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Prostacyclin release and receptor activation: differential control of human pulmonary venous and arterial tone

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1 In human pulmonary vascular preparations, precontracted arteries were more sensitive to the relaxant effect of acetylcholine (ACh) than veins (pD_2 values: 7.25 ± 0.08 ($n = 23$) and 5.92 ± 0.09 ($n = 25$), respectively). Therefore, the role of prostacyclin (PGI_2) was explored to examine whether this mediator may be responsible for the difference in relaxation.

2 In the presence of the cyclooxygenase (COX) inhibitor, indomethacin (INDO), the ACh relaxations were reduced in arteries but not in veins. On the contrary, an inhibitor (L-NOARG) of the nitric oxide synthase blocked preferentially the relaxation in veins.

3 A greater release of 6-keto-$PGF_{1\alpha}$, the stable metabolite of PGI_2, was observed in arterial preparations than in venous preparations when stimulated with either ACh or arachidonic acid (AA).

4 Exogenous PGI_2 produced a reduced relaxant effect in the precontracted vein when compared with the artery. In the presence of the EP_1-receptor antagonist AH6809, the PGI_2 relaxation of veins was similar to arteries.

5 In veins, AA (0.1 mM) produced a biphasic response, namely, a contraction peak (0.4–0.5 g) followed by a relaxation. These contractions in venous preparations were abolished either in the absence of endothelium or in the presence of INDO or an EP_1-receptor antagonist (AH6809, SC19220). In the arterial preparations AA induced only relaxations.

6 In both vascular preparations, COX-1 but not the COX-2 protein was detected in microsomal preparations derived from homogenized tissues or freshly isolated endothelial cells.

7 The differential vasorelaxations induced by ACh may be explained, in part, by a more pronounced production and release of PGI_2 in human pulmonary arteries than in the veins. In addition, while PGI_2 induced relaxation by activation of IP-receptors in both types of vessels, a PGI_2 constrictor effect was responsible for masking the relaxation in the veins by activation of the EP_1-receptor.
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Abbreviations: AA, arachidonic acid; ACh, acetylcholine; COX, cyclooxygenase; INDO, indomethacin; L-NOARG, N^G-nitro-L-arginine; NA, noradrenaline; NO, nitric oxide; NOS, nitric oxide synthase; PG, prostaglandin

Introduction

The vasodilations induced by acetylcholine (ACh) in vessels derived from a number of vascular beds have been associated with the release of relaxant factors, namely, nitric oxide (NO), prostacyclin (PGI_2) and endothelium-derived hyperpolarizing factor (EDHF) (Bunting *et al*., 1976; Furchgott & Zawadzki, 1980; Komori & Suzuki, 1987; Palmer *et al*., 1987). Previous reports have shown that EDHF is not involved in the ACh-induced relaxations of human pulmonary arterial preparations, since treatment of tissues with a combination of NO-synthase (NOS) and cyclooxygenase (COX) inhibitors completely abolished these effects (Norel *et al*., 1996; Lawrence *et al*., 1998). In several species, the relaxant effect of ACh in pulmonary arteries and veins has been reported to be different, since veins are insensitive to this relaxant agonist (De Mey & Vanhoutte, 1982; Gruetter & Lemke, 1986; Ignarro *et al*., 1987; Kemp *et al*., 1997; Shi *et al*., 1997). In contrast, ACh has been reported to relax human pulmonary arteries and veins with the latter tissue being less sensitive (Walch *et al*., 1997). Studies in a variety of human systemic arteries and veins derived from either the same organ or tissue have reported similar results, that is, ACh has no or only a weak relaxant action in veins when compared with arteries (mammary vessels, Lüscher *et al*., 1988; vascular bed of the forearm, Kemme *et al*., 1995; hand vessels, Arner & Högestätt, 1991; and epicardial coronary vessels, Saetrum Opgaard & Edvinsson, 1996).

The exact explanation for the discrepancy observed between the relaxation induced by ACh in human pulmonary arteries and veins has not been elucidated. A variety of events could explain this differential sensitivity to ACh, such as, the enzymatic degradation of ACh, the enzymatic synthesis of

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endothelial mediators NO and/or PGI_2 and the consecutive activation of prostanoid receptors on the underlying smooth muscle. The differential sensitivity to ACh between human pulmonary arteries and veins was not associated with alterations in cholinesterase activity since cholinesterase selective inhibitors do not modify ACh relaxations (Walch *et al*., 1997). The difference in ACh relaxant effects in artery and vein was also not due to a difference of NO sensitivity. Pussard *et al*. (1995) have shown that sodium nitroprusside, an NO donor, relaxed human pulmonary arteries and veins with equal potency. Furthermore, both isolated human pulmonary arteries and veins exhibited the same sensitivities to the relaxant effect of cicaprost, a stable analogue of PGI_2 that is selective for the IP-receptors (Walch *et al*., 1999). However, prostanoids, the metabolites of arachidonic acid (AA) *via* the COX activity may stimulate with variable potency different receptor subtypes. These subtypes include the DP, EP, FP, IP and TP receptors that preferentially respond to PGD_2, PGE_2, $PGF_{2\alpha}$, PGI_2 and TxA_2, respectively. This classification does not exclude the effect, for example, of PGI_2 and TxA_2 on EP receptors (Coleman *et al*., 1994; Wright *et al*., 2001). An activation of some subtypes (TP, EP_1, EP_3, FP) induces vasoconstriction whereas the other receptor subtypes (IP, EP_2, EP_4, DP) are responsible for vasodilation. There are classical selective antagonists for most of these receptor subtypes, for example: BAY u3405 (TP), AH6809 (EP_1, EP_2, DP) and SC19220 (EP_1).

Since the production of endothelial relaxant mediators by human pulmonary veins during ACh-induced relaxation has not been determined, the aim of this study was to investigate the relative contribution of PGI_2 and NO during the ACh-induced relaxation of human pulmonary arteries and veins. This work focused on PGI_2 production and vasoactive effects in these particular vessels.

Methods

Isolated vascular preparations

All research programs involving the use of human tissue were approved and supported by the Medical Ethics Committee at 'Centre Chirurgical Marie Lannelongue'. Human lung tissues were obtained from patients (72 male and 9 female) who had undergone surgery for lung carcinoma. The mean age was 63 ± 1 years. Pulmonary arteries and veins (3–6 mm internal diameter) were cut as rings and set up in 10 ml organ baths containing Tyrode's solution (concentration mM) containing the following: NaCl 139.2, KCl 2.7, $CaCl_2$ 1.8, $MgCl_2$ 0.49, $NaHCO_3$ 11.9, NaH_2PO_4 0.4, glucose 5.5, 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). Changes in force were recorded by an isometric force displacement transducer (Narco F-60) and physiographs (Linseis). Subsequently, preparations were equilibrated (90 min) with bath fluid changes taking place every 10 min.

Contraction/relaxation studies

Some preparations were incubated (30 min) with Tyrode's solution (control) or with Tyrode's solution containing indomethacin (INDO, 1.7 μM), a COX inhibitor and/or the NOS inhibitor, N^G-nitro-L-arginine (L-NOARG; 0.1 mM). Several other rings were incubated with (INDO + L-NOARG) in combination with two K^+ channel blockers (Savage *et al*., 2003; Stoen *et al*., 2003): apamin (100 nM) and charybdotoxin (100 nM). The rings were then contracted with noradrenaline (NA, 10 μM) and increasing concentrations of ACh were added in a cumulative manner to the baths. This effect of ACh was also evaluated in venous preparations after a 30-min incubation without INDO and with the EP_1-receptor antagonists (AH6809, 10 μM or SC19220, 100 μM) or with the TP-antagonist (BAY u3405, 1 μM).

Other rings were incubated (30 min) with Tyrode's solution containing INDO (1.7 μM), BAY u3405 (1 μM), L-NOARG (0.1 mM) and in the presence or absence of AH6809 (30 μM). These vascular preparations were contracted with NA (10 μM) and subsequently cumulative concentrations of PGI_2 were added to the baths.

The effect of AA (10 and/or 100 μM) was measured on isolated vascular preparations at basal tone or after NA-induced precontraction (10 μM). The changes in muscle tone were monitored at 72 s and 15 min after AA stimulation. In several experiments, vascular preparations at basal tone were incubated in the absence or presence of INDO (30 min, 1.7 μM) prior to the addition of AA. In other preparations, the endothelium was mechanically removed by inserting both smooth-edged arms of a dissecting forceps into the lumen of the vessel and gently rolling the moistened preparation between the surface of a forefinger and the forceps for 10 s without undue stretch. The effect of AA was also evaluated in venous preparations after a 30-min incubation with the EP_1-receptor antagonists AH6809 (10 μM) or SC19220 (100 μM).

At the end of the ACh, AA or PGI_2 concentration–effect curves, the maximal relaxation was determined by the addition of papaverine (0.1 μM).

Measurement of 6-keto-$PGF_{1\alpha}$

The release of PGI_2 by human pulmonary vessels was determined by measuring 6-keto-$PGF_{1\alpha}$ levels, that is, the stable metabolite of degradation of PGI_2, in samples obtained from the bath fluid containing vascular rings. Measurements were performed using 6-keto-$PGF_{1\alpha}$ enzyme-immunoassay kits (Stallergènes Laboratories, France).

Preparations were contracted with NA (10 μM) and relaxed with ACh (1 or 10 μM). 6-Keto-$PGF_{1\alpha}$ was measured in samples collected (1) after a 5- and a 10-min incubation of the preparations in Tyrode's solution just prior to the NA stimulation (basal); (2) 10 min after the NA stimulation and (3) 5 min after the ACh stimulation.

In other preparations challenged with AA (0.01 or 0.1 μM), 6-keto-$PGF_{1\alpha}$ was measured in aliquots collected (1) after a 15-min incubation of the preparations in Tyrode's solution prior to the AA stimulation (basal) and (2) 15 min after the AA stimulation.

Isolation of membrane-bound proteins

Microsomal preparations were derived from homogenized vascular tissues or freshly isolated endothelial cells. Endothelial cells were removed from human pulmonary vessels by gently rubbing the luminal surface with a scalpel in ice-cold 50 mM Tris-HCl pH 7.4 buffer containing: disodium EDTA

5 mM, INDO 0.1 mM and phenylmethylsulphonyl fluoride 1 mM. The protocols for isolation of membrane-bound proteins from vessels and endothelial cells were based on the work of Brannon *et al.* (1994). The protein content was determined in each microsomal preparation by the method of Lowry *et al.* (1951) using bovine serum albumin as the standard.

Western blot analysis

In microsomal preparations, the proteins were denatured (100°C, 5 min in the presence of β-mercapto-ethanol). Microsomal preparations (60 μg per lane) were loaded onto a stacking gel (4%). SDS–polyacrylamide gel electrophoresis was performed using a 10% separating gel. After transfer, the nitrocellulose membranes were incubated overnight with COX-1 (ovine) monoclonal antibody, COX-2 (human) monoclonal antibody. Immunoreactive signals were developed using the chemiluminescence reagent (RCL 4000). Ovine COX-1 and COX-2 proteins were used as Western blot standards.

Data analysis

All data are means $\pm$ s.e.m. derived from (n) lung samples and statistical analysis was performed using Wilcoxon's signed-rank test, Student's t-test, Student's paired t-test or ANOVA followed by Dunnett's method with a confidence level of 95%.

- Contraction/relaxation studies. The maximal relaxation (E_{max}) produced by ACh and PGI_2 as well as the half-maximum effective concentration values (EC_{50}) were interpolated from the different agonist concentration–effect curves. The pD_2 values were calculated as the negative log of EC_{50} values. The effects induced by either ACh, PGI_2 or AA were expressed in grams or in % of the papaverine maximal response. The data expressed in grams are positive for the contractions and negative for the relaxations. The data for relaxations expressed as percent of the papaverine relaxation are positive. Since human pulmonary veins sometimes exhibited spontaneous relaxation after precontraction with NA (15–20% of papaverine relaxation after 30 min), the measurements of agonist-induced vascular tone changes were calculated only when inflection points were observed during the dose–response curve.
- Measurement of 6-keto-$PGF_{1\alpha}$. Basal values (10 and 15 min) were subtracted from stimulated values (NA and AA, respectively) and NA-stimulated values were subtracted from ACh-stimulated values.

Compounds

AA, PGI_2, AH6809 (6-isopropoxy-9-oxaxanthene-2-carboxylic acid), antibodies and Western blot standards were purchased from the Cayman Chemical Company, Ann Arbor, MI, U.S.A. RCL 4000 was obtained from Covalab (69007 Lyon, France). All other reagents for SDS–polyacrylamide gel electrophoresis were purchased from Amersham Pharmacia Biotechnology (91898 Orsay, France). BAY u3405 (3(R)-3-(4-fluorophenylsulphonamido)-1,2,3,4-tetrahydro-9-carbazole propanoic acid) was a gift from Bayer, Stokes Poges, U.K. SC19220 (8-chlorodibenz [b,f][1,4] oxazepine-10(11H)-carboxy-(2-acetyl)hydrazide) was a gift from Searle Research and Development, Skokie, IL, U.S.A. Papaverine hydrochloride was obtained from Meram Laboratories (77020 Melun, France). Apamin, charybdotoxin, Tris-HCl, EDTA (ethylenediaminetetraacetic acid), phenylmethylsulphonyl fluoride, bovine serum albumin, NA, ACh, L-NOARG (N^G-nitro-L-arginine) and INDO were purchased from Sigma Chemical Co., St Louis, MO, U.S.A.

Results

Contraction/relaxation studies

The NA-induced contractions were similar in arteries (1.61 ± 0.11 g; $n = 41$) and veins (1.99 ± 0.19 g; $n = 45$). The maximal relaxations determined at the end of the protocols using papaverine (-1.79 ± 0.11 and -2.24 ± 0.19 g in arteries ($n = 41$) and veins ($n = 45$), respectively) were statistically not different.

The data presented in Figure 1 show the relaxant effect of ACh in isolated human pulmonary arteries and veins. Veins were approximately 25-fold less sensitive to the relaxant effect of ACh than arteries (pD_2 values: veins, 5.92 ± 0.09 ($n = 25$) and arteries, 7.25 ± 0.08 ($n = 23$), $P < 0.0001$). The effects of INDO (1.7 μM) and/or L-NOARG (0.1 mM) were determined on the ACh-induced relaxations in paired human pulmonary vessels (Figure 2; Table 1). In arteries, INDO significantly decreased the relaxations induced by ACh whereas L-NOARG modified only the relaxations induced by the highest concentrations of ACh (> 1 μM). In contrast, in veins, L-NOARG significantly decreased the ACh-induced relaxations while INDO slightly, but significantly, increased the maximal relaxations induced by ACh. The combination of both inhibitors abolished the relaxant response induced by ACh in human pulmonary arteries while in veins only 50% of the

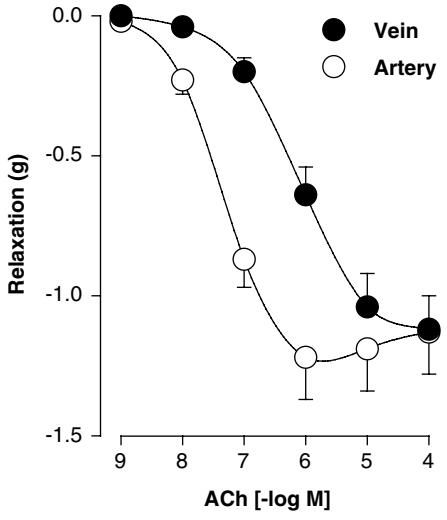

Figure 1 Cumulative concentration–response curves induced by ACh in human pulmonary arteries and veins. The preparations were contracted with NA (10 μM) and cumulative concentrations of ACh were added into the baths. Responses are expressed in grams. Values are means $\pm$ s.e.m. from 23 and 25 different lung samples for arteries and veins, respectively (see text for values significantly different).

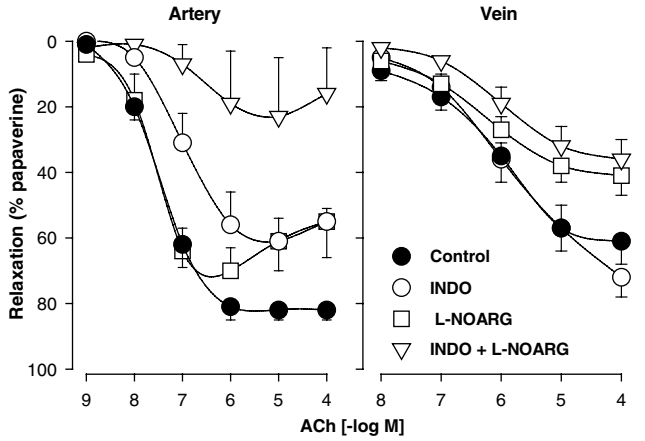

Figure 2 Effect of INDO and L-NOARG on the relaxations induced by ACh in paired isolated human pulmonary vessels. After an incubation (30 min) with Tyrode's solution (Control) or Tyrode's solution containing indomethacin (INDO, 1.7 μM) and/or L-NOARG (0.1 mM), the preparations were contracted with NA (10 μM) and cumulative concentrations of ACh were added into the baths. Responses are expressed as the percent of the relaxation induced with papaverine (0.1 mM). Values are means $\pm$ s.e.m. derived from five paired lung samples for the arteries and five other paired lung samples for the veins (see Table 1 for values significantly different).

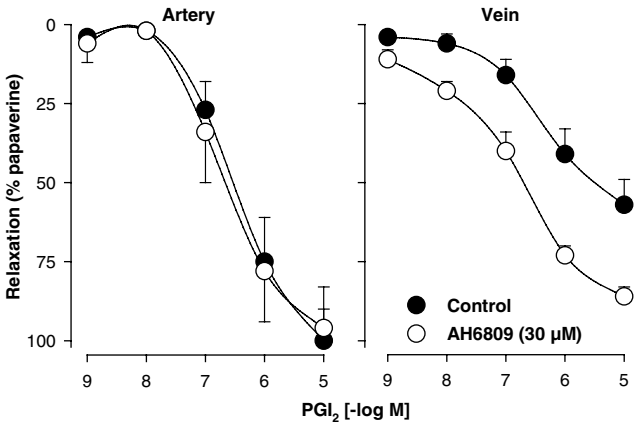

Figure 3 Cumulative concentration–response curves induced by PGI_2 in paired isolated human pulmonary arteries ($n=3$) and veins ($n=4$). All the preparations were treated with BAY u3405 (1 μM). After an incubation (30 min) with Tyrode's solution (Control) or Tyrode's solution containing AH6809 (30 μM), the preparations were contracted with NA (10 μM) and cumulative concentrations of PGI_2 were added into the baths. Responses are expressed as the percent of the relaxation induced with papaverine (0.1 mM) and values are means $\pm$ s.e.m.

response was inhibited (Figure 2; Table 1). This remaining relaxation was not abolished when the venous preparations were incubated with INDO + L-NOARG and the two K^+ channel blockers: apamin and charybdotoxin (10 venous preparations derived from $n=3$ lung samples, data not shown). The effects of L-NOARG (0.1 mM) and/or INDO (1.7 μM) on the basal tone of human pulmonary veins and arteries were variable. These treatments induced frequently small but sustained contractions (<0.5 g; 30 min) primarily in the veins. The NA-induced contractions in pulmonary arteries and veins were not modified by any of the pretreatments mentioned in this report.

In human pulmonary venous preparations (two treated and one control) derived from one lung sample, treatment with either SC19220 (100 μM) or AH6809 (10 μM), increased the ACh maximal relaxation (E_{max}) by 36 and 34% respectively, whereas the pD_2 values were unchanged (preliminary results). In addition, the TP antagonist (BAY u3405) did not modify the ACh-induced relaxation in human pulmonary veins (Control, $E_{max}=37\%$ of papaverine response; BAY u3405 treated, $E_{max}=38\%$ of papaverine response; $n=1$ lung sample, two preparations).

PGI_2 relaxed dose-dependently human pulmonary arteries and veins contracted with NA and similar sensitivities to PGI_2 were observed (pD_2 values: arteries, 6.29 ± 0.10 ($n=3$) and veins, 6.29 ± 0.09 ($n=4$). However, the relaxant curve and the maximal relaxation induced by PGI_2 in the human pulmonary vein were significantly reduced when compared with the artery (Figure 3). Subsequent to AH6809 treatment the relaxant effect of PGI_2 was potentiated in veins, while unchanged in arteries. Therefore, in the presence of the EP_1 antagonist, the PGI_2 relaxant effect was similar in vein and artery (Figure 3).

In human pulmonary arteries, AA applied to elevated or basal tone-induced relaxations (Figure 4 and 5). The relaxations were unaffected by treatment with INDO in the precontracted arteries (Figure 4) or in preparations devoid of endothelium (Figure 5). In human pulmonary vein, AA stimulation of elevated or basal tone-induced a transient peak contraction followed by a relaxation (Figure 4 and 5). In the NA precontracted preparations, the contractions induced by

Table 1 Effect of INDO and L-NOARG on the relaxations induced by ACh in paired human pulmonary arteries or veins precontracted with NA

	Arteries		*Veins*	
	pD_2 values	E_{max}	*pD_2 values*	E_{max}
Control	7.42 ± 0.12	87 ± 0.3	6.07 ± 0.15^b	62 ± 7^b
INDO	6.90 ± 0.21^a	61 ± 09^a	5.83 ± 0.24^b	72 ± 6^a
L-NOARG	7.61 ± 0.15	74 ± 06^a	6.28 ± 0.26^b	$42\pm6^{a,b}$
INDO + L-NOARG	NC	28 ± 16^a	5.77 ± 0.17	37 ± 7^a

The maximal relaxations (E_{max}) induced by ACh are expressed as percent of the relaxation produced with papaverine (0.1 mM). Values are means $\pm$ s.e.m. derived from five lung samples.
[a]Data significantly different ($P<0.05$) from control values (ANOVA + Dunnett's method).
[b]Data significantly different ($P<0.05$) from corresponding values obtained in arteries (Student's t-test). NC = not calculable.

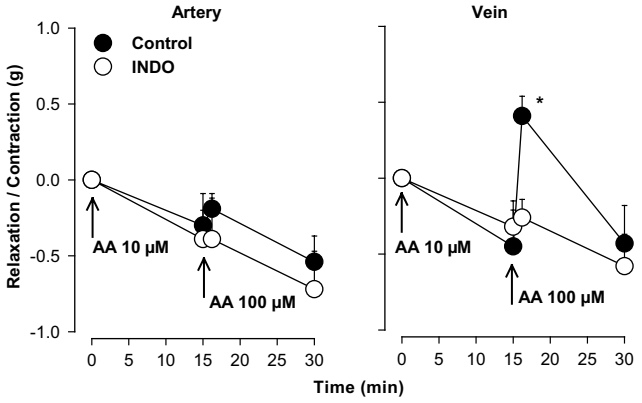

Figure 4 Cumulative concentration–response curves induced by AA in human pulmonary arteries and veins precontracted with NA (10 μM). Responses are expressed in grams. Values are means $\pm$s.e.m. from 5–6 paired lung samples. *Indicates data significantly different ($P<0.05$) from values obtained 72 s after AA (100 μM) stimulation in arteries without treatment or in veins treated with INDO or from values measured 15 min after AA (10 μM) stimulation in veins (Student's paired t-test).

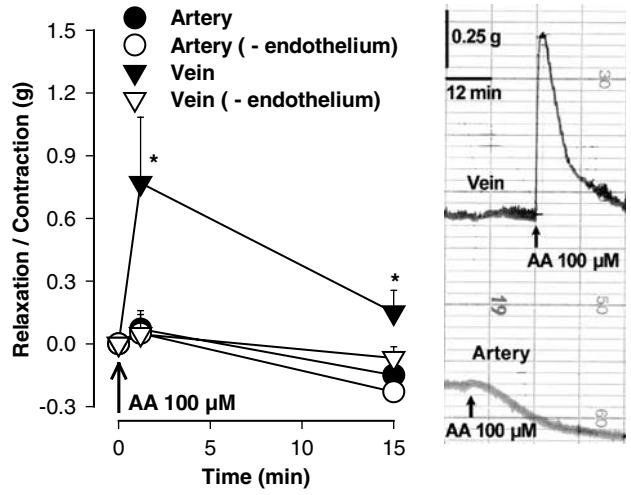

Figure 5 Physiological effects of AA (100 μM) on the basal tone of human pulmonary arteries and veins with or without endothelium. Responses are expressed in grams. Left panel, values are means $\pm$s.e.m. from seven paired lung samples for arteries and veins. *Indicates data significantly different ($P<0.05$) from appropriate control (Wilcoxon's signed rank test). Right panel, representative tracing of the effects induced by AA (100 μM) in two vascular preparations derived from the same lung sample.

AA were abolished by treatment of veins with INDO (Figure 4). In venous preparations at resting tone, the AA-induced contractions were significantly inhibited either by the different EP_1-receptor antagonists (AH6809 or SC19220, $n=4$; Figure 6) or in the absence of endothelium (Figure 5).

Measurement of 6-keto-PGF$_{1\alpha}$

The basal production of 6-keto-$PGF_{1\alpha}$ was similar in human pulmonary arteries and veins (16 ± 02 and 18 ± 01 pg mg^{-1} of wet tissue in arteries ($n=15$) and veins ($n=14$), respectively), after a 10-min incubation. The NA stimulation provoked approximately a two-fold increase in the 6-keto-$PGF_{1\alpha}$ production in both type of vessels (40 ± 06 and 36 ± 04 pg mg^{-1}

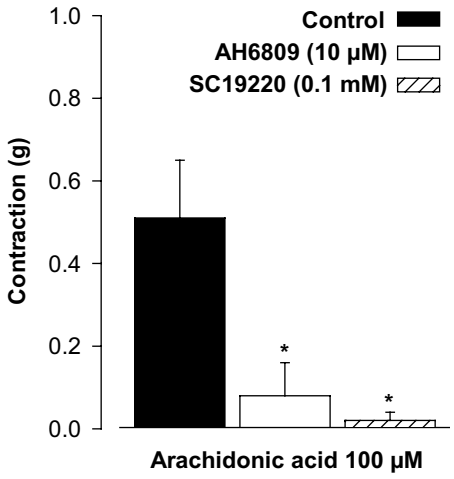

Figure 6 Inhibition of the response induced by AA (100 μM) on the basal tone of human pulmonary veins with prostanoid receptor antagonists (SC19220 or AH6809; 30 min incubation). Responses are expressed in grams. Values are means $\pm$s.e.m. from four paired lung samples. *Indicates data significantly different ($P\leqslant0.05$) from appropriate control (Student's paired t-test).

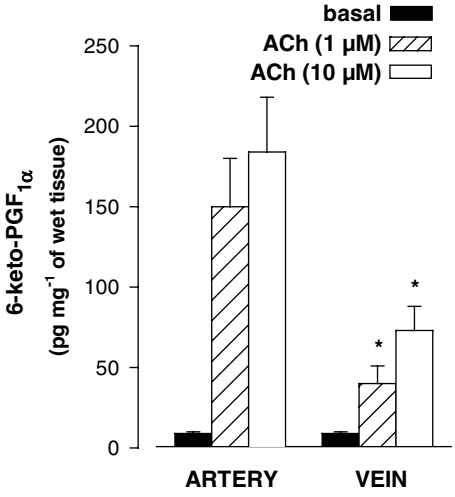

Figure 7 Release of 6-keto-$PGF_{1\alpha}$ by isolated human pulmonary vessels stimulated with ACh. The preparations were contracted with NA (10 μM) and relaxed with ACh (1 or 10 μM). 6-Keto-$PGF_{1\alpha}$ was measured in aliquots collected (1) after a 5-min incubation of the preparations with the bath fluid prior to the NA stimulation (basal) and (2) 5 min after the ACh stimulation. Stimulated (NA) values were subtracted from stimulated values (ACh). The 6-keto-$PGF_{1\alpha}$ quantities were expressed as pg mg^{-1} of tissue wet weight. Values are means $\pm$s.e.m. derived from 14–15 (basal), 9 (ACh 1 μM) or 10 (ACh 10 μM) lung samples. *Indicates data significantly different ($P<0.05$) from similar values obtained in arteries (Student's paired t-test).

of wet tissue in arteries ($n=15$) and veins ($n=14$), respectively, after 10 min incubation; $P<0.0001$). A significant increase of 6-keto-$PGF_{1\alpha}$ production was also observed after challenge with ACh in the precontracted human pulmonary vessels (Figure 7). While the stimulation with ACh (1 μM) induced a 17-fold increase of 6-keto-$PGF_{1\alpha}$ production by human pulmonary arteries, only a four-fold increase was observed under the same conditions in veins. A stimulation with ACh (10 μM) also provokes a significantly greater release of 6-keto-$PGF_{1\alpha}$ in arteries than in veins. Finally, the productions of

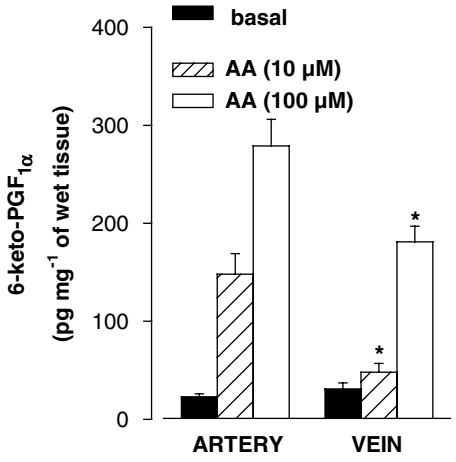

Figure 8 Release of 6-keto-$PGF_{1\alpha}$ by isolated human pulmonary vessels stimulated with AA. The preparations were challenged with AA (10 or 100 μM). 6-Keto-$PGF_{1\alpha}$ was measured in aliquots collected (1) after a 15-min incubation of the preparations with the bath fluid just prior to the AA stimulation (basal) and (2) 15 min after the AA stimulation. Basal values were subtracted from stimulated values. The 6-keto-$PGF_{1\alpha}$ quantities were expressed as pg mg^{-1} of tissue wet weight. Values are means $\pm$ s.e.m. derived from 11 lung samples. *Indicates data significantly different ($P<0.05$) from similar values obtained in arteries (Student's paired t-test).

6-keto-$PGF_{1\alpha}$ by human pulmonary vessels were measured after challenge with AA (10 and 100 μM); the productions were, respectively, 3- and 1.5-fold greater in arteries than in veins (Figure 8). The production of 6-keto-$PGF_{1\alpha}$ induced by AA (100 μM) was significantly and similarly reduced by 68 ± 30 and $56\pm13\%$, in arteries and veins, respectively ($n=4$) after endothelium removal.

Western blot analysis

In microsomal preparations derived from homogenized vascular tissues, the COX-1 protein was detected and expression was similar between arteries and veins ($n=6$). In contrast, the COX-2 protein was not detectable in either type of vessels. Similar data were obtained from microsomal preparations derived from endothelial cells ($n=3$; Figure 9).

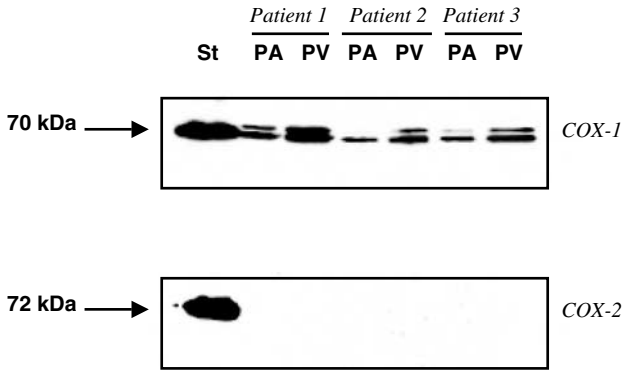

Figure 9 Western blot analysis of endothelial COX-1 and COX-2 proteins in human pulmonary arteries (PA) and veins (PV) obtained from three different patients. SDS–PAGE was performed on microsomal preparations derived from endothelial cells freshly isolated from human pulmonary vessels. Ovine COX-1 and COX-2 proteins were used as standard (St) for Western blots. Films were exposed 30 min on membranes. Arrows indicate the molecular weight of COX proteins in kDa.

Discussion

The data (present report) show a reduced sensitivity of isolated human pulmonary veins to the relaxant effect of ACh when compared with arteries. The results obtained with L-NOARG suggest that the endogenous production of NO may be greater in human pulmonary veins than in arteries. These data suggest that the difference in ACh sensitivities of the human pulmonary vessels were not associated with NO. In contrast, the decreased production and release of PGI_2 by veins may account for this difference. In addition, prostacyclin may be the AA metabolite responsible for the contraction of human pulmonary vein but not artery *via* the activation of the EP_1-receptor. These observations suggest that PGI_2 differential metabolism and vasoactive effects may, in part, be responsible for the reduced sensitivity to the relaxant effect of ACh in human pulmonary veins when compared with arteries.

Previous investigations have shown that porcine (Bina *et al*., 1998; Bäck *et al*., 2002) and ovine (Tzao *et al*., 2001) pulmonary vessels exhibited greater NOS activity in veins than in arteries. These observations are supported by the different physiological results obtained with L-NOARG on the relaxations induced by ACh in human pulmonary veins when compared with arteries. The effect of L-NOARG in the arterial preparations only unmasked the contractile activity of ACh (Norel *et al*., 1996). In the veins since there is no contraction induced by ACh (Walch *et al*., 1997), the reduced ACh relaxation observed in the presence of L-NOARG was entirely associated with the reduced production of NO. L-NOARG has also been reported to completely abolish the bradykinin-induced relaxation in porcine pulmonary veins, but only partially in arteries (Félétou *et al*., 1995) data that further support a preferential NO regulation in pulmonary veins when compared with pulmonary arteries. These results suggest that NO release plays a greater role in the control of human pulmonary venous smooth muscle tone than in pulmonary arteries which has also been reported for ovine pulmonary vessels (Bansal *et al*., 1993). Therefore, NO metabolism cannot explain why human pulmonary arteries are more sensitive to the relaxant effect of ACh than veins.

The endothelial relaxant response to ACh of some rodent vessels may be inhibited by the release of TxA_2, a potent vasoconstrictor (Altiere *et al*., 1986; Kato *et al*., 1990). However, previous investigations with human pulmonary or umbilical vessels have shown that there was no difference between artery and vein in the quantities of TxA_2 released (Mehta & Roberts, 1983; Maddox *et al*., 1985; Bjoro *et al*., 1986; 1987). In addition, preliminary results (present report) showed that the TP antagonist (BAYu3405) did not modify the ACh induced relaxation in human pulmonary veins. Similarly, PGE_2 would also not be a candidate to explain the difference in ACh-induced relaxation between these vessels. PGE_2 may activate EP_1 (contractant) and an EP (relaxant) receptors in human pulmonary veins (Walch *et al*., 1999; 2001) as well as EP_3 (contractant) receptor in the arteries (Qian *et al*., 1994). In addition, the quantities of PGE_2 released are the same in both pulmonary preparations (Carter *et al*., 1984;

Norel *et al*., 1991). These data together may explain why Walch *et al*. (1999) reported PGE_2-induced relaxations in human pulmonary veins whereas arteries do not relax. Thus, PGE_2 may not play a role in the greater ACh-induced relaxation of human pulmonary arteries.

PGI_2 release may be a more relevant candidate to explain the difference in sensitivities of human pulmonary vessels to relax after ACh stimulation. This suggestion was supported by the measurement of 6-keto-$PGF_{1\alpha}$, the stable metabolite of endogenous PGI_2. A greater level of 6-keto-$PGF_{1\alpha}$ was found in artery (17- to 20-fold *versus* basal) when compared with the release in vein (four- to eight-fold *versus* basal), after stimulation with ACh (present report). In human pulmonary arterial preparations, Schellenberg *et al*. (1986) demonstrated a 15-fold release of 6-keto-$PGF_{1\alpha}$ during a 15-min stimulation with histamine. In contrast, there are no reports of 6-keto-$PGF_{1\alpha}$ release by human pulmonary veins after other receptorial activation. However, in human umbilical vessels after hypoxic stimulation, arteries release ($1.5\times$) more 6-keto-$PGF_{1\alpha}$ than veins (Bjoro *et al*., 1987). Furthermore, AA stimulation of isolated bovine pulmonary arteries and endothelial cells derived from human pulmonary arteries exhibited a greater release of 6-keto-$PGF_{1\alpha}$ when compared with the respective venous preparations (Johnson, 1980; Ignarro *et al*., 1985).

In addition to the enhanced arterial production and release of PGI_2, equivalent concentrations of exogenous PGI_2 induced greater relaxations in arteries when compared with the veins even in the presence of the TP antagonist (control curves Figure 3). The potentiation effect of the EP_1-receptor antagonist AH6809 on the exogenous PGI_2-induced relaxations suggests the activation of the EP_1-receptor described in the human pulmonary veins (Walch *et al*., 2001) but not in the arteries (Qian *et al*., 1994). The results obtained in human pulmonary veins suggest that exogenous PGI_2 induced vasorelaxation *via* the IP-receptors and this effect is counterbalanced by the activation of the EP_1-receptors. Similarly, the relaxations induced by ACh *via* endogenous release of PGI_2 were also increased in human pulmonary veins treated with the EP_1 antagonists (AH6809, SC19220). Other studies have indicated the ability of PGI_2 to activate EP_1-receptor in human cells (Walsh & Kinsella, 2000) or to contract guinea-pig smooth muscle *via* the EP_1-receptor (Dong *et al*., 1986; Lawrence *et al*., 1992). This dual control of the venous tone *via* EP_1- and/or IP-receptor in the human lung seems also present in the human hand vein (Arner & Hogestätt, 1991). In the latter tissue ACh or the IP-/EP_1- selective agonist (iloprost) are less potent to induce relaxations than in the arteries.

Exogenous AA induced contractions in a variety of venous preparations isolated from dog or rabbit while arterial preparations were unaffected (Miller & Vanhoutte, 1985; Pratt *et al*., 1996; Rouaud *et al*., 1999). A similar result was obtained with the human pulmonary vascular preparations (present report) either at the basal or elevated tone. The venous contractions were abolished in the presence of the EP_1-receptor antagonists, INDO or the absence of endothelium. One explanation for these results is the endothelial metabolism of AA to PGI_2 and the subsequent activation of EP_1-receptor. This interpretation corroborates with the results obtained with exogenous PGI_2 or ACh in the human pulmonary veins.

The endogenous PGI_2 production and the receptors activated by this prostanoid may also explain the variable effects of the COX inhibitor (INDO) in the human pulmonary vessel during ACh vasorelaxation. INDO significantly shifted to the right the ACh-induced relaxation curves obtained in human pulmonary arteries by a factor of three-fold (present report). In contrast, this treatment significantly increased the maximal relaxations induced by ACh in human pulmonary veins. INDO treatment of ACh-stimulated arteries inhibited the release of endogenous PGI_2 thereby preventing the activation of the IP-receptors and consequently reducing vasorelaxation. In the human pulmonary veins, inhibition of the endogenous PGI_2 production by INDO affects the stimulation of IP- and EP_1-receptors. Therefore, both relaxation and contraction that are produced by PGI_2 in the veins after ACh stimulation in the presence of INDO are inhibited. Thus, the only resulting effect of INDO in the veins is a slight increase in the maximal relaxation produced by ACh.

In human pulmonary arteries (present report), the combination of both inhibitors (L-NOARG and INDO) completely abolished the ACh-induced relaxation. This inhibitory effect was markedly different than from the results observed after treatment with each inhibitor separately. Similar results were described in human internal mammary arteries in response to ACh challenge (Yang *et al*., 1991). Such data suggest that NO may regulate the COX pathway in human pulmonary arteries as has been reported for other vascular endothelium (Salvemini *et al*., 1996; Davidge, 2001). The observation that NO or $OONO^-$ may inhibit prostacyclin synthase (PGIS) and the release of PGI_2 has also been shown in human saphenous veins (Barker *et al*., 1996), HUVEC (Camacho *et al*., 1998), bovine endothelial cells (Doni *et al*., 1988) and aortic preparations (Zou & Ullrich, 1996). Since NO production appears more pronounced in human pulmonary veins when compared with the arteries, this kind of PGIS-control may explain in part the reduced production of PGI_2 by the veins. However, the combination of treatment (INDO + L-NOARG) completely abolished the ACh-induced relaxations in human pulmonary arteries but not in the veins. These results suggested the involvement of an endothelial mediator independent of the COX and NOS pathways in the veins. In addition, the results obtained with apamin and charybdotoxin in the veins provide evidence for the lack of involvement of EDHF in these relaxations.

The present data demonstrated that COX-2 protein was not expressed in human pulmonary arteries and veins suggesting that the PGI_2 released was primarily due to COX-1 enzymatic activity. These data are in agreement with the results obtained by Ait Said *et al*. (2002) in human pulmonary microvascular endothelial cells where COX-2 expression was observed only in the presence of proinflammatory stimuli.

The present report suggest that the ACh-induced relaxations in human pulmonary vessels are principally mediated by the endothelial release of NO and PGI_2. The reduced relaxations produced by ACh in the human pulmonary veins in comparison with arteries may be explained by the lower production and release of PGI_2 in the veins. In addition, the relaxant response induced by the released PGI_2 in the veins is masked by an activation of the EP_1-receptor associated with the contraction.

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