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Differential role of vasoactive prostanoids in porcine and human isolated pulmonary arteries in response to endothelium-dependent relaxants

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1 The pig is increasingly being used in medical research, both as a model of the human cardiovascular system, and as a possible source of organs for xenotransplantation. However, little is known about the comparative functions of the vascular endothelium between porcine and human arteries. We have therefore compared the effects of two endothelium-dependent vasorelaxants, acetylcholine (ACh) and the Ca^{2+}-ATPase inhibitor, cyclopiazonic acid (CPA) on the porcine and human isolated pulmonary artery using isometric tension recording.

2 ACh and CPA produced endothelium-dependent relaxations of both the human and porcine pulmonary arteries.

3 In the porcine pulmonary artery, the cyclo-oxygenase inhibitor, flurbiprofen had no effect on relaxations to ACh (E_{max}: control $67.8 \pm 8.8\%$ versus $72.4 \pm 9.5\%$ ($n=11$)) or CPA (E_{max}: control $79.6 \pm 5.0\%$ versus $94.0 \pm 10.6\%$ ($n=7$)). The nitric oxide synthase inhibitor, L-NAME converted relaxations to both ACh and CPA into contractile responses (maximum response: ACh $30.0 \pm 11.1\%$ ($n=10$); CPA $80.4 \pm 26.2\%$ ($n=8$) of U46619-induced tone). These contractile responses in the presence of L-NAME were abolished by flurbiprofen.

4 In the human pulmonary artery, L-NAME and flurbiprofen partly attenuated relaxations to ACh (E_{max}: control: $45.1 \pm 12.1\%$; flurbiprofen: $33.4 \pm 13.5\%$; L-NAME: $10.1 \pm 7.2\%$) and CPA (E_{max}: control: $78.1 \pm 5.5\%$; flurbiprofen: $69.6 \pm 7.2\%$; L-NAME $37.9 \pm 10.7\%$ of U46619-induced tone). These responses were abolished by the combination of both inhibitors.

5 We have demonstrated that while the release of nitric oxide is important in responses to endothelium-dependent vasorelaxants in both human and porcine pulmonary arteries, in the human arteries, there is an important role for vasorelaxant prostanoids whilst in the porcine arteries, vasoconstrictor prostanoids are released.

Keywords: Acetylcholine; cyclopiazonic acid; pulmonary artery; endothelium; nitric oxide; prostanoids; man; pig

Introduction

In the last decade, considerable research has focused on the pig as a species for cardiovascular studies (Attinger & Cahill, 1960; Rendas *et al*., 1978; Lee, 1986; White & Bloor, 1986) and as a potential donor of organs for transplantation into man-xenotransplantation (Bach, 1992; Lawson & Platt, 1996). Central to both areas of research has been a need for a greater understanding of the comparative properties of the vascular endothelium between the pig and man.

The vascular endothelium produces several vasoactive substances in response to a wide range of hormones and physical deformation, e.g. shear stress. Among these mediators are the short-acting dilator substances, nitric oxide, prostacyclin and endothelium-derived hyperpolarizing factor (EDHF), (Moncada *et al*., 1991; Mombouli & Vanhoutte, 1997), and the vasoconstrictor hormones endothelin, prostaglandin H_2 and thromboxane A_2 (Lüscher *et al*., 1992; Rubanyi & Polokoff, 1994). Based on studies of isolated blood vessels and vascular beds of the rat, guinea-pig and rabbit, it is clear that the relative importance of these endothelium-derived vasoactive factors, and the receptors linked to their release, varies considerably both within and between vascular beds (Lüscher *et al*., 1992; Gardiner & Bennett, 1993). Much less is known about the extent of heterogeneity in the release of endothelium-derived vasoactive factors in man, but studies on the human isolated coronary artery (Stork & Cocks, 1994) and mesenteric artery (Martinez *et al*., 1994) and on blood flow in the human forearm (Haynes & Webb, 1994) have provided evidence for varying contribution by nitric oxide, EDHF, vasodilator prostanoids and endothelin. With respect to the heart, a possible donor organ for xenotransplantation, large coronary arteries from man (Stork & Cocks, 1994) and the pig (von der Weid & Bény, 1992; Kilpatrick & Cocks, 1994) share similar pharmacological properties. Firstly, bradykinin, substance P and A23187, but not acetylcholine elicit endothelium-dependent relaxations. Secondly, both nitric oxide and EDHF have been shown to contribute to these responses.

Presently, it is not clear whether the vasculature of other organs from the pig exhibit similar properties to those of man. In the case of the porcine pulmonary arteries, however, acetylcholine produces endothelium-dependent relaxations with nitric oxide as the major contributor with negligible contribution from other vasodilator substances (Kovitz *et al*., 1993). In the present study, we have therefore undertaken a comparative pharmacological study on isolated segments of the human and porcine pulmonary artery. This has been done by examining the effects of removal of the endothelium and the addition of cyclooxygenase and nitric oxide synthase inhibitors

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Table 1 Comparison of responses to 60 mM KCl and to the thromboxane-mimetic, U46619 in endothelium intact (E(+)) and endothelium-denuded (E(−)) segments of the porcine and human isolated pulmonary arteries

	Porcine pulmonary artery		*Human pulmonary artery*	
	E(+)	*E(−)*	*E(+)*	*E(−)*
60 mM KCl (g. wt)	3.86±0.50 (n=11)	3.66±0.54 (n=11)	1.63±0.42 (n=6)	0.89±0.23 (n=6)
U46619-induced tone (% of KCl response)	53.9±4.0 (n=15)	54.2±7.8 (n=15)	108.6±12.9 (n=6)	122.1±15.7 (n=6)

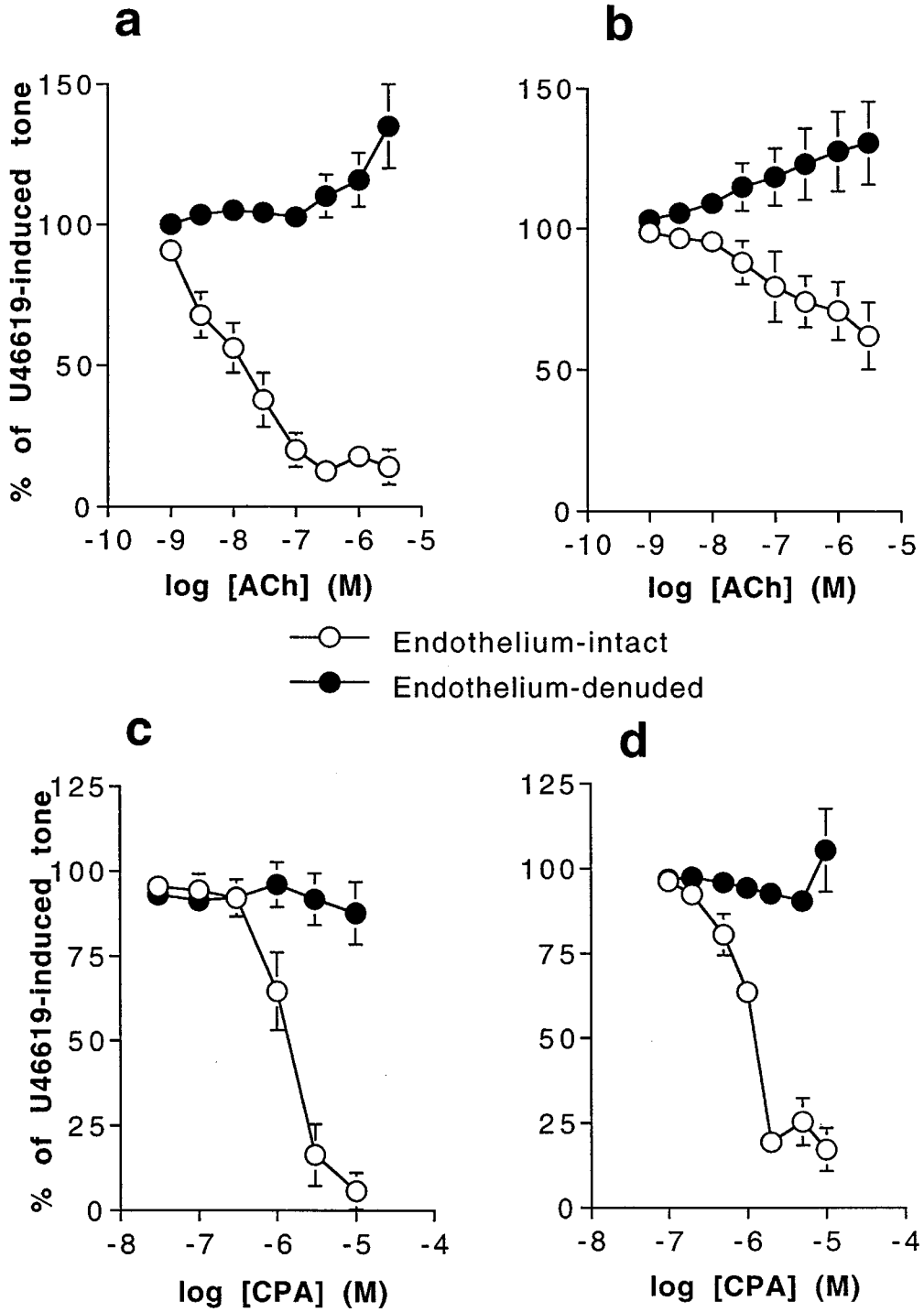

Figure 1 Comparison of the cumulative concentration-response curves for acetylcholine (ACh) and cyclopiazonic acid (CPA) in endothelium-intact and endothelium-denuded segments of the porcine isolated pulmonary artery (a and c, respectively) and the human isolated pulmonary artery (b and d, respectively), preconstricted with the thromboxane-mimetic, U46619. Responses have been expressed as a percentage of U46619-induced tone and are shown as the means±s.e.mean of 6–8 observations.

on responses to the receptor agonist, acetylcholine and the non-receptor activator, cyclopiazonic acid (Lüscher *et al*., 1992; Higuchi *et al*., 1996) in the two vascular preparations.

Methods

Lungs removed from pigs recently killed in the abattoir or segments of lungs from humans after lobectomies or pneumonectomies from lung carcinoma patients (within 1 h) were transported back to the laboratory in ice-cold, modified Krebs-Henseleit (K-H) solution. Pulmonary arteries were located running next to bronchial airways, and carefully followed, cutting away any branches. The arteries were placed in 20 ml of K-H solution containing 2% ficoll, which had previously been gassed for 5–10 min with 95% O_2 : 5% CO_2, and stored overnight at 4°C; ficoll was used to prevent osmotic swelling of the cells (Lot *et al*., 1993).

The following day, vessels (approximately 2–4 mm in diameter) were cleaned of excess connective tissue and cut into 5–6 mm wide segments. The endothelium of some segments was removed by gently rubbing the lumen of the vessel with a roughened metal rod. Two stainless steel wires (0.2 mm thick) were then placed in the artery, one being linked to a glass support while the other was connected by cotton to a Grass force-displacement transducer (Model FT03) connected to a Grass Polygraph. The segments were then placed in an isolated organ bath containing 5 ml K-H solution maintained at 37°C and gassed with 95% O_2 : 5% CO_2.

Experimental protocol

An initial resting tension of 2 and 4 g wt. was applied to each segment of human and porcine pulmonary arteries respectively, 30 min after equilibration. The final resting tension was approximately 1 and 2 g wt. in the human and porcine pulmonary arteries, respectively, after a further 30 min. The preparations were then stimulated with 60 mM KCl until reproducible responses were obtained. Endothelium-denuded and endothelium-intact segments of the human and porcine pulmonary arteries were then preconstricted with the thromboxane mimetic, U46619 (1–50 nM), to produce a degree of tone equivalent to approximately 80% of the response to 60 mM KCl. Once a stable response was achieved, preparations were exposed to increasing concentrations of either acetylcholine or cyclopiazonic acid (CPA).

In a separate set of experiments, the effects of acetylcholine and CPA against U46619-induced tone were examined in endothelium-intact segments of arteries from both species, but in the presence of either the cyclo-oxygenase inhibitor, flurbiprofen (1 μM), the nitric oxide synthase inhibitor, L-NAME (N^G-nitro-L-arginine methyl ester, 100 μM) or a combination of both agents. Control experiments with acetylcholine and CPA in the absence of flurbiprofen and L-NAME were also conducted. In each instance, the final vasoconstrictor tone was approximately 80% of that produced by 60 mM KCl, but since both enzyme inhibitors influenced vascular tone, the order in which they were added was different. L-NAME was added prior to the induction of vascular tone with U46619, while flurbiprofen was added only after the thromboxane mimetic. The effect of acetylcholine and CPA were examined only after the inhibitor had been allowed to equilibrate for 40 min and vasoconstrictor tone was stable. Finally, the effect of acetylcholine on U46619-induced tone, in the presence of 100 μM L-NAME was also examined in endothelium-intact and endothelium-denuded segments of the porcine isolated pulmonary artery.

Data analysis

Unless otherwise stated, the effects of the putative vasorelaxants have been expressed in terms of the percentage of U46619-induced tone prior to the addition of the drug and are shown as the means ± s.e.mean. In some experiments, the negative logarithm of the concentration of drug required to produce 50% of the maximum response (pD_2) were determined by interpolation while the maximum response (E_{max}) was expressed as a percentage of the U46619-induced tone. Differences between mean values have been examined using unpaired Students *t*-tests, while a two-way ANOVA was used for a comparison of concentration-response curves. If $P < 0.05$, then the difference between the means was considered to be statistically significant.

Drugs and solutions

The following chemicals were used: potassium chloride (purchased from AnalaR), U46619 (9,11-dideoxy-9α,11α-methanoepoxy Prostaglandin $F_{2\alpha}$ methyl acetate), flurbiprofen, N^G-nitro-L-arginine methyl ester (L-NAME), acetylcholine chloride and cyclopiazonic acid (all purchased from Sigma Chemical Co, Dorset, U.K.). Stock solutions of U46619 (10 mM) and flurbiprofen (1 mM) were prepared in absolute ethanol while cyclopiazonic acid (10 mM) was dissolved in dimethylsulphoxide. Further dilutions of all drugs were made in distilled water. The composition of the K-H solution in mM

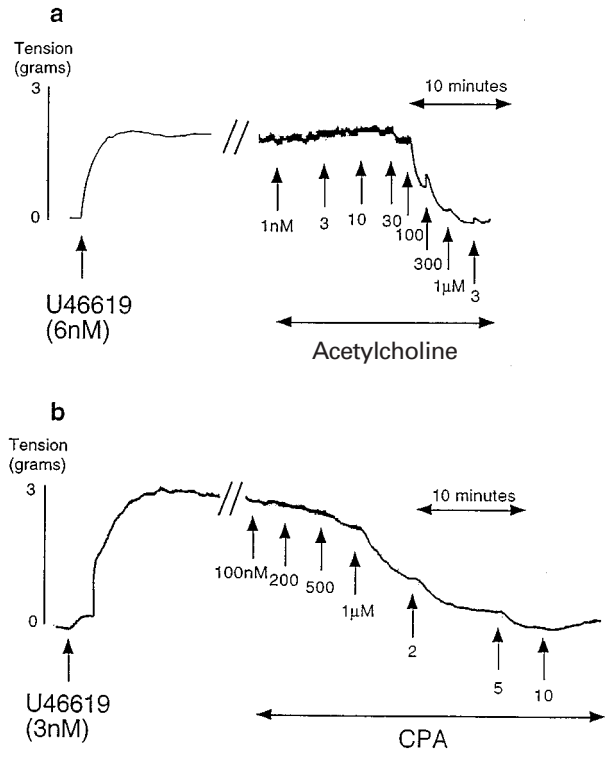

Figure 2 Representative traces of the application of increasing concentrations of (a) acetylcholine (ACh) and (b) cyclopiazonic acid (CPA) in endothelium-intact segments of the porcine isolated pulmonary artery. Comparison of the time-course of responses to each of these agents clearly show a much faster response to the addition of acetylcholine than to CPA. Responses to acetylcholine and CPA in the human isolated pulmonary arteries had similar time-courses to those represented here.

was: NaCl 119; KCl 4.7; $MgSO_4$ $7H_2O$ 1.17; $NaHCO_3$ 24; $CaCl_2$ 1.25; KH_2PO_4 1.17; Glucose 5.5.

Results

Comparison of endothelium-intact and -denuded segments

There was no statistically significant difference between contractions produced by KCl (60 mM) or in the level of tone induced by U46619 (1–30 nM) between endothelium-intact and endothelium-denuded segments of the porcine isolated pulmonary artery. Similar results were obtained for the human isolated pulmonary artery (Table 1).

Acetylcholine (1 nM–3 μM) produced concentration-dependent relaxations of U46619-induced contractions in endothelium-intact segments of both the porcine pulmonary artery (E_{max}: 86.1±6.2% of U46619-induced tone, $n=8$) and of the human pulmonary artery (E_{max}: 45.3±12.1%, $n=8$), although the maximum response to acetylcholine in the human pulmonary artery was significantly lower than that in the porcine pulmonary artery (Figure 1a and b). In the absence of the endothelium, acetylcholine (0.3–3 μM) elicited contractions in both preparations (PPA: 34.8±15.0% contraction of U46619-induced tone, $n=8$; HPA: 32.2±14.4% contraction, $n=6$; Figure 1a and b). Thus, acetylcholine is an endothelium-dependent relaxant.

In endothelium-intact segments, cyclopiazonic acid (CPA), (0.3–10 μM) elicited concentration-dependent relaxations of U46619-induced tone in both the porcine pulmonary artery (E_{max}: 94.3±5.4%, $n=8$) and in the human pulmonary artery (E_{max}: 87.8±5.3%, $n=6$; Figure 1c and d). The maximum responses to CPA were comparable between the two species,

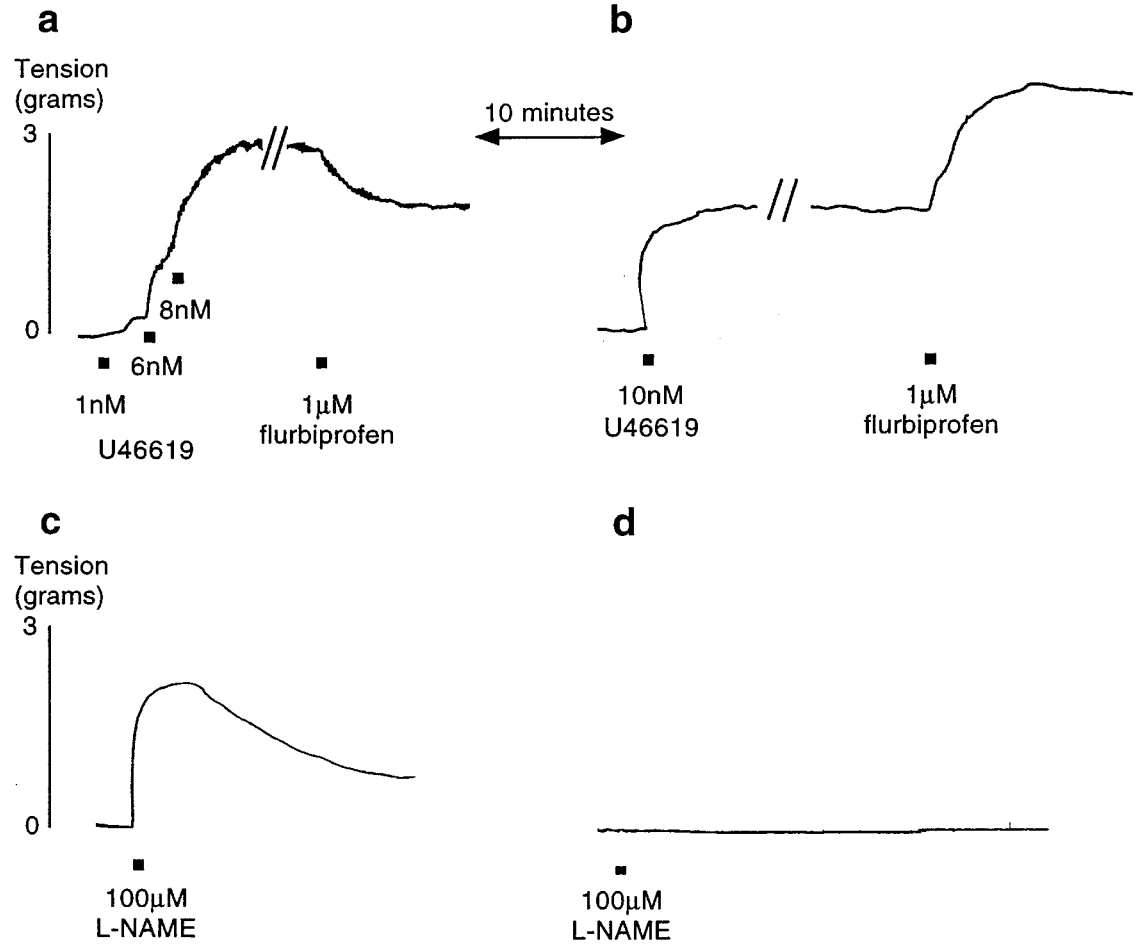

Figure 3 Representative traces of the effects of 1 μM flurbiprofen on U46619-induced tone and 100 μM L-NAME on baseline tone in endothelium-intact segments of the porcine isolated pulmonary artery (a and c, respectively) and the human isolated pulmonary artery (b and d, respectively).

Table 2 Comparison of the responses to the cyclo-oxygenase inhibitor, flurbiprofen (1 μM) and the nitric oxide synthase inhibitor, L-NAME (100 μM) in endothelium-intact segments of the porcine and human isolated pulmonary arteries

	Porcine pulmonary artery	*Human pulmonary artery*
1 μM flurbiprofen (% of U46619-induced tone)	52.5±4.1 relaxation ($n=37$)	31.4±10.1 contraction ($n=15$)
100 μM L-NAME (% of response to 60 mM KCl)	99.8±19.1 contraction ($n=15$) (waned to 46.8±9.3)	no response ($n=15$)

All responses have been expressed as the means±s.e.mean of n observations.

whereas the time-course of the responses to CPA was markedly slower than those to acetylcholine in both species (Figure 2). CPA did not affect U46619-induced tone in endothelium-denuded segments of either species. Thus, CPA is an endothelium-dependent relaxant.

Effect of inhibition of cyclo-oxygenase and nitric oxide synthase

Flurbiprofen (1 μM) inhibited U46619-induced tone of the porcine pulmonary artery, but enhanced U46619-induced tone of the human pulmonary artery (Figure 3 and Table 2). In the case of the porcine pulmonary artery, sufficient U46619 was then added to re-establish tone equivalent to approximately 80% of the response to 60 mM KCl).

As shown in Figure 4a and c, 1 μM flurbiprofen did not alter the endothelium-dependent relaxations to acetylcholine (pD_2: control 6.66 ± 0.22, flurbiprofen 6.84 ± 0.15 ($n=11$)) or to CPA (pD_2: control 5.59 ± 0.13, flurbiprofen 5.81 ± 0.11 ($n=7$)) in the porcine pulmonary artery (see Table 3). In the human pulmonary artery, 1 μM flurbiprofen also had no significant effect on responses to acetylcholine (pD_2: control 6.42 ± 0.34, flurbiprofen 6.22 ± 0.32, ($n=8$); Figure 4b and Table 3). In contrast, however, the presence of 1 μM flurbiprofen caused a statistically significant attenuation of CPA-induced relaxations of the human pulmonary artery (pD_2: control 5.84 ± 0.04, flurbiprofen 5.55 ± 0.07 ($n=6$); Figure 4d and Table 3).

The nitric oxide synthase inhibitor, L-NAME (100 μM), produced a large contraction in endothelium-intact segments of the porcine pulmonary artery (Figure 3). However, in endothelium-intact segments of the human pulmonary artery, L-NAME (100 μM) did not affect baseline tone (Table 2 and Figure 3). In each case, sufficient U46619 was again added to establish similar vasoconstrictor tone (approximately 80% of the response to 60 mM KCl) prior to the addition of the 'relaxants'.

In endothelium-intact segments of the porcine pulmonary artery, L-NAME (100 μM) abolished relaxations to both aceylcholine and CPA. Under these conditions, L-NAME uncovered concentration-dependent contractile responses to both acetylcholine and CPA (Figure 5a and c; Table 3). The contractile response to low concentrations of acetylcholine (3–300 nM), in the presence of 100 μM L-NAME, was not observed in the absence of the endothelium ($n=8$; Figure 6).

As shown in Figure 5a and c, while the presence of 100 μM L-NAME converted acetylcholine and CPA from relaxant to constrictor agents in the porcine isolated pulmonary artery, no constrictor response was observed in the presence of the combination of 100 μM L-NAME and 1 μM flurbiprofen. Indeed, this combination of enzyme inhibitors uncovered a further small relaxant response to both agents (Table 3).

It is noteworthy that the addition of 1 μM flurbiprofen to L-NAME-exposed segments caused a reduction in vasoconstrictor tone from $45.4 \pm 8.0\%$ to $9.5 \pm 3.6\%$ of the response to 60 mM KCl ($n=25$).

In endothelium-intact segments of the human pulmonary artery, 100 μM L-NAME significantly attenuated responses to both acetylcholine and CPA (Figure 5b and d; Table 3). The presence of both 1 μM flurbiprofen and 100 μM L-NAME, however, abolished the remaining relaxation responses to acetylcholine and CPA seen in the presence of L-NAME alone (Figure 5b and d; Table 3).

Discussion

The starting point for the present study was a desire to compare the properties of the arterial endothelium of human and porcine pulmonary arteries, with the aim to (i) determine the endothelium-derived factor(s) that modulate vascular tone and (ii) provide greater insight into the subcellular mechanisms responsible for their release. Two agents were chosen for this purpose: acetylcholine as a known vasorelaxant that acts at endothelial muscarinic receptors (Crawley *et al*., 1990; Liu *et al*., 1992), and CPA, which is known to block endoplasmic Ca^{2+}-dependent ATPase and promote the entry of extracellular Ca^{2+} ions (Goerger & Riley, 1989; Zhang *et al*., 1994). The influx of Ca^{2+} ions is thought to elevate endothelial Ca^{2+} ion concentration and, therefore, stimulate the release of endothelium-derived vasoactive factors (Higuichi *et al*., 1996; Kamata *et al*., 1996). Taken together, several observations from the present study highlight superficial similarities in the pulmonary vasculature of the pig and man.

First, in agreement with earlier reports on the human and porcine pulmonary artery, acetylcholine inhibited U46619-induced tone in unrubbed preparations (Greenberg *et al*., 1987; Dinh-Xuan *et al*., 1990; Liu *et al*., 1992; Kovitz *et al*., 1993). Second, removal of the endothelium converted acetylcholine into a weak vasoconstrictor, providing support for the presence of muscarinic receptors on the underlying vascular smooth muscle, as noted by McCormack *et al*. (1988) and Norel *et al*. (1996). Third, CPA produced a slow inhibition of U46619-induced tone in unrubbed segments of both preparations. Finally, removal of the endothelium abolished these relaxations to CPA. This is the first report of the ability of CPA to produce endothelium-dependent relaxations in a

Table 3 Comparison of the maximum responses to ACh and CPA in the absence and presence of the cyclo-oxygenase inhibitor, flurbiprofen (1 μM) and the nitric oxide synthase inhibitor, L-NAME (100 μM) in endothelium-intact segments of the porcine and human isolated pulmonary arteries

	Porcine pulmonary artery (E_{max})		*Human pulmonary artery (E_{max})*	
	ACh	*CPA*	*ACh*	*CPA*
Control (% of U46619-induced tone)	67.8 ± 8.8 relaxation	79.6 ± 5.0 relaxation	45.1 ± 12.1 relaxation	78.1 ± 5.5 relaxation
1 μM flurbiprofen (% of U46619-induced tone)	72.4 ± 9.5 relaxation	94.0 ± 10.6 relaxation	33.4 ± 13.5 relaxation	69.6 ± 7.2 relaxation
100 μM L-NAME (% of U46619-induced tone)	30.0 ± 11.1 contraction	80.4 ± 26.2 contraction	10.1 ± 7.2 relaxation	37.9 ± 10.7 relaxation
1 μM flurbiprofen + 100 μM L-NAME (% of U46619-induced tone)	11.6 ± 3.6 relaxation	25.9 ± 14.2 relaxation	18.7 ± 14.3 contraction	2.5 ± 9.1 contraction

All responses have been expressed as the means ± s.e.mean of 6–11 observations.

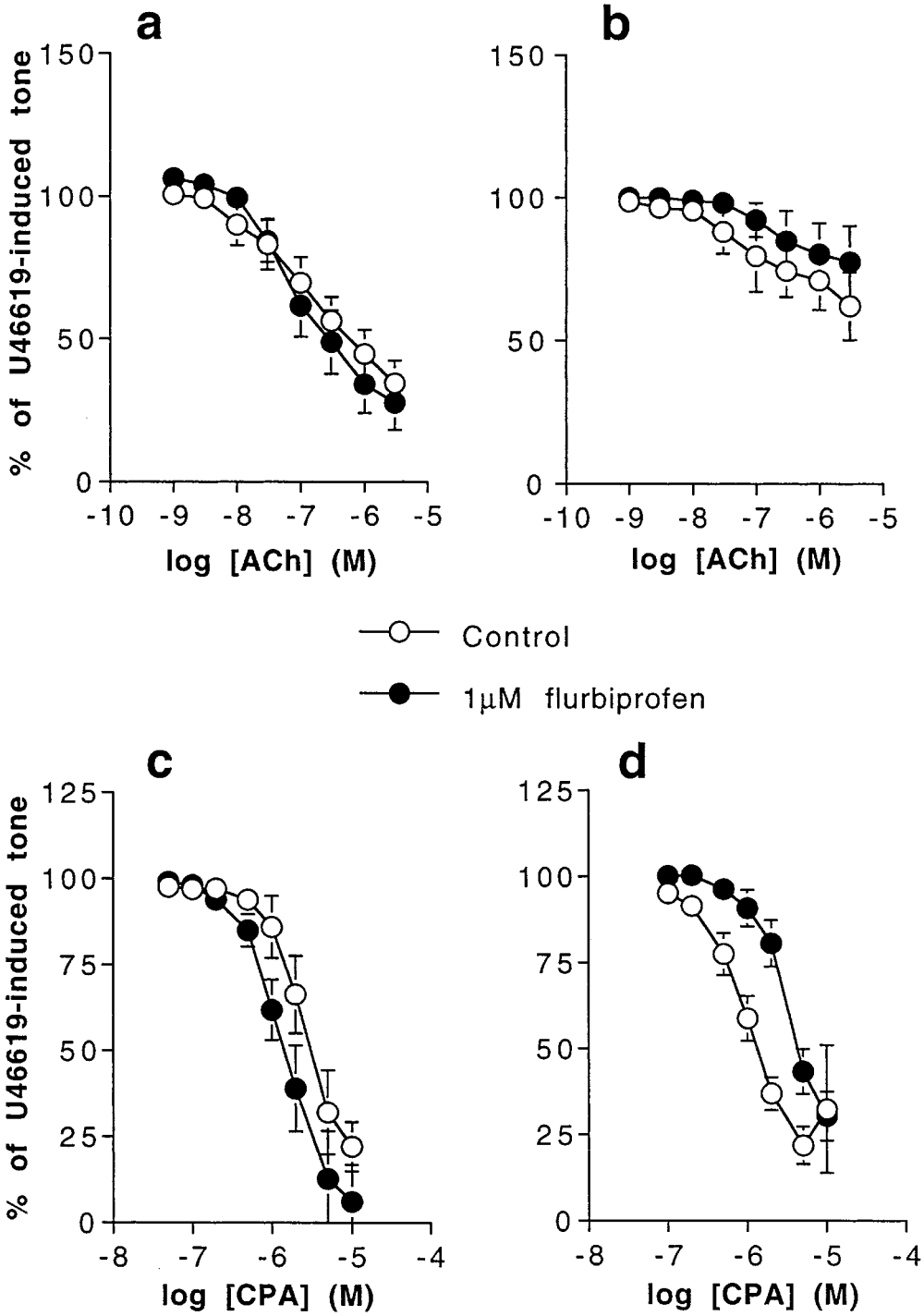

Figure 4 Effects of 1 μM flurbiprofen (a cyclo-oxygenase inhibitor) on cumulative concentration-response curves to acetylcholine (ACh) and cyclopiazonic acid in endothelium-intact segments of the porcine isolated pulmonary artery (a and c, respectively) and the human isolated pulmonary artery (b and d, respectively) preconstricted with the thromboxane-mimetic, U46619. Responses have been expressed as a percentage of U46619-induced tone and are shown as the means ± s.e.mean of 6–11 observations.

human artery and indicates that in man, as in other species, Ca^{2+} ions play a pivotal role in the release of endothelium-derived vasoactive factors.

The role of nitric oxide and prostanoids in responses to acetylcholine and CPA

Our systematic examination of the effect of L-NAME, an inhibitor of nitric oxide synthase (Moncada *et al*., 1991), and flurbiprofen, an inhibitor of cyclo-oxygenase (Crook *et al*., 1976; Nozu, 1978) has provided evidence for a contribution of both nitric oxide and a prostanoid in acetylcholine- and CPA-induced responses in the two preparations.

In the human pulmonary artery, L-NAME merely attenuated endothelium-dependent relaxations, thereby providing evidence for the contribution of at least two dilator substances. Since flurbiprofen alone also reduced responses to CPA in this preparation, both nitric oxide and a prostanoid are implicated. Qualitatively similar effects of flurbiprofen were obtained against responses to acetylcholine, although this did

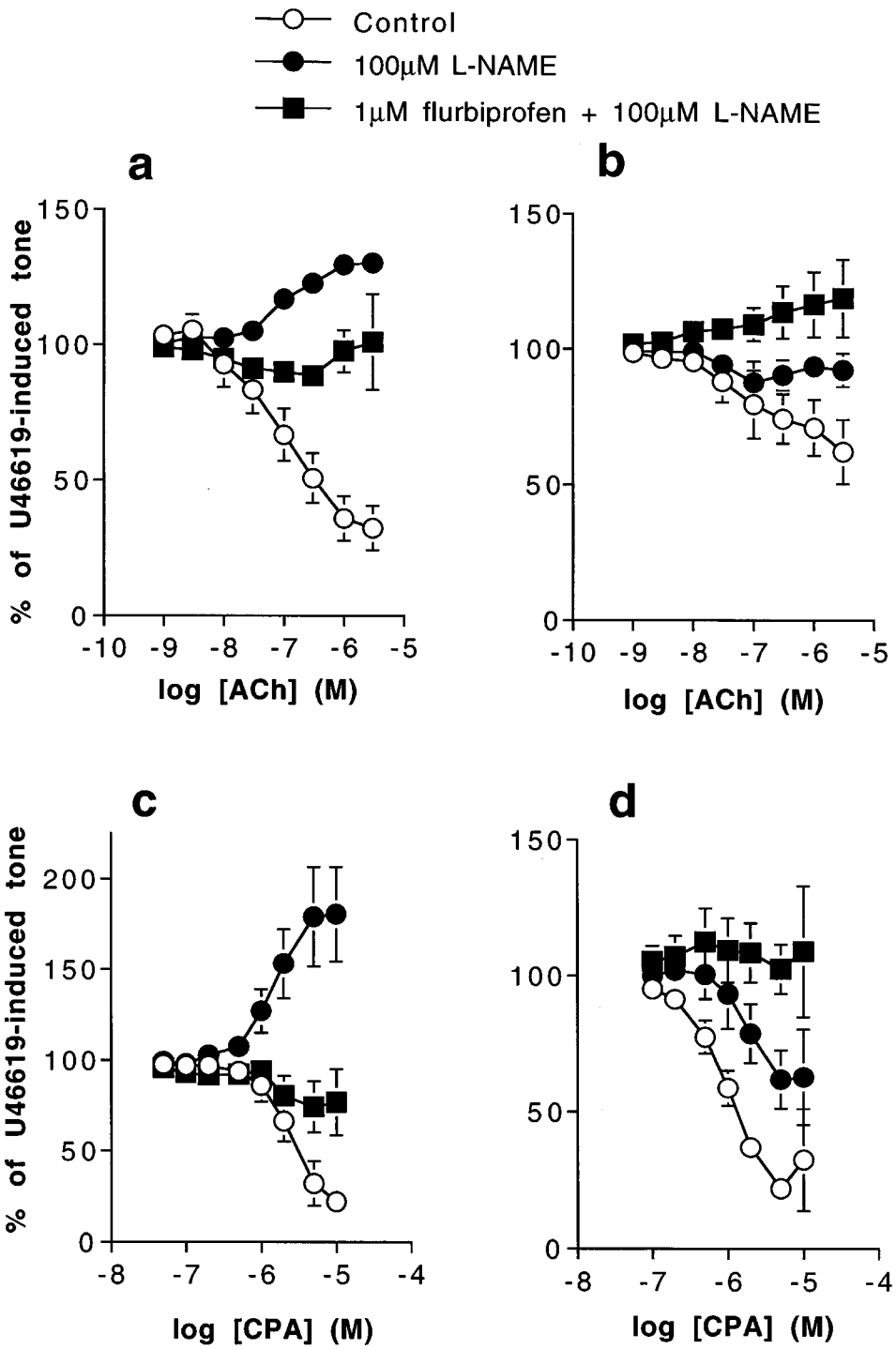

Figure 5 The effect of 100 μM L-NAME alone, and the combination of 1 μM flurbiprofen and 100 μM L-NAME on responses to increasing concentrations of acetylcholine (ACh) and cyclopiazonic acid (CPA) in endothelium-intact segments of the porcine isolated pulmonary artery (a and c, respectively) and the human isolated pulmonary artery (b and d, respectively). Responses have been expressed as a percentage of U46619-induced tone and are shown as the means$\pm$s.e.mean of 5–10 observations.

not reach statistical significance. The involvement of both nitric oxide and prostanoids in the human pulmonary artery is further supported by the fact that the combination of L-NAME and flurbiprofen had a greater effect on the response to CPA than either inhibitor alone, which is similar to that reported by Norel *et al.* (1996) in this preparation. In contrast to this report, two earlier studies concluded that vasorelaxant prostanoids were not implicated in endothelium-dependent relaxations of the human pulmonary artery (Greenberg *et al.*, 1987; Dinh-Xuan *et al.*, 1990), since indomethacin alone had no effect on responses to acetylcholine. However, we found that the involvement of prostanoids in the responses to acetylcholine and CPA was more evident after the inhibition of NO synthase, highlighting the value of our systematic approach.

In contrast to the human pulmonary artery, in the porcine pulmonary artery, nitric oxide appears to be the sole endothelium-derived vasorelaxant released by acetylcholine and CPA. While L-NAME abolished relaxations to both agents in endothelium-intact preparations, and uncovered an

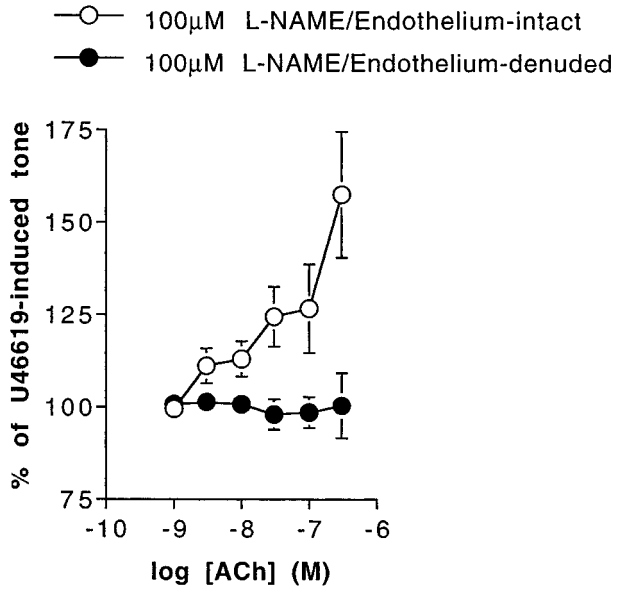

Figure 6 The effect of acetylcholine (ACh) on U46619-induced tone in the presence of 100 μM LNAME in endothelium-intact and endothelium-denuded segments of the porcine isolated pulmonary artery. Responses have been expressed as a percentage of U46619-induced tone and are shown as the means$\pm$s.e.mean of 8 observations.

endothelium-dependent contractile response, flurbiprofen failed to attenuate the relaxation responses to either agent. Thus, there is no evidence for the involvement of vasodilator prostanoids. However, in the presence of L-NAME, flurbiprofen abolished the contractile responses to acetylcholine and CPA and converted the response to CPA into a weak relaxation response. Taken together, there is strong evidence for the release of vasoconstrictor prostanoids from the porcine pulmonary endotheluim (at least in the presence of L-NAME), by both receptor and non-receptor stimulants. Since qualitatively similar observations have been made with both bradykinin and A23187 in this preparation (unpublished observations) this appears to be a general property of the porcine pulmonary endothelium. While there are reports of endothelium-dependent contractions to agonists in other isolated blood vessels (Shirahase *et al*., 1987; Katusic *et al*., 1988; Buzzard *et al*., 1993; Altiere *et al*., 1994), and observations consistent with this in man (Vallance *et al*., 1989; Collier & Vallance, 1990), this is the first report of such responses in the porcine isolated pulmonary artery. In an earlier study, Kovitz *et al*. (1993) failed to detect similar responses to acetylcholine in this preparation, but this may have been due to the routine inclusion of a cyclo-oxygenase inhibitor in the bathing medium. Again, the importance of our systematic approach is further highlighted by observations in the porcine coronary artery, where it has been demonstrated that the inclusion of the cyclo-oxygenase inhibitor, indomethacin, produced a statistically significant enhancement of the response to the α_2-adrenoceptor agonist, UK14304, implicating the release of vasoconstrictor prostanoids (Barber & Miller, 1997).

Hitherto, interest in prostanoid production by either cultured endothelial cells (Hong & Deykin, 1982; White & Martin, 1989; Richards *et al*., 1991) or isolated blood vessels (Zellers *et al*., 1994; Razzuk & Zellers, 1995) from the pig has focused almost exclusively on prostacyclin. It is clear from the functional data presented above, however, that the production of vasoconstrictor prostanoids by the porcine vascular endothelium is also worthy of attention.

The ability of the combination of flurbiprofen and L-NAME to practically abolish endothelium-dependent relaxations in the pulmonary vessels of both species contrast sharply with that found in other blood vessels, and serves to underline the heterogeneous nature of the vascular endothelium. For example, in coronary vessels of both the pig and man there is a major contribution to endothelium-dependent relaxations by a non-prostanoid, non-nitric oxide, hyperpolarizing factor (von der Weid & Bény, 1992; Kilpatrick & Cocks, 1994; Stork & Cocks, 1994).

The role of nitric oxide and prostanoids at rest

Examination of the effect of flurbiprofen and L-NAME on pulmonary vascular tone highlighted further differences between the two preparations. In the porcine pulmonary artery, flurbiprofen inhibited both U46619- and L-NAME-induced contractions. The latter observation is similar to those reported in both the splenic (Lot *et al*., 1993) and coronary (Nakaike *et al*., 1995) arteries of the pig, and indicates the involvement of a vasoconstrictor prostanoid. In the human pulmonary artery, however, flurbiprofen increased U46619-induced tone and produced a contraction in the presence of L-NAME (data not shown). Both observations suggest a major role for vasorelaxant prostanoids and agree with earlier reports indicating that another cyclo-oxygenase inhibitor, indomethacin, caused contractions in the human pulmonary artery (Hadhazy *et al*., 1983; Schellenberg *et al*., 1987; Ortiz *et al*., 1992). Thus, the action of flurbiprofen alone indicates that the function of vascular prostanoids differs between pig and man.

In the case of L-NAME alone, endothelium-dependent contractions were noted in the porcine pulmonary artery, but not in the corresponding human vessel. The former finding raises the possibility that basal nitric oxide exerts an inhibitory, autocrine effect on other endothelium-derived factors, i.e. L-NAME causes disinhibition of endothelial prostanoid release. This is certainly consistent with the findings of Barker *et al*. (1996), who noted the potential for nitric oxide to exert an inhibitory effect on vascular prostanoid production in the human saphenous vein. While quantitatively similar observations have been observed in both the porcine splenic and coronary arteries (see above) and the human isolated mammary artery (Yang *et al*., 1991), it is noteworthy that only small, but variable, contractions have been reported for nitric oxide synthase inhibitors in other studies on the human pulmonary artery (Crawley *et al*., 1990; Ortiz *et al*., 1992). This implies that the autocrine and/or paracrine effects of basal nitric oxide in the pulmonary vascular bed is of greater functional significance in the pig compared to man. In support of this proposition, McCormack (1990) reported that cultured endothelial cells from the human pulmonary artery failed to release EDRF under basal conditions.

Taken together, the above observations indicate that the pulmonary endothelium in man and the pig have the capacity to produce and release nitric oxide and prostanoids. Since this occurs in response to CPA, as well as acetylcholine, both vasoactive factors appear to be regulated by intracellular Ca^{2+} ions. In one crucial respect, however, the endothelial cells differ, inasmuch as the prostanoid from the human pulmonary artery is a relaxant, while that from the pig is a vasoconstrictor. Further experiments are clearly warranted on vessels from these two species, to establish whether the repertoire of vasoactive factors released in response to

pathological stimuli, e.g. inflammatory or bacterial products, are also different. Finally, these findings have important implications, not only for the future use of the porcine vasculature as a suitable model for cardiovascular studies, but also for the prospect of using the transgenic pig for the purpose of xenotransplantation.

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