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RESEARCH PAPER

Differential reactivity of human mammary artery and saphenous vein to prostaglandin E_2: Implication for cardiovascular grafts

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BACKGROUND AND PURPOSE

Human internal mammary arteries (IMA) and saphenous veins (SV) are frequently used for coronary artery bypass graft surgery. Intra- and postoperatively, the bypass grafts are exposed to inflammatory conditions, under which there is a striking increase in the synthesis of prostaglandin E_2 (PGE_2). In this context, the physiological response of these vascular grafts to PGE_2 is highly relevant. The aim of this study was thus to characterize the PGE_2 receptor subtypes (EP_1, EP_2, EP_3 or EP_4) involved in modulation of the vascular tone in these two vessels.

EXPERIMENTAL APPROACH

Rings of IMA and SV were prepared from 48 patients. The rings were mounted in organ baths for isometric recording of tension, and a pharmacological study was performed, together with associated reverse transcriptase PCR and immunohistochemistry experiments.

KEY RESULTS

PGE_2 induced contractions of IMA (E_{max} = 1.43 ± 0.20 g; pEC_{50} = 7.50 ± 0.10); contractions were also observed with the EP_3 receptor agonists, sulprostone, 17-phenyl-PGE_2, misoprostol or ONO-AE-248. In contrast, PGE_2 induced relaxation of the precontracted SV (E_{max} = –0.22 ± 0.02 g; pEC_{50} = 7.14 ± 0.09), as did the EP_4 receptor agonist, ONO-AE1-329. These results were confirmed by the use of selective EP receptor antagonists (GW627368X, L-826266, ONO-8713, SC-51322) and by molecular biology and immunostaining.

CONCLUSIONS AND IMPLICATIONS

PGE_2 induced potent and opposite effects on the human vascular segments used for grafting, namely vasoconstriction of the IMA and vasodilatation of the SV via EP_3 and EP_4 receptors respectively. These observations suggest that EP_3 and EP_4 receptors could constitute therapeutic targets to increase vascular graft patency.

Abbreviations

CABG, coronary artery bypass graft; COX, cyclooxygenase; IMA, internal mammary artery; PG, prostaglandin; SV, saphenous vein

Introduction

Prostanoids [prostaglandins (PG) and thromboxane] are produced by endothelial and smooth muscle cells in the vascular wall and are derived from arachidonic acid via cyclooxygenase (COX) activity. Two isoforms of this enzyme have been described. The constitutive isoform, COX-1, is present in normal physiological conditions, but under local inflammatory conditions, such as in atherosclerosis (Schonbeck *et al.*, 1999), aneurysm (Bayston *et al.*, 2003) and after surgical interventions or with increased shear stress, an inducible isoform, COX-2, is expressed in the vascular wall. The presence or absence of COX-2 activity leads to different profiles of the locally released prostanoids.

In most models of vascular inflammation, prostacyclin and PGE_2 are the dominant prostanoids detected since microsomal PGE synthase-1 is coexpressed and/or colocalized with COX-2 (Cipollone *et al.*, 2003). These prostanoids are produced *ex vivo* in higher concentrations by human internal mammary artery (IMA) than by the saphenous vein (SV) (Chaikhouni *et al.*, 1986; Bishop-Bailey *et al.*, 1998). The production of PGE_2 in IMA and SV is considerably increased (20-fold and 700-fold respectively) under inflammatory conditions induced by 48 h exposure to a cytokine mixture (Bishop-Bailey *et al.*, 1998). In the human vascular wall, PGE_2 is involved in the processes of cell proliferation (Proudfoot *et al.*, 1999) and migration (Jiang *et al.*, 2004) and may also control vascular smooth muscle tone (Qian *et al.*, 1994). These observations are in accordance with a key role for PGE_2 in the control of both vascular wall structure and vascular tone.

PGE_2 can activate four prostanoid receptor subtypes (EP_1, EP_2, EP_3, EP_4; nomenclature follows Alexander *et al.*, 2009). EP_2 and EP_4 receptors are involved in vascular wall remodelling, where their appearance or increased expression have been detected in different pathologies such as atherosclerosis (Takayama *et al.*, 2002; Sussmann *et al.*, 2004) and abdominal aortic aneurysm (Bayston *et al.*, 2003). The EP receptor subtypes activated by PGE_2 are also implicated in the control of vascular smooth muscle tone. EP_1 or EP_3 receptors have been associated with vasoconstriction, while the activation of EP_2 or EP_4 receptors is preferentially associated with vasorelaxation (Norel, 2007).

Coronary artery bypass graft (CABG) surgery involves the use of IMA and/or SV to bypass the stenosed coronary arteries (Goldman *et al.*, 2004). The vascular reactivity of IMA and SV to different endogenous and pharmacological agents in the immediate postoperative period is not well documented. Both the inflammatory response and the numerous pharmacological agents administered, such as analgesic and anti-inflammatory drugs (COX-1 and COX-2 inhibitors) and aspirin (Mangano, 2002) or exogenous catecholamines could interfere with endogenous prostanoid biology and modulate IMA and SV vascular reactivity. Moreover, in the years following CABG, patients are exposed to many other drugs that can potentially interfere with IMA and SV vascular reactivity as well as prostanoid biology. In addition, SV grafts undergo profound remodelling that compromise their long-term viability (Dashwood, 2009; Jorapur *et al.*, 2009), and the underlying mechanisms are poorly understood. Given the complex biological roles of PGE_2, and the fact that there is little or no information available concerning the effects of PGE_2 on the vascular tone of human IMA and SV, the aim of the present study was to compare these effects and to characterize the EP receptor subtypes implicated. We found that PGE_2 mediated opposing effects in these two vessels: contraction in IMA (via EP_3 receptors) and relaxation in SV (via EP_4 receptors).

Methods

Isolated vascular preparations

All research programs involving the use of human tissue were approved and supported by the Ethics Committee of INSERM (the French National Institute for Health and Medical Research**),** and these tissues are considered as surgical waste in accordance with French ethical laws (L.1211-3–L.1211-9). Human IMA and SV were obtained from patients (42 male and 6 female) who had undergone surgery for coronary bypass. The mean age was 67 ± 1 years.

Physiological responses (contraction/relaxation studies)

Human vessels (2–4 mm internal diameter) were cut into rings and mounted in 10 mL organ baths containing Tyrode's solution gassed with 5% CO_2 in O_2 at 37°C and pH 7.4. Each ring was initially stretched to an optimal load (1.5–2 g). In some preparations, the endothelium was mechanically removed (Walch *et al.*, 1997). Changes in force were recorded by an isometric force displacement transducer (Narco F-60) connected to a physiograph (Linseis). Only one concentration–response curve was performed with each vascular ring.

After an equilibration period (90 min), all the vascular rings were contracted (first contraction) with noradrenaline (1 $\mu mol \cdot L^{-1}$; inducing 75% of noradrenaline maximal contraction). When the preparations reached a plateau, the bath fluid was renewed several times until the preparations returned passively to their initial resting tone. Subsequently, in order to avoid any physiological effect induced by endogenous PGs, nitric oxide (NO) or TP receptor activation, the preparations were incubated for 30 min with Tyrode's solution containing the COX inhibitor (indomethacin, 1.7 $\mu mol \cdot L^{-1}$), the NO synthase inhibitor (N^G-nitro-L-arginine, L-NOARG, 0.1 $mmol \cdot L^{-1}$) and a TP antagonist (BAY u3405, 10 $\mu mol \cdot L^{-1}$). In addition to this treatment, some preparations were exposed to other selective antagonists [ONO-8713 (10 $\mu mol \cdot L^{-1}$; EP_1 receptor antagonist), SC-51322 (1, 10 $\mu mol \cdot L^{-1}$; $EP_1 > EP_3$ receptor antagonist), GW627368X (10 $\mu mol \cdot L^{-1}$; EP_4 receptor antagonist) or L-826266 (0.3, 3 $\mu mol \cdot L^{-1}$; EP_3 receptor antagonist)] during this incubation period.

After this incubation period, the vascular rings were precontracted (relaxation protocol) or not (contraction protocol) with noradrenaline (1 $\mu mol \cdot L^{-1}$) before the addition in a cumulative fashion of increasing concentrations of different prostanoids or synthetic analogues to the organ baths. At the end of some protocols, in the absence of any response to a prostanoid, a noradrenaline-induced contraction (1 $\mu mol \cdot L^{-1}$) was repeated.

Reverse transcriptase PCR (RT-PCR) analysis

The human vascular preparations were disrupted in guanidinium isothiocyanate, using a Polytron apparatus. RNAs were isolated using ultracentrifugation on CsCl or with a Tissue RNA kit (OMEGA bio-tek). The identification of the EP receptors transcripts was performed by RT-PCR using 32–38 and 22 cycles for glyceraldehyde 3-phosphate dehydrogenase (GAPDH). RT-PCR products were separated by electrophoresis on 1.2% agarose gels, stained with ethidium bromide and visualized under UV illumination. The primers used to amplify the EP_1 receptor were forward primer (5′-ggtatcatggtggtgtcgtg-3′) and reverse primer (5′-ggcctctggttgtgcttaga-3′). Primers used to amplify the EP_2 receptor were: forward primer (5′-ccacctcattctcctggcta-3′) and reverse primer (5′-cgacaaca gaggactgaacg-3′); those for the EP_3 receptor were forward primer (5′-cttcgcataactggggcaac-3′) and reverse primer (5′-tctccgtgtgtgtcttgcag-3′); those for the EP_4 receptor were forward primer (5′-tggtatgtgggctggctg-3′) and reverse primer (5′-gaggacggtggcgagaat-3′); and for GAPDH were forward primer (5′-atcaccatcttccaggagcg-3′) and reverse primer (5′-cctgcttcaccaccttcttg-3′). The size markers were purchased from New England Biolabs, Ipswich, MA, USA.

Measurement of PGE_2

The release of PGE_2 was measured in samples collected after a 15 min incubation of the vascular rings mounted in the organ bath in Tyrode's solution without any treatment, just prior to (basal) and 15 min after the first noradrenaline stimulation. Measurements were performed using PGE_2 enzyme-immunoassay kits (Cayman Chemical, Ann Arbor, MI, USA).

Immunohistochemistry

Transverse sections (5 μm) of human vessels were obtained from paraffin-embedded preparations. The sections were submitted to a high-temperature (80°C) antigen unmasking technique using Vector's solution (H-3300). Cayman or Alpha Diagnostic International (ADI) rabbit polyclonal antibodies directed against the human EP_1, EP_2, EP_3 or EP_4 (C-Term) receptor subtypes were used as primary antibodies (1/100, overnight 4°C). In addition, some sections were incubated with a Cayman anti-EP primary antibody and the respective blocking peptide. Biotinylated anti-rabbit was the secondary antibody, and peroxidase Vectastain® Elite ABC kits (Burlingame, CA, USA) were used for detection, followed by haematoxylin treatment for cell nuclei staining.

Data analysis

Acquisition and processing of the physiological data (contraction/relaxation) was performed with the IOX software (EMKA Technologies, Paris, France). The effects induced by the different agonists were expressed in grams (g) or normalized (%) with respect to the first noradrenaline precontraction. The values presented are positive for the contractions and negative for the relaxations. Where possible, a four-parameter logistic equation of the form:

$$E = \frac{E_{max}[A]^{nH}}{EC_{50}{}^{nH} + [A]^{nH}}$$

was fitted to the data obtained from each organ bath protocol to provide estimates of the maximal effect (E_{max}) produced by 10 μmol·L^{-1} of the EP receptor ligands (A), the half-maximum effective concentration values (EC_{50}) as well as Hill slope (nH) parameters. The pEC_{50} values were calculated as the negative log of EC_{50} values.

All data are means ± s.e.mean derived from (*n*) different patients, and statistical analysis on the curves were performed using one-way or two-way ANOVA followed by Student–Newman–Keuls test or Student's *t*-test for the pEC_{50} values with a confidence level of 95%.

Materials

The selectivity of the compounds indicated in parenthesis below is derived from the International Union of Basic and Clinical Pharmacology Committee on Receptor Nomenclature and Drug Classification. ONO-AE-248 [11,15-O-dimethyl-PGE_2] (EP_3 receptor agonist), ONO-8713 [4-[2-[*N*-isobutyl-*N*-(2-furylsulfonyl)amino]-5-trifluoromethyl-phenoxymethyl] cinnamic acid] (EP_1 receptor antagonist), ONO-AE1-259 (EP_2 receptor agonist) and ONO-AE1-329 (EP_4 receptor agonist) were gifts from Ono Pharmaceutical Co., Ltd. (Chuo-ku, Osaka, Japan) BAY u3405 [3(*R*)-3-(4-fluorophenylsulphonamido)–1,2,3,4-tetrahydro-9-carbazole propanoic acid] was a gift from Bayer (Stoke Poges, UK). L-826266 (2*E*)-N-[(5-bromo-2-methoxy-phenyl)sulfonyl]–3-[5-chloro-2-(2-naphthylmethyl)phenyl] acrylamide (EP_3 receptor antagonist) was a gift from Merck (Kirkland, Canada) and GW627368X (*N*-{2-[4-(4,9-diethoxy-1-oxo-1, 3-dihydro-2H-benzo[*f*]isoindol-2-yl)phenyl]acetyl} benzene sulphonamide) (EP_4 receptor antagonist) from GlaxoSmithKline (Stevenage, UK). Misoprostol ($EP_{2\text{-}4}$ receptor agonist), sulprostone ($EP_{1,3}$ receptor agonist), 17-phenyl-PGE_2 ($EP_{1,3}$ receptor agonist) and iloprost (IP and EP_1 receptor agonist) were purchased from Cayman Chemical, SC-51322 ($EP_1 > EP_3$ receptor antagonist) from Biomol and noradrenaline (Plymouth Meeting, PA, USA), EGTA, N^G-nitro-L-arginine (L-NOARG; NO synthase inhibitor) and indomethacin (cyclooxygenase inhibitor) from Sigma Chemical Co (St. Louis, MO, USA).

Results

Effect of the EP receptor agonists and antagonists on vascular tone

Human IMA and SV both contracted after noradrenaline (1 μmol·L^{-1}) stimulation. These noradrenaline-induced contractions were 1.47 ± 0.15 g ($n = 26$) in IMA and 0.64 ± 0.14 g ($n = 11$) in SV. In contrast, PGE_2 induced contractions only in IMA and relaxations only in human SV (Figure 1; Tables 1 and 2). In addition, PGE_2 induced an additional concentration-dependent contraction on the plateau of the noradrenaline-precontracted IMA preparations (E_{max} = 106 ± 36% and pEC_{50} = 7.50 ± 0.30; $n = 3$).

The selective $EP_{1/3}$ (sulprostone, 17-phenyl-PGE_2) or EP_3 (ONO-AE-248, misoprostol) receptor agonists produced concentration-dependent contractions of IMA rings (Figure 1A; Table 1). With the exception of sulprostone, the other EP receptor agonists were unable to contract SV preparations (Figure 1B; Table 2). However, the contraction induced by sulprostone was completely abolished in absence of extracellular calcium in the SV but not in IMA (Tables 1 and 2). The EP_4

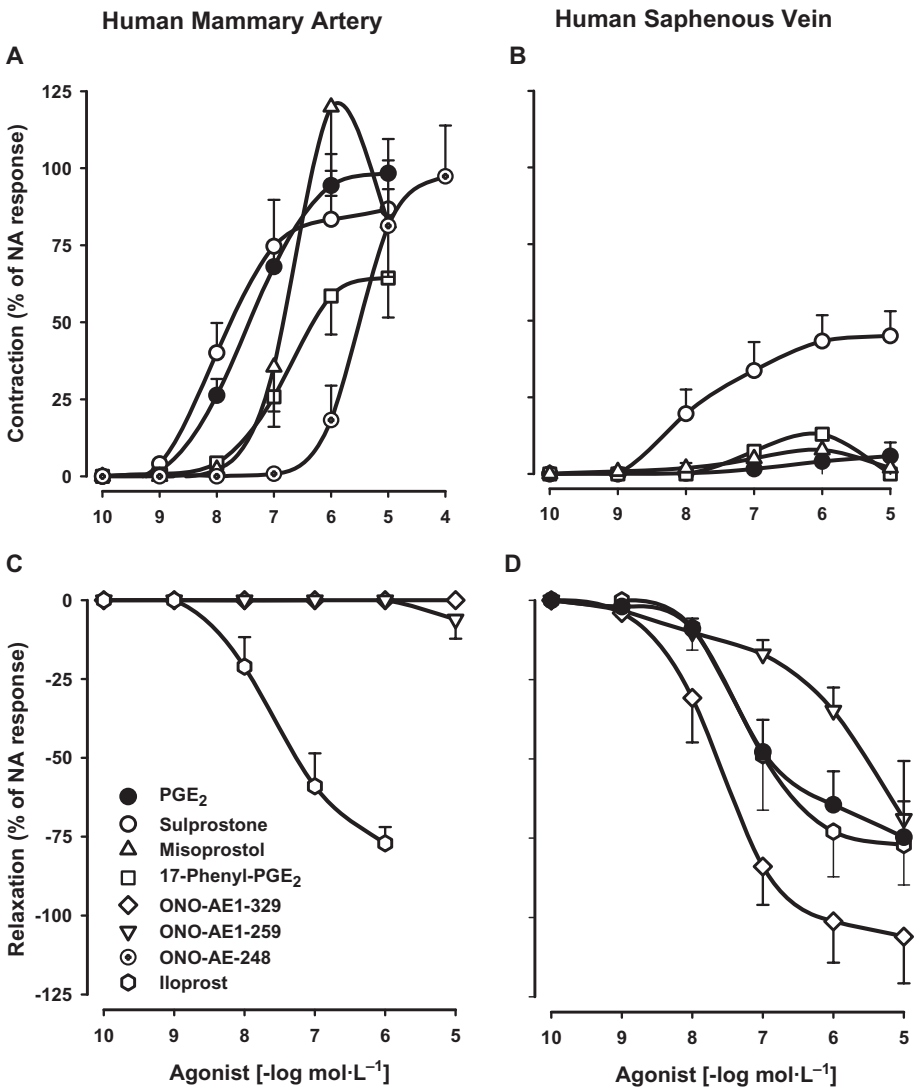

Figure 1

Cumulative concentration–response curves induced by EP receptor agonists in human mammary artery **(**A, C**)** and saphenous vein **(**B, D**).** These effects were measured on preparations with **(**C, D**)** or without **(**A, B**)** precontraction (noradrenaline, 1 μmol·L^{-1}). All preparations were treated (30 min) with BAY u3405 (10 μmol·L^{-1}), indomethacin (1.7 μmol·L^{-1}) and L-NOARG (0.1 mmol·L^{-1}) before establishing concentration–response curves. Values are means ± s.e.mean (see Tables 1 and 2 for *n*, pEC_{50} and E_{max} values), nH were not significantly different from 1.

receptor agonist (ONO-AE1-329) produced a concentration-dependent relaxation of SV, while this effect was only observed with the high concentration of ONO-AE1-259, the EP_2 receptor agonist (Figure 1D; Table 2). Finally, iloprost, the IP/EP_1 receptor agonist, induced only concentration-dependent relaxation of IMA and SV with or without noradrenaline-precontraction (Figure 1C and D; Table 1).

The use of the selective EP receptor antagonists confirmed the previous results obtained with the different selective agonists. The contraction of IMA induced by PGE_2 was significantly inhibited by a low dose of the selective EP_3 receptor antagonist (L-826266), while the high concentration of the EP_1 receptor antagonist (ONO-8713) was ineffective (Figure 2; Table 1). Another EP_1 receptor antagonist (SC-51322, 1 μmol·L^{-1}) was also ineffective, whereas at a higher nonselective (between EP_1/EP_3 receptors) concentration (10 μmol·L^{-1}), this antagonist caused a concentration-related rightward shift (sevenfold) of the PGE_2 concentration–effect curves (Table 2). In these vascular preparations, L-826266 (0.3 μmol·L^{-1}; 3 μmol·L^{-1}) caused a rightward shift (5- and 19-fold respectively) of the PGE_2 concentration–effect curve with a pK_B value of 7.23 ± 0.34 (n = 6). Finally, the SV relaxation induced by ONO-AE1-329 was significantly blocked by GW627368X, a selective EP_4 receptor antagonist (Table 2).

RT-PCR analysis of EP receptors in IMA and SV

RT-PCR analysis revealed the accumulation of transcripts corresponding to EP_3 and EP_4 but not to EP_1 and EP_2 receptor subtypes in the IMA RNA preparations derived from two different patients. However, the RT-PCR products for the EP_1 receptor were strongly detected in human pulmonary vein

Table 1

Effect of prostanoid receptor agonists on the muscular tone of isolated human mammary artery (IMA)

Treatment	Agonist	pEC_{50}	E_{max} (% noradrenaline)	*n*
IMA with basal tone				
	PGE_2	7.51 ± 0.10	98 ± 11	19
SC-51322 (1 μmol·L^{-1}; EP_1 > EP_3 antagonist)	PGE_2	7.07 ± 0.07	53 ± 23	5
SC-51322 (10 μmol·L^{-1})	PGE_2	6.66 ± 0.23*	57 ± 25	4
ONO-8713 (10 μmol·L^{-1}; EP_1 antagonist)	PGE_2	7.24 ± 0.19	93 ± 21	5
L-826266 (0.3 μmol·L^{-1}; EP_3 antagonist)	PGE_2	6.80 ± 0.22*	77 ± 10	6
L-826266 (3 μmol·L^{-1})	PGE_2	6.23 ± 0.27*	47 ± 09*	6
without BAY u3405 (TP antagonist)	PGE_2	6.98 ± 0.44	152 ± 23*	5
without endothelium	PGE_2	7.47 ± 0.17	77 ± 16	4
	Sulprostone (EP_3 > EP_1)	7.80 ± 0.12	87 ± 16	11
EGTA (50 μmol·L^{-1}) and without calcium	Sulprostone	7.93 ± 0.43	95 ± 19	5
	17-phenyl-PGE_2 ($EP_{1,3}$)	6.76 ± 0.15	64 ± 13	5
	Misoprostol ($EP_{2\text{-}4}$)	6.86 ± 0.06	120 ± 29	5
	ONO-AE-248 (EP_3)	5.64 ± 0.22	97 ± 16	4
	Iloprost (IP > EP_1)	7.41 ± 0.37	−39 ± 11	3
Precontracted IMA				
NA precontraction	Iloprost	7.46 ± 0.24	−77 ± 05	5
NA precontraction	ONO-AE1-329 (EP_4)	NC	0 ± 0	4
NA precontraction	ONO-AE1-259 (EP_2)	NC	−6 ± 06	3

IMA rings were incubated for 30 min with indomethacin (1.7 μmol·L^{-1}), L-NOARG (0.1 mmol·L^{-1}), BAY u3405 (10 μmol·L^{-1}) and with (or without) one of the indicated treatments. The selectivity of the different compounds is shown in brackets. The maximal effects (E_{max}), induced by the agonists (10 μmol·L^{-1}) are expressed as % of the noradrenaline (NA, 1 μmol·L^{-1}) contraction. Positive and negative E_{max} values indicate contraction or relaxation respectively. The half-maximum effective concentrations are presented as pEC_{50} (NC, not calculable). Values are means ± s.e.mean, derived from cumulative concentration–response curves induced by EP agonists and from (*n*) different patients.* indicates data significantly different from respective control values (same agonist without the indicated treatment in the first column; $P < 0.05$, ANOVA).

RNA preparations with the same primers (data not shown), and therefore, the human pulmonary vein was included as a positive control for the EP_1 receptor. EP_1, EP_3 and EP_4 transcripts and to a lesser extent EP_2 receptor transcripts were detected in SV preparations derived from four different patients. In Figure 3, the different EP receptor transcripts involved in the control of IMA and SV muscular tone are presented.

Measurement of PGE_2

The release of PGE_2 into the organ bath fluid by vascular rings was greater in human IMA than in SV. Noradrenaline (1 μmol·L^{-1}) stimulation induced a twofold increase in PGE_2 production in both types of vessel after a 15 min incubation period (Figure 4).

Immunohistochemistry

Representative results of immunohistochemical experiments derived from 46 sections from 11 patients are presented in Figure 5. They were performed on human IMA and SV preparations using polyclonal antibodies for the EP_1, EP_2, EP_3 or EP_4 receptor subtypes. In the absence of primary antibody or in the presence of EP receptor antibodies incubated with their respective blocking peptide (data not shown), results were similar, that is no staining was observed. The EP_3 receptor subtype was strongly detected in the IMA, while a very low staining was observed with the EP_1 antibody in these preparations. The EP_2 and EP_4 receptor subtypes were strongly detected in the SV (Figure 5). EP_2, EP_3 and EP_4 receptor staining was mainly observed within the media, over the smooth muscle cells (Figure 5).

Discussion and conclusion

In the present report, PGE_2 induced only contractions of IMA, whereas only relaxations were induced by this same PG in SV. The former response was associated with EP_3 receptor activation in the IMA and the latter via EP_4 receptor activation in the SV. These pharmacological results were confirmed by immunolocalization of these receptors in the smooth muscle layer and by the identification of the corresponding mRNA in these preparations. Furthermore, endogenous PGE_2 production was greater in IMA when compared with SV especially when vessels were stimulated with noradrenaline. This last result confirms and extends previous reports obtained with these vascular rings (Bishop-Bailey *et al.*, 1997).

The involvement of the EP_3 receptor in IMA contraction was supported by the following observations. First, ONO-AE-248 and misoprostol, selective EP_3 receptor agonists, always

Table 2

Effect of prostanoid receptor agonists on the muscular tone of isolated human saphenous vein (SV)

Treatment	Agonist	pEC_{50}	E_{max} (% noradrenaline)	*n*
SV with basal tone				
	PGE_2	NC	6 ± 04	6
without BAY u3405 (TP antagonist)	PGE_2	NC	29 ± 12	3
	Misoprostol (EP_{2-4})	NC	2 ± 04	5
	17-phenyl-PGE_2 ($EP_{1,3}$)	NC	0 ± 15	3
	Sulprostone ($EP_3 > EP_1$)	7.48 ± 0.23	45 ± 08	7
EGTA (50 μmol·L^{-1}) and without calcium	Sulprostone	NC	9 ± 03*	3
Precontracted SV				
NA precontraction	PGE_2	7.14 ± 0.09	−75 ± 11	4
NA precontraction	ONO-AE1-329 (EP_4)	7.65 ± 0.15	−106 ± 15	5
NA precontraction and GW627368X (10 μmol·L^{-1}; EP_4 antagonist)	ONO-AE1-329	<5	−95/−132	2
NA precontraction	ONO-AE1-259 (EP_2)	<5	−69 ± 18	5
NA precontraction	Iloprost ($IP > EP_1$)	7.09 ± 0.21	−77 ± 13	4

SV rings were incubated for 30 min with indomethacin (1.7 μmol·L^{-1}), L-NOARG (0.1 mmol·L^{-1}), BAY u3405 (10 μmol·L^{-1}) and with (or without) one of the indicated treatments. The selectivity of the different compounds is shown in brackets. The maximal effects (E_{max}) induced by the agonists (10 μmol·L^{-1}) are expressed as % of the noradrenaline (NA, 1 μmol·L^{-1}) contraction. Positive and negative E_{max} values indicate contraction or relaxation, respectively. The half-maximum effective concentrations are presented as pEC_{50} (NC, not calculable). Values are means ± s.e.mean, derived from cumulative concentration–response curves induced by EP receptor agonists and from (*n*) different patients. * indicates data significantly different from respective control values (same agonist without the indicated treatment in the first column; $P < 0.05$, ANOVA).

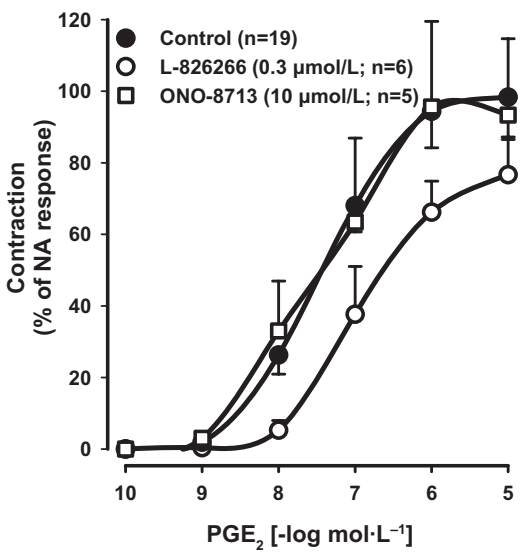

Figure 2

Effects of selective $EP_{1/3}$ receptor antagonists in human mammary artery. Cumulative concentration–response curves induced by PGE_2 in presence of an EP_3 receptor antagonist (L-826266) or an EP_1 receptor antagonist (ONO-8713). Responses were expressed as a percentage of the noradrenaline (NA, 1 μmol·L^{-1}) contraction. Values are means ± s.e.mean derived from (*n*) different patients (see Table 1 for pEC_{50}, E_{max} values and statistics). All preparations were also incubated with indomethacin (1.7 μmol·L^{-1}), BAY u3405 (10 μmol·L^{-1}) and L-NOARG (0.1 mmol·L^{-1}).

induced contractions in IMA. Second, the potency ranking for previously documented agonists was sulprostone >17-phenyl-PGE_2. These results support the notion that this vasoconstriction followed activation of EP_3 receptors rather than that of EP_1 receptors (Qian *et al.*, 1994). In addition, L-826266, the selective antagonist for the EP_3 receptor, inhibited the contraction induced by PGE_2 in IMA, while the selective EP_1 receptor antagonists (SC-51322, ONO-8713) were ineffective. In addition, the IP/EP_1 vasoconstriction agonist, iloprost, failed to contract the human IMA, suggesting that the EP_1 receptor was not involved. In some publications (Cracowski *et al.*, 2001; Wiley and Davenport, 2002), a contractile effect of PGE_2 in human IMA had been reported, but the receptor involved was not determined. We demonstrate here that this effect is mediated via activation of the EP_3 receptor, a result confirmed by the detection of EP_3 receptor transcripts and protein in IMA preparations. Furthermore, the TP receptor is also stimulated by PGE_2, since the IMA contraction induced by PGE_2 was significantly increased in the absence of the TP receptor antagonist, BAY u3405. The vasoconstrictive effects of PGE_2 on IMA are similar to those observed in human pulmonary artery and vein, mediated by EP_3 and EP_1 receptor activation respectively (Qian *et al.*, 1994; Walch *et al.*, 2001; Norel *et al.*, 2004). In precontracted IMA, PGE_2 induces a cumulative contraction, and no relaxation was observed as previously shown by Wiley and Davenport (2002). This effect of PGE_2 and the absence of vasodilatation produced by the selective $EP_{2/4}$ receptor agonists (ONO-AE1-259, ONO-AE1-329) suggest the absence of functional EP_2 or EP_4 receptors in the IMA smooth muscle.

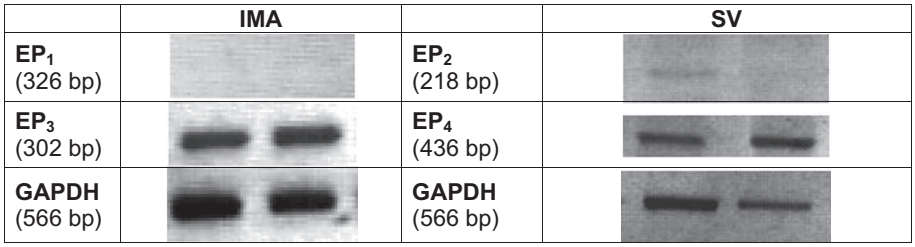

Figure 3

In the human mammary arteries (IMA; $n = 2$), RT-PCR experiments showed the presence of transcripts corresponding to EP_3 receptors and GAPDH, whereas transcripts corresponding to EP_1 receptors were not detectable. In saphenous veins (SV; $n = 2$ representative from $n = 4$), the EP_4 receptor and GAPDH transcripts were detected at higher levels than those for the EP_2 receptor.

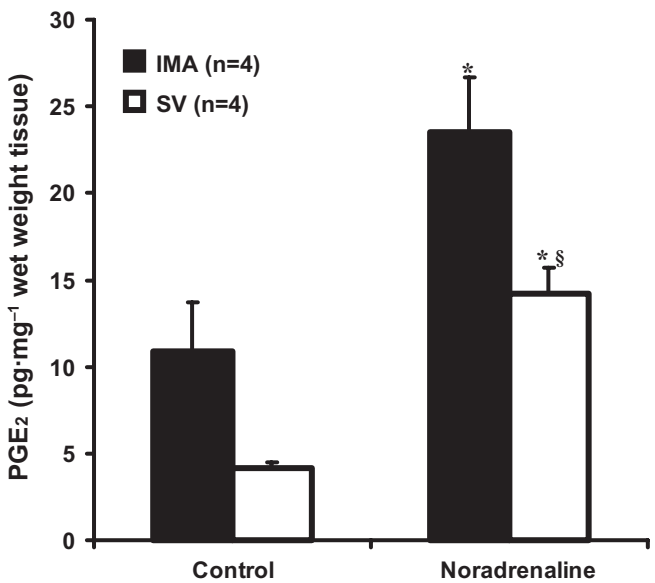

Figure 4

Release of PGE_2 by human isolated mammary artery (IMA) and saphenous vein (SV) mounted in the organ bath system. PGE_2 was measured in bath fluid aliquots collected after a 15 min incubation period of the preparations before (control) and after noradrenaline (1 µmol·L^{-1}) stimulation. PGE_2 quantities were expressed as pg·mg^{-1} of wet weight tissue. Values are means ± s.e.mean derived from (n) different patients.* indicates data significantly different ($P < 0.05$) from similar values obtained in control and § indicates data significantly different ($P < 0.05$) from the corresponding value obtained in IMA (one-way ANOVA).

The results obtained in SV are opposed to those observed in IMA. PGE_2 and the $EP_{1/3}$ receptor agonists (except sulprostone) did not modify the basal tone in SV, suggesting the absence of EP_1 or EP_3 receptor involvement in the control of SV muscular tone. PGE_2 relaxed precontracted human SV, and these preparations were at least 380 times less sensitive to the EP_2 receptor agonist, ONO-AE1-259, than to the EP_4 receptor agonist, ONO-AE1-329. These effects were NO-independent, and the relaxation induced by ONO-AE1-329 was blocked by the selective EP_4 receptor antagonist, GW627368X. These results suggest the involvement of the EP_4 receptor in PGE_2-induced relaxation of SV, a proposal also supported by our immunohistochemical and molecular biology experiments. This EP_4 receptor–mediated relaxation is similar to that observed in human arteries (cerebral, uterine) (Baxter *et al.*, 1995; Davis *et al.*, 2004) and pulmonary veins (Foudi *et al.*, 2008). Although the EP_2 receptor subtypes were detected by immunohistochemistry, very few EP_2 receptor transcripts were detected in SV, and ONO-AE1-259 did not modify SV vascular tone. These results suggest the non-involvement of the EP_2 receptor in PGE_2-induced relaxation in SV and a nonspecific staining in the SV with this EP_2 antibody.

The effects of PGE_2 on isolated preparations of human IMA and SV that we observed are different from those reported in most of the previous comparative studies with other agonists (Luscher *et al.*, 1988; Cracowski *et al.*, 1999). In these investigations, the vasodilator agonists were more potent on human IMA, while the contractile agonists were either equipotent in both types of vessel or more effective on human SV. These differences have been interpreted to explain the higher patency of IMA coronary bypass grafts compared with SV grafts (Goldman *et al.*, 2004).

Two other pharmacological conclusions can be drawn from our results. The concentration-dependent relaxation induced by iloprost is in agreement with the presence of the IP receptor on the smooth muscle cells of IMA and SV. A similar result has been previously described on both preparations with PGI_2 (Yang *et al.*, 1989). On the other hand, sulprostone was the only $EP_{3/1}$ receptor agonist inducing contraction of both vessels, and this effect was completely abolished in the SV after depletion of calcium and in the presence of EGTA. The contraction of IMA was not affected by this treatment in accordance with the activation of a classical EP_3 receptor inhibiting the cAMP pathway (Norel, 2007). In the SV, the calcium-dependent contraction induced by sulprostone is similar to that observed in guinea pig aorta or rat femoral artery, which involves a Ca^{2+} influx–Rho–kinase pathway (Shum *et al.*, 2003; Hung *et al.*, 2006). However, in our experiments, the SV contractions induced by sulprostone appeared not to be mediated via EP receptor activation since PGE_2, misoprostol and 17-phenyl-PGE_2 were all without effect.

The present characterization of the EP_3 and EP_4 receptor subtypes in the IMA and SV, respectively, could be relevant to several clinical situations. On the one hand, the presence

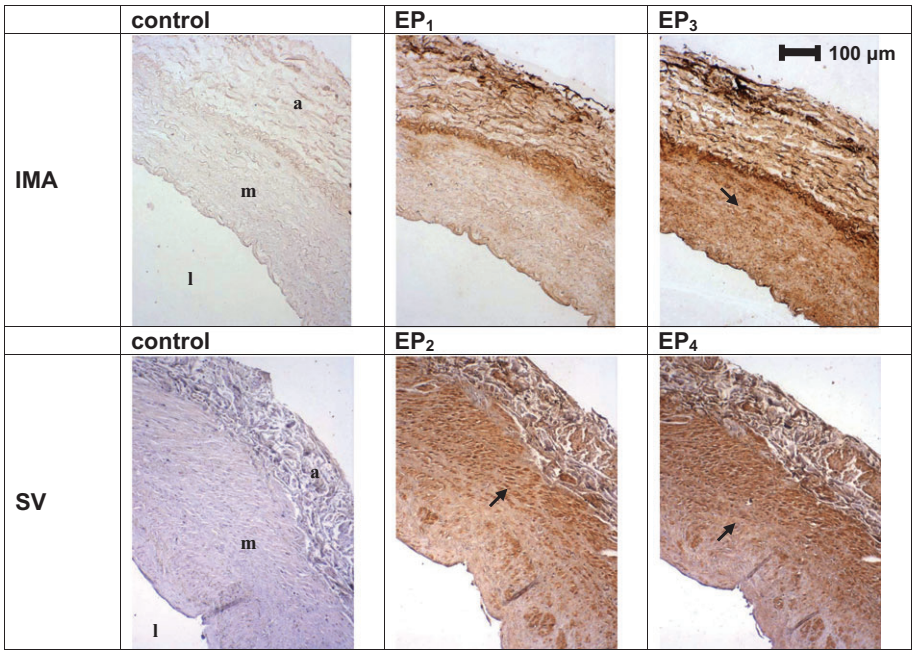

Figure 5

Immunohistochemistry was performed on serial sections of human vessels without (control) or with anti-EP antibodies. Significant staining (black arrow) was mainly detected in the medial smooth muscle layer of the human mammary artery (IMA) with the EP_3 receptor antibody (ADI 1/100) and in the saphenous vein (SV) with both anti-EP_2 and anti-EP_4 receptor antibodies (Cayman 1/100). Weak staining or nonspecific staining was observed in the IMA with the anti-EP_1 receptor antibody (Cayman 1/100). Photomicrographs show representative results from $n = 11$ different patients and 46 paraffin sections analysed. a, adventitia; m, media; l, lumen. Magnification × 10.

of the relaxant EP_4 receptor in the SV could be targeted as a therapeutic approach in the frequently observed development of SV graft aneurysm (Almanaseer *et al.*, 2005). On the other hand, synthetic EP receptor agonists (misoprostol, sulprostone, gemeprost), used in different clinical settings such as abortion, labour induction and gastrointestinal disorders are associated with several adverse cardiovascular side effects (hypertension, myocardial infarction, death) (Ulmann *et al.*, 1992; Cocco *et al.*, 1998; Lauer and Berentelg, 2000; Turan *et al.*, 2003) for which EP_3-mediated vasoconstriction could be responsible. More generally, an antagonist with a dual affinity toward TP and EP_3 receptors could provide a new therapeutic approach to cardiovascular disorders.

In conclusion, we demonstrate that PGE_2 mediated opposing effects in two human vascular preparations: contraction in IMA and relaxation in SV. These results have potential clinical implications since these vascular segments are the most frequently used as grafts during coronary artery bypass. The roles of PGE_2, its antagonists and therapeutic analogues may require further attention and experimental studies in order to improve graft patency.

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Conflicts of interest

None.

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