acting as a vasoconstrictor agent, promotes endothelial dysfunction (Blanco-Rivero *et al.*, 2005; Xavier *et al.*, 2008). In addition, when added to noradrenaline pre-contracted resistance arteries, exogenous PGI_2 produced a biphasic response, which was characterized by an initial contractile response followed by a relaxation (Xavier *et al.*, 2008). In these

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resistance arteries, the vasodilator response to PGI_2 was partially 'masked' by its action on TP receptors while the PGI_2 contractile response was inhibited by a TP receptor antagonist. Previous results in prostanoid research have revealed that PGI_2 can bind not only to the IP or TP receptors but also to the PGE_2 (EP) receptors (Narumiya *et al*., 1999; Breyer *et al*., 2001), suggesting crosstalk between PGI_2 and the PGE family. Four different subtypes of EP receptors, EP_1, EP_2, EP_3 and EP_4, are known to exist in the vasculature. EP_1 and EP_3 mediate vasoconstriction, while EP_2 and EP_4 mediate vasodilation. These prostanoid receptors have all been described in both vascular endothelial and smooth muscle cells (Breyer *et al*., 2001; Alfranca *et al*., 2006; Norel, 2007; Tang and Vanhoutte, 2008).

Most of the studies investigating the vasoconstrictor action of PGI_2 have focused their experiments on conductance vessels (Levy, 1978; 1980; Davis *et al*., 1980; Williams *et al*., 1994; Zhao *et al*., 1996; Gluais *et al*., 2005) and have revealed that the vasoconstrictor response to PGI_2 is mediated by TP receptor activation and is negatively modulated by endothelium-derived nitric oxide (NO) (Gluais *et al*., 2005). However, at present, there are no published studies on the effect of PGI_2 on resistance arteries, and this could be especially relevant as these arteries have a very important role in the regulation of vascular resistance. Moreover, the role of EP receptor subtypes in the vascular actions of PGI_2 remains unknown.

In this study we have used pharmacological agents to investigate the mechanism of the vasoconstrictor effect of PGI_2 in rat mesenteric resistance arteries and the role of endothelium in this effect.

Methods

Animal

This investigation conforms to the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996) and with the directives 609/86 CEE and R.D. 233/88 of the *Ministerio de Agricultura, Pesca y Alimentación* (registration No. EX-021U) of Spain. Male Sprague-Dawley rats (6 months old) were obtained from colonies maintained at the Animal Quarters of the *Facultad de Medicina* of the *Universidad Autónoma de Madrid*. Rats were housed with a constant room temperature, humidity, 12 h light/dark cycle and free access to tap water and standard rat chow.

Vascular reactivity study

After death by CO_2 inhalation, the mesenteric vascular bed was removed from Sprague-Dawley rats and placed in cold (4°C) Krebs-Henseleit solution (KHS; in mM: 115 NaCl, 2.5 $CaCl_2$, 4.6 KCl, 1.2 KH_2PO_4, 1.2, $MgSO_4.7H_2O$, 25 $NaHCO_3$, 11.1 glucose and 0.03 EDTA).

For reactivity experiments the third-order branch of the mesenteric arcade (318 ± 6.3 μm internal diameter) was dissected from the mesenteric bed, cleaned of connective tissue and cut into segments of approximately 2 mm in length. Two tungsten wires (40 μm diameter) were introduced through the lumen of the segments and mounted in a small vessel chamber myograph (Danish Myo Technology A/S, Äarhus, Denmark) to measure isometric tension as described by Mulvany and Halpern (1977). After a 30 min equilibration period in oxygenated (95%O_2/5% CO_2) KHS at 37°C and pH 7.4, segments were stretched to their optimal lumen diameter for active tension development. This was determined based on the internal circumference-wall tension ratio of the segments by setting their internal circumference, L_0, to 90% of what the vessels would have if they were exposed to a passive tension that was equivalent to the tension produced by a transmural pressure of 100 mmHg (Mulvany and Halpern, 1977).

Experimental protocols

After a 30 min equilibration period, arteries were exposed twice to 120 mM KCl to check their functional integrity. Thirty minutes later, the arteries were contracted with a submaximal concentration of noradrenaline (10 μM), and then acetylcholine (1 μM) was added to relax the noradrenaline-contracted arteries. A relaxation equal to or greater than 80% was considered evidence of the functional integrity of the endothelium. After 30 min, cumulative concentration–response curves were generated for PGI_2 (1 nM–10 μM) on each ring. In other experiments, the PGI_2 curves were constructed in arteries without endothelium. The endothelium was removed by gently rubbing the intimal surface with a human hair. The effectiveness of endothelium removal was confirmed by the inability of acetylcholine (1 μM) to relax noradrenaline-contracted arteries.

To determine the participation of NO in the response to PGI_2, arteries were incubated with N^G-nitro-L-arginine methyl ester [L-NAME, 100 μM, a non-selective NO synthase (NOS) inhibitor]. In another set of experiments, the role of endothelium-derived hyperpolarizing factor (EDHF) on the PGI_2-induced contraction was analysed. For this, the effect of a calcium-activated potassium channel blockade, produced by apamin (1 μM) plus charybdotoxin (0.1 μM), on the PGI_2 response was analysed in arteries pretreated with L-NAME. The effect of apamin plus charybdotoxin on the PGI_2 response was also analysed in arteries without endothelium.

The participation of TP and EP receptors on the response to PGI_2 was analysed in arteries pre-incubated with the TP receptor antagonist SQ29548 (1S-[1α,2β{5z},3β,4β])7-(3-[{2-(phenylamino)carbonyl}hydrazino]methyl)-7-oxabicyclo [2.2.1]hept-2-yl-5-heptenoate; 1 μM; Sprague *et al*., 1980) or the non-selective EP receptor antagonist AH6809 (6-isopropoxy-9-oxoxanthene-2-carboxylic acid; 30 μM; Abramovitz *et al*., 2000; Tang *et al*., 2008) respectively. To analyse the participation of EP_1 receptors in the response to PGI_2, arteries with or without endothelium were pre-incubated with the selective EP_1 receptor antagonist SC19220 (8–chloro–dibenz[b,f][1,4]oxazepine-10(11H)–carboxylic acid 2–acetylhydrazide; 100 μM; Tang *et al*., 2008). The effect of SC19220 was also analysed in arteries with endothelium pretreated with L-NAME or L-NAME plus apamin plus charybdotoxin.

The effects of SQ29548, SC19220 or AH6809 on the concentration–response curves to the thromboxane A_2 mimetic, U46619, were also analysed.

In another set of experiments, responses to PGI_2 (0.01 nM–10 μM) was analysed in noradrenaline pre-contracted arteries. Concentration–response curves to PGI_2 were obtained from arteries with or without endothelium and in absence or presence of SQ29548, AH6809 or SC19220.

Release of NO

In order to study the effect of PGI_2 on NO release, the second, third and fourth branches of mesenteric artery were incubated for 60 min in 3 mL of a buffer containing in mM: 119 NaCl, 20 HEPES, 4.6 KCl, 1 $MgSO_4.7H_2O$, 0.15 $Na_2HPO_4.12H_2O$, 0.4 KH_2PO_4, 5 $NaHCO_3$, 1.2 $CaCl_2.2H_2O$, 5.2 glucose, at 37°C (stabilization period). Afterward, arteries were incubated with the fluorescent probe 4,5-diaminofluorescein (2 μM) for 45 min, and medium was collected to measure basal NO release. Once the organ bath was refilled, PGI_2, acetylcholine or 2-(N,N-Diethylamino)-diazenolate-2-oxide.diethylammonium salt (DEA-NONOate) was added cumulatively (10 nM–10 μM) at 2 min intervals. The medium was collected only at the end of the concentration–response curve to acetylcholine or PGI_2. The fluorescence of the medium was measured at room temperature using a spectrofluorimeter (LS50 Perkin Elmer Instruments, FL WINLAB Software, Waltham, MA, USA) with excitation wavelength set at 492 nm and emission wavelength at 515 nm. The stimulated NO release was calculated by subtracting the basal NO release from that evoked by PGI_2. Also, blank measurement samples were collected from the medium without mesenteric segments in order to subtract background emission. Some assays were performed in the presence of L-NAME (100 μM), SQ29548 (1 μM), SC19220 (100 μM), SQ29548 plus SC19920, AH6809 (30 μM) or the IP receptor antagonist RO1138452 (4,5-dihydro-1H-imidazol-2-yl)-[4-(4-isopropoxybenzyl)phenyl] amine; 1 μM; Jones *et al*., 2006). The effect of SQ29548 or SC19220 was also analysed in the presence of RO1138452. The effect of AH6809 or SC19220 was also analysed in the presence of L-NAME. These inhibitors did not affect the fluorescence signal *per se*. The amount of NO released was expressed as arbitrary units per milligram of tissue.

Statistical analysis

Results are expressed as mean ± SEM for the number of rats indicated. Differences were analysed using two way analysis of variance (ANOVA) for the concentration–response curves to PGI_2 and for experiments of NO release, using the Graphpad Prism 4.0 Software (San Diego, CA, USA). A *P*-value of less than 0.05 was considered to be significant.

Materials

The drugs used were: PGI_2, l-noradrenaline hydrochloride, acetylcholine chloride, L-NAME hydrochloride, DEA-NONOate, apamin, charybdotoxin, SC19220, AH6809 (Sigma; St. Louis, MO, USA), SQ29548 and RO1138452 (Cayman Chemical Company, Ann Arbor, MI, USA). Stock solutions (10 mM) of drugs were made in distilled water; except for noradrenaline, which was dissolved in a NaCl (0.9%)-ascorbic acid (0.01% w/v) solution; PGI_2 and SQ29548, which was solubilized in ethanol and SC19220, AH6809 and RO1138452, which were solubilized in DMSO (dimethyl sulphoxide). All stock solutions were kept at –20°C, and appropriate dilutions were made in KHS on the day of the experiment.

Results

Prostacyclin caused cumulative concentration-dependent contractions in untreated segments of rat mesenteric resistance arteries with and without endothelium (Figures 1A and 2). Endothelium removal significantly increased the contractions to PGI_2 (Figure 1A). Pretreatment of the mesenteric arteries with L-NAME also increased the contraction to PGI_2 (Figure 1A). This effect was enhanced in presence of L-NAME plus apamin plus charybdotoxin (Figure 1A). Pretreatment with the three-drug combination increased the PGI_2-induced contraction to levels as high as those observed in the segments without endothelium (Figure 1A). In arterial segments without endothelium, the PGI_2-induced contraction remained unmodified in the presence of L-NAME or apamin plus charybdotoxin (Figure 1B).

In arteries with endothelium, SQ29548, AH6809 or SC19220 almost abolished the contractions induced by PGI_2 (Figure 3). The inhibitory effect of either AH6809 (Figure 3A) or SC19220 (Figure 3B) on the PGI_2-induced contraction was partially prevented in arteries pretreated with L-NAME or L-NAME plus apamin plus charybdotoxin. On the other hand, the inhibitory effect of SQ29548 on the PGI_2-induced contraction was unaltered by pre-incubation with L-NAME or L-NAME together with apamin plus charybdotoxin (results not shown). In presence of L-NAME, SC19220 prevented enhancement of the PGI_2 vasoconstriction produced by charybdotoxin plus apamin (Figure 3C).

As observed in segments with endothelium, pretreatment of de-endothelialized arterial segments with SQ29548 abolished the contractions induced by PGI_2 (Figure 4). In contrast, in segments without endothelium, SC19220 or AH6809 only partly reduced the contractions to PGI_2 (Figure 4).

U46619, the synthetic TP receptor agonist, induced concentration-dependent contractions in rat mesenteric resistance arteries, and these contractions were abolished in the presence of SQ29548 (Figure 4B). SC19220 or AH6809 slightly shifted the concentration–response curve to U46619 rightward without any changes in the maximum response (Figure 5B).

In arterial segments pre-contracted with noradrenaline, PGI_2 induced a concentration-dependent relaxation (Figure 5A) that was significantly reduced after endothelium removal (Figure 5B). In segments with endothelium, SQ29548, SC19220 or AH6809 all increased relaxation to PGI_2, and this effect was higher in the presence of either SC19220 or AH6809 (Figure 5A). Endothelium removal completely abolished the effects of SQ29548, SC19220 and AH6809 on PGI_2-induced relaxation (Figure 5B).

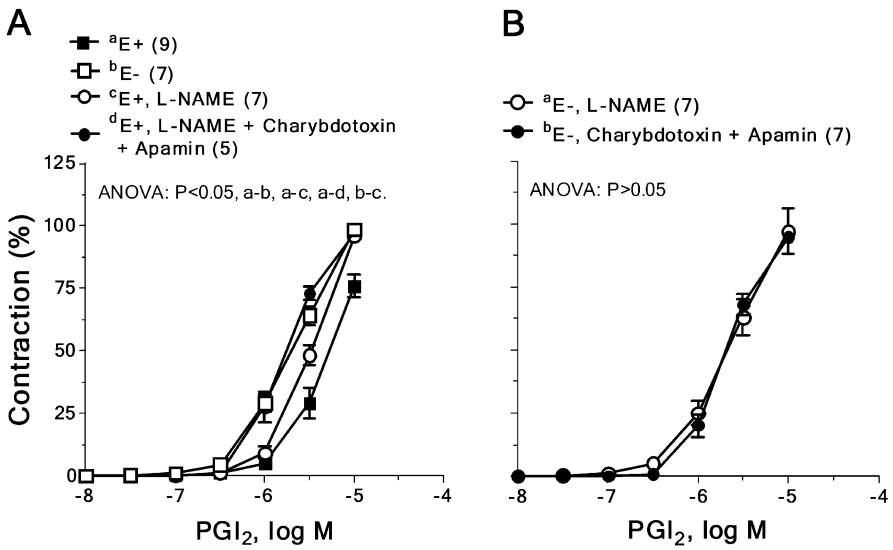

Figure 1 (A) Effect of N^G-nitro-L-arginine methyl ester (L-NAME), L-NAME plus apamin plus charybdotoxin or endothelium removal (E–) on the concentration-dependent vasoconstriction to prostacyclin (PGI_2) in segments of mesenteric resistance arteries from Sprague-Dawley rats. (B) Effect of L-NAME or L-NAME plus apamin plus charybdotoxin on contractile responses to PGI_2 in mesenteric resistance arteries without endothelium (E–). Results (means ± SEM) are expressed as a percentage of the response elicited by KCl. Number of animals (*n*) used is indicated in parentheses.

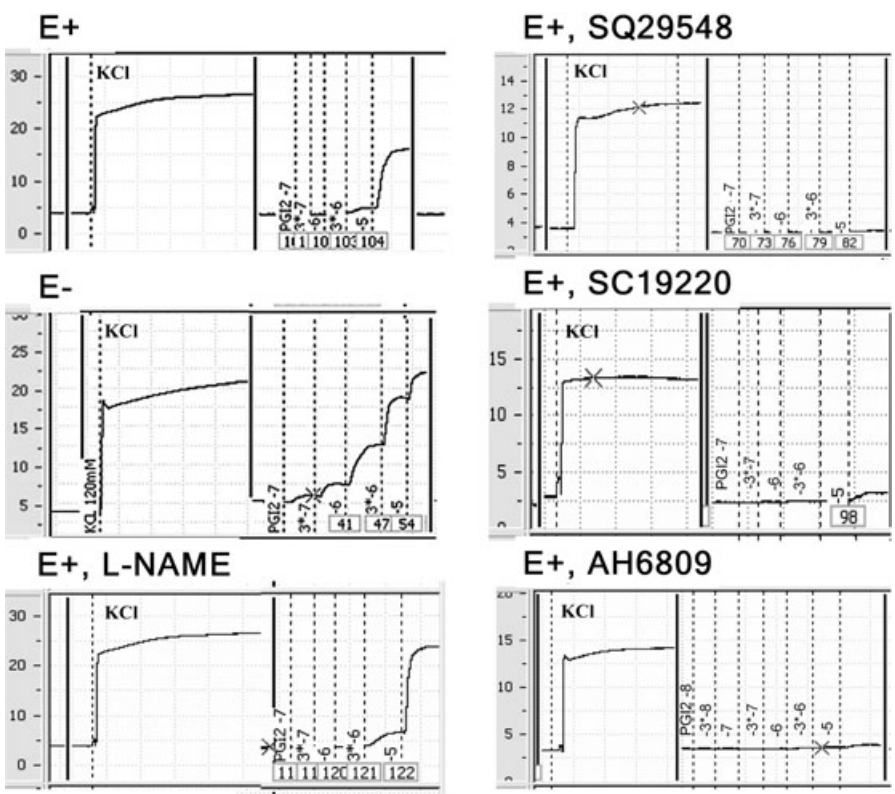

Figure 2 Typical tracings of tension recordings of segments of mesenteric resistance arteries (with, E+, or without, E–, endothelium) exposed to cumulative concentrations of PGI_2. L-NAME, N^G-nitro-L-arginine methyl ester; PGI_2, prostacyclin.

Release of NO

Prostacyclin induced NO release in mesenteric arterial segments from Sprague-Dawley rats that was inhibited by the IP receptor antagonist, RO1138452 (Figure 6), and the NOS inhibitor, L-NAME (Figure 7A). This PGI_2-induced NO release was increased in arteries pretreated with SQ29548, SC19220, AH6809 or SQ29548 plus SC19220, and it was higher in the presence of AH6809 or SQ29548 plus SC19220 (Figure 6). The increase in NO release induced by these drugs was inhibited when the arterial segments were co-incubated with

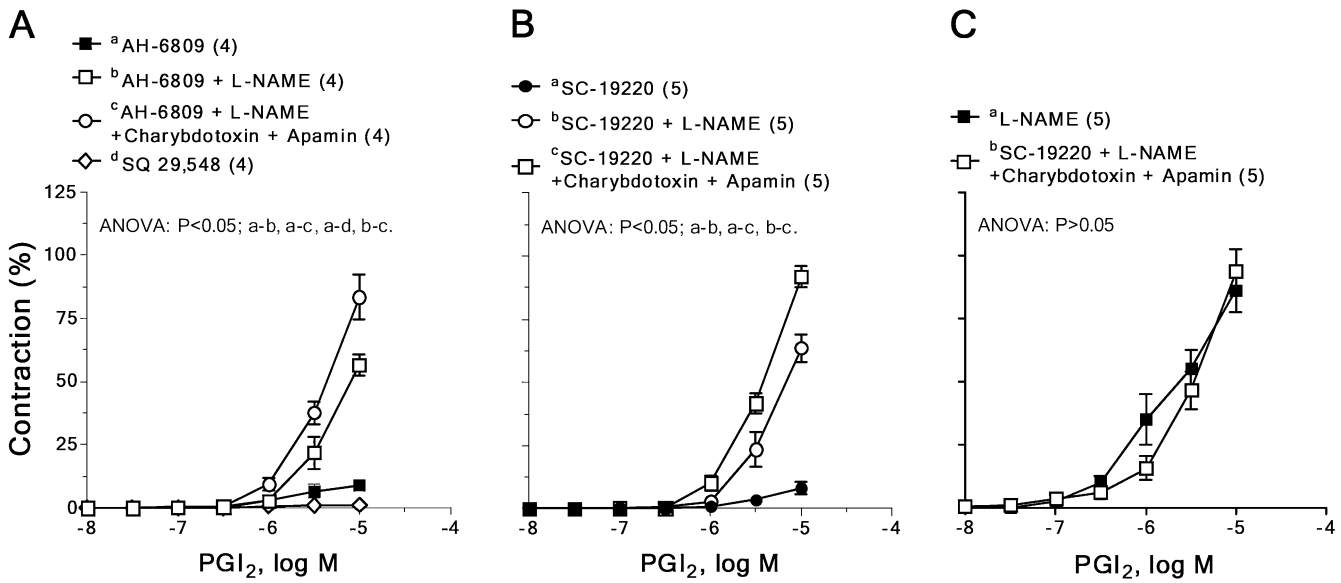

Figure 3 (A) Effect of SQ29548 or AH6809 in the absence or the presence of N^G-nitro-L-arginine methyl ester (L-NAME) or L-NAME plus apamin plus charybdotoxin on the concentration-dependent vasoconstriction to prostacyclin (PGI_2) in segments of mesenteric resistance arteries with endothelium. (B) Effect of SC19220, in the absence or the presence of L-NAME or L-NAME plus apamin plus charybdotoxin, on the concentration-dependent vasoconstriction to PGI_2 in mesenteric resistance arteries with endothelium. (C) Effect of L-NAME plus apamin plus charybdotoxin on the response to PGI_2 in arteries pretreated with SC19220. Results (means ± SEM) are expressed as a percentage of the response elicited by KCl. Number of animals (*n*) used is indicated in parentheses.

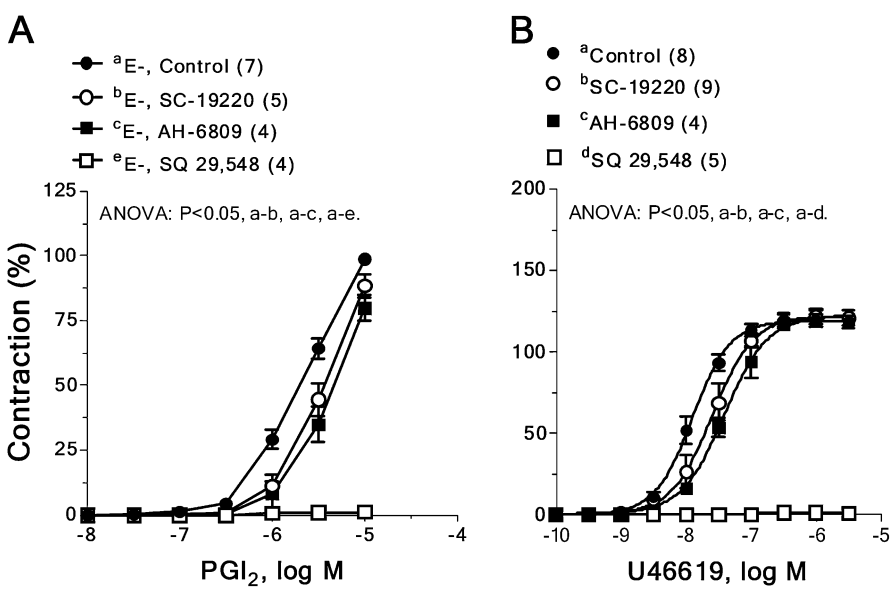

Figure 4 (A) Effect of SQ29548, AH6809 or SC19220 on the concentration-dependent vasoconstriction to prostacyclin (PGI_2) in segments of mesenteric resistance arteries, without endothelium. (B) Effect of SQ29548, AH6809 or SC19220 on the concentration-dependent vasoconstriction to the thromboxane A_2 mimetic, U46619, in arterial segments. Results (means ± SEM) are expressed as a percentage of the response elicited by KCl. Number of animals (*n*) used is indicated in parentheses.

RO1138452 (Figure 6) or L-NAME (Figure 7B). Acetylcholine produced NO release in rat mesenteric resistance arteries (Figure 7A). This NO release was inhibited in the presence of L-NAME (Figure 7A). The NO donor DEA-NONOate also produced a significant fluorescent signal when added to arterial segments pretreated with the fluorescent probe 4,5-diaminofluorescein (results not shown).

Discussion and conclusions

Prostacyclin is commonly referred to as an endothelium-dependent vasodilator factor, but it can also evoke contraction in many vascular beds (Levy, 1978; 1980; Davis *et al.*, 1980; Williams *et al.*, 1994; Zhao *et al.*, 1996; Gluais *et al.*, 2005; Xavier *et al.*, 2008). Results obtained in the current

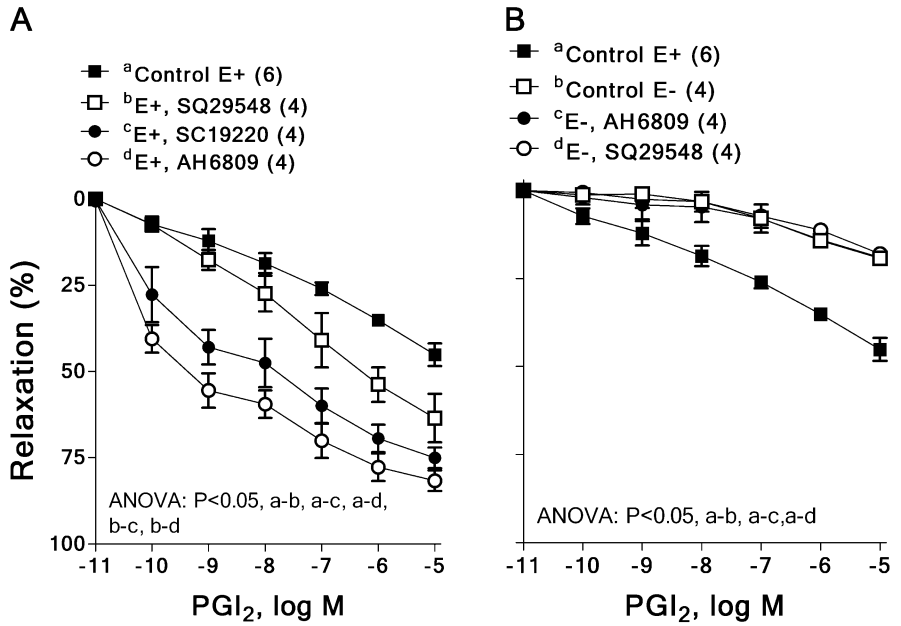

Figure 5 (A) Effect of SQ29548, SC19220 or AH6809 on the concentration-dependent relaxation to prostacyclin (PGI_2) in noradrenaline-pre-contracted segments of mesenteric resistance arteries with endothelium (E+). (B) Effect of endothelium removal on the relaxation to PGI_2 and the effect of either AH6809 or SQ29548 on relaxation to PGI_2 in mesenteric resistance arteries without endothelium (E–). Results are expressed as means ± SEM. Number of animals (*n*) used is indicated in parentheses.

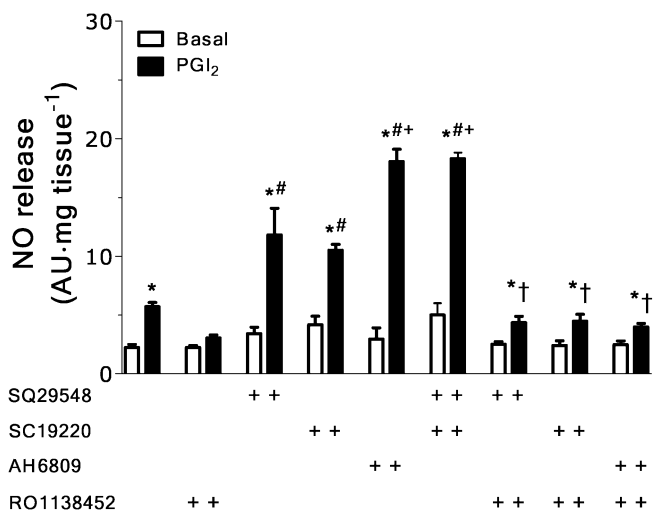

Figure 6 Effects of RO1138452, AH6809, SC19220 alone or plus RO1138452, or SQ29548 alone or in the presence of RO1138452, on basal and prostacyclin (PGI_2)-induced nitric oxide (NO) release in segments of mesenteric resistance arteries. Results (means ± SEM) are expressed as arbitrary units (AU) per milligram of tissue. ANOVA: *$P < 0.05$ versus basal; #$P < 0.05$ versus control; +$P < 0.05$ versus control, SQ29548, or SC19220; †$P < 0.05$ versus SQ29548, SC19220 or AH6809. $n = 4–5$ animals in each group.

study demonstrated that in a concentration-dependent manner, PGI_2 caused contraction in segments of mesenteric resistance arteries from Sprague-Dawley rats. This contraction was significantly higher in arterial segments without endothelium, suggesting that the endothelial cells decrease vasoconstriction to PGI_2 in resistance vessels. This endothelial effect seems to be partially related to NO release, as the concentration–response curve to PGI_2 was shifted to the left in the presence of the NOS inhibitor L-NAME, as previously reported in conductance vessels (Gluais *et al.*, 2005). To analyse if the observed modulating effect of NO on the PGI_2-induced contraction was due to its basal release or to the ability of PGI_2 to release NO, we measured the amount of this endothelium-derived factor in the absence and presence of PGI_2. The results obtained here corroborate previous studies in conductance vessels showing that PGI_2 can induce the release of endothelial NO (Shimokawa *et al.*, 1988). This NO release was inhibited by L-NAME. In agreement with previous reports (Ray *et al.*, 2002; Ray and Marshall, 2006), our results also demonstrate that in mesenteric resistance arteries the PGI_2-induced NO release is mediated by IP receptor activation.

The fact that the potentiation of the PGI_2-induced contraction in segments without endothelium was higher than in segments pre-incubated with L-NAME indicates the participation of other endothelium-derived vasodilating factors in the effects of PGI_2. One of these is presumably EDHF, as the combination of apamin plus charybdotoxin (Félétou and Vanhoutte, 2006) did produce a significant increase in the contractile response to PGI_2 in L-NAME-treated arteries, the magnitude of which approached the level observed after endothelium removal. Although it is known that PGI_2 can directly activate smooth muscle potassium channels (Corriu *et al.*, 2001; Orie *et al.*, 2006), our results demonstrate that the effect of apamin and charybdotoxin in the contraction to PGI_2 was dependent on the presence of the endothelium, which is consistent with inhibition of EDHF-mediated actions (Edwards and Weston, 2004).

There is substantial evidence in the literature that PGI_2 induces vasoconstriction through TP receptor activation

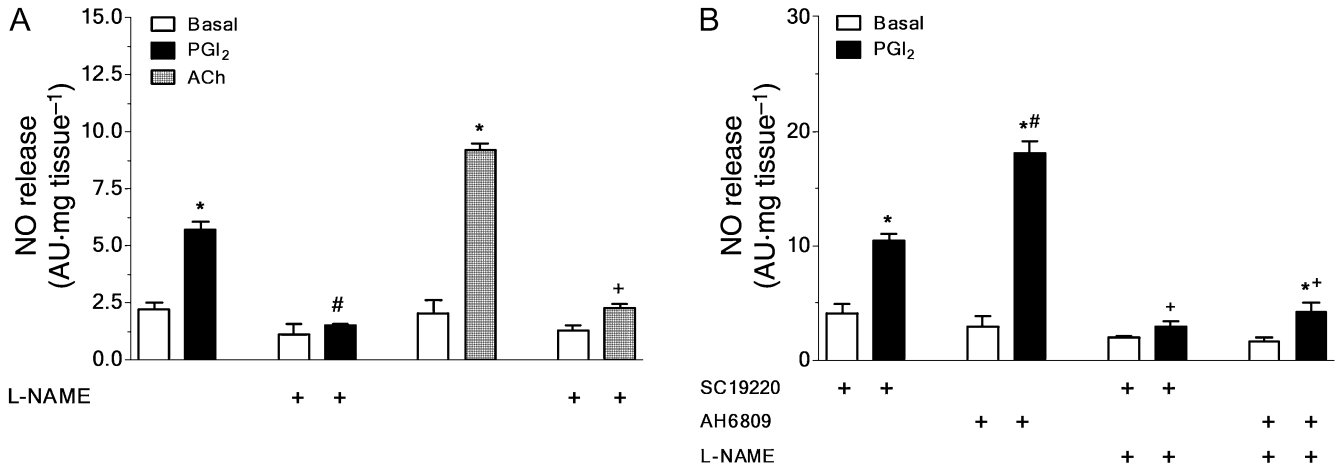

Figure 7 (A) Effect of N^G-nitro-L-arginine methyl ester (L-NAME) (100 μM) on nitric oxide (NO) release evoked by prostacyclin (PGI_2) or acetylcholine (ACh) in segments of mesenteric resistance arteries. (B) Effect of L-NAME on the increased PGI_2-induced NO release produced by AH6809 in arterial segments. Results (means ± SEM) are expressed as arbitrary units (AU) per milligram of tissue. ANOVA: *$P < 0.05$ versus basal; #$P < 0.05$ versus PGI_2; +$P < 0.05$ versus ACh/L-NAME. n = 4–5 animals in each group.

(Williams *et al.*, 1994; Zhao *et al.*, 1996; Gluais *et al.*, 2005; Xavier *et al.*, 2008). On the other hand, it has also previously been reported that PGI_2 can bind not only to the IP or TP receptor but also to other prostanoid receptors, such as the EP receptors (Narumiya *et al.*, 1999; Breyer *et al.*, 2001). Interactions of other cyclooxygenase-derived prostanoids, such as PGE_2, with different vascular prostanoid receptors have also been previously reported (Tang *et al.*, 2008), raising the possibility that the vasoconstriction to PGI_2 could be modulated by different prostanoid receptors.

In segments of mesenteric resistance arteries with endothelium, contraction to PGI_2 was prevented by the TP receptor antagonist SQ29548, implying that in these arteries PGI_2 mediates contraction mainly through activation of TP receptors. Surprisingly, in the presence of the selective EP_1 receptor antagonist SC19220, or the non-selective EP receptor antagonist, AH6809, which has equal affinity for the EP_1, EP_2 and EP_3 receptors, the contraction induced by PGI_2 was decreased as much as in the presence of SQ29548. Some reports have been demonstrated that these drugs present a partial antagonism at TP receptors (Tang *et al.*, 2008). Therefore, in our study we tested the possibility that the effect of AH6809 or SC19220 on the PGI_2 contractile response could be attributed to their antagonism at the TP receptor. Our results demonstrated that either SC19220 or AH6809 slightly reduced the contractile response to the TP receptor agonist, U46619, which is consistent with their partial antagonism at the TP receptor, as previously reported (Tang *et al.*, 2008). However, the effect of these drugs on the U46619-induced contraction demonstrates that their effect on PGI_2-induced contraction is not only due to a TP receptor antagonism.

As vascular endothelium expresses various subtypes of prostanoid receptors (Alfranca *et al.*, 2006) and PGI_2 can bind to some of these receptors, the present findings imply that the effect of the EP receptor antagonist on the contractile response to PGI_2 could be due to alterations in the endothelial function. Considering that the contractile response to PGI_2 in arteries without endothelium was only slightly reduced by either AH6809 or SC19220, which is explained by their partial antagonism to the TP receptors, we hypothesized that the effect of the EP antagonists on the PGI_2-induced contraction in the arteries with endothelium could be explained by the vasodilator action of the endothelial cells. In light of the evidence, we performed some experiments to clarify this hypothesis and identify the possible mediators involved. To assess the participation of NO on the effect of SC19220, PGI_2 curves were performed in presence of this EP_1 antagonist plus L-NAME. These conditions partially restored the contractile response to PGI_2, leading us to speculate that the NO released by PGI_2 was enhanced after the blockade of EP_1 receptors. Supporting this hypothesis is the fact that the PGI_2-induced NO release was enhanced in the presence of either SC19220 or AH6809. These results therefore indicate that in mesenteric resistance arteries PGI_2 induces NO release through IP receptor activation, and this release is counterbalanced by an activation of EP_1 receptors by the prostanoid. It is important to note that the magnitude of the effect of AH6809 on NO release was higher than that observed in the presence of SC19220. The reasons for this effect are unknown, but observations suggest a role for other EP receptors, probably EP_3 and/or EP_4. When stimulated, EP_3 receptors decrease intracellular levels of cyclic-AMP, reduce endothelial NOS (eNOS) activity and induce vasoconstriction (Zhang and Hintze, 2006), whereas EP_4 receptor activation increases eNOS activity, NO release and produces vasodilation (Hritovska *et al.*, 2007).

Consistent with the results obtained in untreated arterial segments, the vasodilator response to PGI_2 in noradrenaline-pre-contracted segments was reduced by endothelium removal and enhanced in presence of AH6809, SC19220 or SQ29548, and this effect was higher in the presence of either AH6809 or SC19220. On the other hand, in segments without endothelium, neither AH6809 nor SQ29548 was able to produce an increase in PGI_2-induced relaxation.

As PGI_2 also binds to TP receptors, we investigated the role of these receptors in the NO released by this prostanoid. The NO production in response to PGI_2 was also enhanced in the presence of SQ29548, or in the presence of SC19220,

suggesting a counter-regulatory effect by these receptors on NO release induced by PGI_2. This result reinforces previous reports showing that activation of TP receptors decreases endothelial NO production and promotes endothelial dysfunction (Ashton and Ware, 2004; Alfranca *et al.*, 2006). The fact that the PGI_2-induced NO release was further increased in presence of both SQ29548 and SC19220 suggest that the modulation of TP and EP_1 receptors in this release occurs through an independent mechanism.

Various studies have demonstrated that vasodilation to PGI_2 is partially dependent on endothelial NO release (Armstead, 1995; Shimokawa *et al.*, 1988) and may be 'masked' by the contractile effect that it produces through smooth muscle TP receptor activation (Zhao *et al.*, 1996). Results from the current study reveal that the vasodilatory effect of PGI_2 may also be 'masked' by its action on endothelial EP and TP receptors, which decreases NO release. The present findings do not permit further speculation as to what causes the reduction in NO release in response to activation of TP and EP_1 receptors. However, one possible explanation is that endothelial EP and TP activation can desensitize the IP receptor and cause its internalization (Wilson *et al.*, 2007). Thus the NO release and dilator effects of PGI_2 can be enhanced by blocking the activation of either TP or EP_1 receptors. In line with this hypothesis, blockade of EP_1 receptors enhanced the effect of iloprost in the isolated rabbit lung (Schermuly *et al.*, 2007) and guinea-pig aorta (Clapp *et al.*, 1998). Furthermore, it has been reported that IP and TP receptors dimerize and cause cross desensitization (Wilson *et al.*, 2007). The results presented here reveal an additional mechanism that could contribute to the endothelial dysfunction associated with enhanced PGI_2 release (Blanco-Rivero *et al.*, 2005; Xavier *et al.*, 2008). To our knowledge this is the first study to demonstrate that PGI_2 triggers NO production in mesenteric arterial tissue from Sprague-Dawley rats and that EP and TP receptors modulate this effect.

As we had functional evidence that PGI_2 induced the release of EDHF in segments of mesenteric resistance arteries from Sprague-Dawley rats, we also analysed the contribution of EDHF to PGI_2 vasodilation after EP_1 receptor antagonism. The results obtained here demonstrated that in arteries pretreated with SC19220, L-NAME plus apamin and charybdotoxin produced an additional left-ward displacement in the PGI_2-induced contraction that was similar in magnitude to the displacement observed in arteries without endothelium. Additionally, SC19220 prevented this additional displacement on the PGI_2 curves produced by L-NAME plus apamin plus charybdotoxin. These functional results confirm the hypothesis that the release of EDHF induced by PGI_2 is also antagonized by the activation of EP_1 receptors.

We recently estimated that, in rat mesenteric resistance arteries, the amount of PGI_2 released per milligram of tissue in response to acetylcholine was approximately 0.05 μmol (Xavier *et al.*, 2008), indicating that the local concentration of PGI_2 could be much higher than the levels released in plasma, thus producing a paracrine effect on the vascular wall. Moreover, we and others have presented consistent evidence that this locally released PGI_2 effectively acts as a vasoconstrictor agent in the process of endothelial dysfunction (Blanco-Rivero *et al.*, 2005; Gluais *et al.*, 2005; Xavier *et al.*, 2008). This makes the identification of the possible mechanism behind the vasoconstrictor effects of PGI_2 very relevant to the investigation of vasoactive substances that occur with diseases associated with cardiovascular risk, as therapeutic targets.

The findings presented here collectively suggested that PGI_2 activated smooth muscle cell TP receptors in mesenteric resistance arteries of Sprague-Dawley rats, and produced vasoconstriction, modulated by the endothelium through activation of TP and EP_1 receptors. In addition, these results have demonstrated that PGI_2 released EDHF and, through activation of IP receptors, induced NO release from endothelium that was antagonized by TP and EP receptor activation. The ability of PGI_2 to stimulate EP_1 and TP receptors and decrease NO release may be important in some situations in which PGI_2 has been implicated.

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Statement of conflicts of interest

None.

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