British Journal of Pharmacology (1997) 121, 441–450 © 1997 Stockton Press All rights reserved 0007–1188/97 $12.00

Evidence for specific regional patterns of responses to different vasoconstrictors and vasodilators in sheep isolated pulmonary arteries and veins

*B.K. Kemp, †J.J. Smolich & *,[1]T.M. Cocks

*Department of Pharmacology, The University of Melbourne, Parkville, Victoria 3052 and †Institute of Reproduction and Development, Monash Medical Centre, Melbourne, Victoria, Australia

1 Responses of large (5–7 mm in diameter) and medium sized (3–4 mm in diameter) branches of sheep isolated intrapulmonary arteries and veins and three groups of small pulmonary arteries (200, 500 and 1000 μm diameter) to the vasoconstrictors endothelin-1, 5-hydroxytryptamine (5-HT), noradrenaline and the thromboxane A_2 mimetic, U46619, were examined. Also, relaxation responses to the endothelium-dependent vasodilators, acetylcholine (ACh), bradykinin and ionomycin and the endothelium-independent vasodilator, sodium nitroprusside (SNP), were studied to determine their predominant site of action within the pulmonary vasculature.

2 Endothelin-1 was the most potent vasoconstrictor tested in all vessels. The maximum response to endothelin-1, expressed as a percentage of the maximum contraction to KCl depolarization, did not differ significantly between the different vessels. By contrast, pulmonary arteries greater than 200 μm in diameter failed to contract to U46619, whereas U46619 was a potent constrictor of large and medium-sized veins.

3 5-HT caused similar contractions in all arteries >200 μm in diameter, but the maximum response was significantly diminished in smaller arteries. By contrast, the maximum response to noradrenaline was progressively attenuated with decreasing arterial diameter. Both 5-HT and noradrenaline caused poor contractions in veins. Pulmonary veins were less sensitive to 5-HT than arteries and at low concentrations 5-HT caused relaxation. No change in sensitivity to noradrenaline was noted between the arteries and veins.

4 Relaxation responses to bradykinin and ionomycin decreased progressively along the pulmonary vascular tree and were nearly absent in large veins. Also, ACh was a poor relaxing agent of large and medium-sized arteries and failed to mediate any relaxation response in other vessel segments. Surprisingly the smallest arteries examined ($\sim$200 μm in diameter) failed to relax to ionomycin, bradykinin and SNP. However, both the sensitivity and maximum relaxation to SNP were similar in all other arterial and venous segments.

5 In conclusion, marked regional differences in reactivity to both vasoconstrictors and vasodilators occur in arterial and venous segments of the sheep isolated pulmonary vasculature. Such specialization may have important implications for the regulation of resistance in this low tone vascular bed.

Keywords: Pulmonary circulation; pulmonary vascular reactivity; pulmonary vasoconstriction; pulmonary vasodilatation; pulmonary artery; pulmonary vein

Introduction

Pulmonary vascular tone is influenced by both circulating and locally released vasodilators and vasoconstrictors (for reviews see Barer, 1976; Bergofsky, 1980; Said, 1982). However, studies in isolated perfused lungs have suggested that many vasoconstrictor and vasodilator agents have non-uniform effects in the various compartments of the pulmonary vascular tree. For example, in the dog isolated perfused lung, 5-hydroxytryptamine (5-HT) elevates pulmonary vascular resistance via an increase in arterial resistance (Linehan & Dawson, 1983), which occurs mainly in large arteries (Bradley *et al*., 1993). By contrast both noradrenaline (Linehan & Dawson, 1983) and the thromboxane A_2 mimetic, U46619 (Barman *et al*., 1989) increase pulmonary vascular resistance via a predominant increase in venous resistance. Similarly, the endothelium-dependent vasodilator, bradykinin, decreases arterial resistance more than venous resistance (Levine *et al*., 1973), whereas another endothelium-dependent vasodilator, acetylcholine (ACh), decreases large artery resistance, has no effect on small artery resistance and increases both small and large vein resistances in the dog isolated perfused lung (Barman *et al*., 1989).

One possible explanation for the differing responses to vasoconstrictors and vasodilators evident in arterial and venous compartments of isolated perfused lungs is regional variation in vascular reactivity. Indeed, differences in reactivity to vasodilator and vasoconstrictor stimuli have been observed between large isolated pulmonary arteries and veins (Joiner *et al*., 1975; Gruetter & Lemke, 1986; Toga *et al*., 1992) and large and small isolated pulmonary arteries (Leach *et al*., 1992). However, given that no *in vitro* study to date has examined responses to vasoconstrictors and vasodilators in vessel segments obtained from along the length of the pulmonary vascular tree, it is not clear if these differences simply reflect a general heterogeneity in pulmonary vascular reactivity, or are related to a distinct pattern of responses to vasoconstrictor and vasodilator agents in the pulmonary vasculature.

Therefore, the aim of the present study was to determine the sites of action of selected vasoconstrictors and vasodilators within the sheep pulmonary vasculature by comparing their reactivity in isolated large, medium-sized and small pulmonary arteries and large and medium-sized pulmonary veins. In addition, given that *in vitro* studies allow further division of the pulmonary vasculature than can be achieved with isolated perfused lungs, we also determined if responses to vasoactive agents different within a given segment of the pulmonary

[1] Author for correspondence.

vasculature. This was most easily addressed in the arterial portion of the pulmonary circulation where we examined the response to vasodilators and vasoconstrictors in five different sized pulmonary arteries.

Our results indicate that in the sheep isolated pulmonary vasculature, responses to both vasoconstrictor and vasodilator stimuli differ markedly between arterial and venous segments and also within the arterial vasculature.

Methods

Tissue source

Lungs from adult, mixed breed sheep were obtained from a local abattoir, approximately 5–10 min after the animal had been killed and exsanguinated. The lungs were placed in cold (4–6°C), oxygenated Krebs solution and transported within 15 min to the laboratory. After careful exposure to the intrapulmonary vascular network, the main (large) and a first-order (medium-sized) branch of the pulmonary artery and main (large) and a first order (medium-sized) tributary of the pulmonary vein were excised and placed in oxygenated Krebs solution. Small pulmonary arteries (100–1000 μm in diameter), situated next to bronchi, were identified and dissected from a segment of lung base under a dissecting light microscope.

Mounting of vessels in the organ bath and myograph

Large and medium-sized pulmonary arteries and veins were cut into 3 mm ring segments and the endothelium removed from a number of vessels by gently rotating the segments around the tips of fine forceps. All rings were then suspended on 350 μm diameter stainless steel wire hooks, in 25 ml jacketed glass organ baths. The upper hook was suspended from a force transducer (model FTO3C, Grass, Quincy, MA) to measure changes in isometric tension, which were amplified and recorded on dual channel flat-bed recorders (W & W Scientific instruments, Basel Switzerland). The lower hook was fixed to a support leg attached to a micrometer which was used to measure the distance between the two wires (see Normalization section). Small pulmonary arteries (<1 mm in diameter) were cut into 2 mm lengths and mounted on 40 μm wires in a small vessel Mulvany-Halpern myograph as previously described (Angus *et al*., 1988). As for the organ bath, one wire in the myograph was attached to a force transducer and the other to a micrometer. All vessel segments were maintained in physiological Krebs solution at 37°C and continuously bubbled with carbogen (95% O_2, 5% CO_2). The Krebs solution was composed of (in mM): Na^+ 144, K^+ 5.9, Ca^{2+} 2.5, Mg^{2+} 1.2, Cl^- 128.7, HCO_3^- 25, SO_4^{2-} 1.2, $H_2PO_4^-$ 1.2 and glucose 11, pH 7.4.

Normalization procedure

In order to compare the reactivity of arteries and veins with different internal diameters, the internal circumferences of the vessels were normalized to defined distending pressures (see Mulvany & Halpern, 1977; Angus *et al*., 1986) before the construction of concentration-response curves. This procedure set the arteries and veins at a passive tension equivalent to 90% of their internal circumference (0.9 L_{20} or 0.9 L_{10}) if they had been relaxed and perfused with a transmural pressure of 20 mmHg and 10 mmHg, respectively. These pressures were chosen as they approximate those measured in the pulmonary trunk of conscious sheep (Kemp *et al*., 1995) and pulmonary venous segments of the intact dog (Brody *et al*., 1968). Briefly, the vessels were stretched successively and corresponding measurements of force (F) and internal circumference (L) were made. L was determined from the formula: $L=(\pi+2)d+2f$ where d is the diameter of the wires and f is the separation of the wires. Successive F values were used to calculate wall tension (T) from the relationship $T=F/2g$ where g is vessel length. The tension (T) and circumference (L) values at the end of each stretch were then fitted to the exponential equation $T=AexpBL$ where A and B are constants for that particular vessel. The wall tension (T) (at 0.9 L_{20} and 0.9 L_{10} for arteries and veins, respectively) was then used to estimate the equivalent transmural pressure (P), assuming a thin walled sphere from the Laplace relation $T=r.P$ where radius (r) is $L/2\pi$. After the vessel had been stretched to the desired pressure the vessel was then set to a passive tension equivalent to 0.9 of the internal circumference at that pressure. The internal circumference (L) measurement, L_{20} or L_{10}, was then used to determine the internal diameter of the vessel, D_{20} or D_{10}.

Experimental protocol

Organ bath experiments Following normalization, vessel segments were left to equilibrate for 1 h and responses to either vasoconstrictors or vasodilators were then assessed.

Vasoconstrictor responses Cumulative (0.5 log unit) concentration-contraction responses to endothelin-1, 5-hydroxytryptamine (5-HT), noradrenaline and the thromboxane A_2 mimetic, U46619, were obtained in separate rings of large and medium-sized arteries and veins. Noradrenaline responses were obtained in the presence of propranolol (1 μM) to prevent β-adrenoceptor-mediated smooth muscle relaxation. When the maximum response to each constrictor was obtained, KCl (75 mM) was added to record the maximum contraction (F_{max}) of each tissue. Only one concentration-contraction curve to any one agonist was obtained in each ring of tissue.

Table 1 Sheep isolated pulmonary arteries and veins–summary of initial vessel parameters at normalization

	Number of rings	*D* (mm)	*P* (mmHg)	F_{max} (g)	F_{max}/D (gmm)
Large pulmonary artery	71	6.62±0.17	13.3±0.2	10.9±0.6 (24)	1.55±0.07 (24)
Medium-sized pulmonary artery	59	3.66±0.14	12.9±0.2	6.0±0.5** (28)	1.49±0.10 (28)
Small pulmonary arteries					
~1000 μm in diameter	14	0.92±0.04	12.1±1.2	0.94±0.11** (14)	1.04±0.14* (14)
~500 μm in diameter	14	0.44±0.03	13.9±0.9	0.52±0.09** (14)	1.14±0.19 (14)
~200 μm in diameter	13	0.17±0.02	13.7±1.3	0.20±0.05** (13)	1.13±0.18 (13)
Medium-sized pulmonary vein	57	3.24±0.15	7.0±0.1	4.8±0.4** (25)	1.33±0.12 (25)
Large pulmonary vein	58	5.39±0.19	6.9±0.2	7.6±0.7** (20)	1.22±0.12 (20)

D is internal diameter of the vessel estimated for the corresponding transmural pressure. P is the equivalent transmural pressure needed to distend the vessel at 90% of its internal circumference at its respective transmural pressure. Values in parentheses refer to the number of vessels in which F_{max} was obtained. *$P<0.05$, **$P<0.01$ compared with value in large pulmonary artery, Dunnett's modified *t* statistical test.

Vasodilator responses Endothelium-dependent and -independent relaxation responses were examined in large and medium-sized arteries and veins precontracted with a submaximal concentration of endothelin-1 (3 nM) which contracted vessels to approximately 50% F_{max}. Once the endothelin-1 contraction had reached a stable plateau, cumulative concentration-relaxation responses to the endothelium-dependent vasodilators, acetylcholine (ACh), bradykinin and the calcium ionophore, ionomycin, were obtained. Concentrations of ionomycin >0.3 μM were not used since contractions occurred at these concentrations. When the maximum relaxation to each endothelium-dependent vasodilator was reached the endothelium-independent vasodilator, sodium nitroprusside (SNP: 10 μM), was added to obtain maximum relaxation in all vessel segments. Relaxation responses to bradykinin and ionomycin were expressed as a percentage of the maximal relaxation to SNP and responses to ACh as a percentage of precontracted force. Cumulative-concentration relaxation responses to SNP were also obtained in separate arteries and veins and results expressed as a percentage reversal of the endothelin-1 contraction. Only one concentration-relaxation response curve to endothelium-dependent and -independent dilators was obtained for any one ring of tissue.

Myograph experiments Following normalization, small pulmonary arteries were left resting at 0.9 L_{20} for 30 min before being contracted with an isotonic KCl (124 mM) depolarizing physiological solution (KPSS). The composition of the KPSS solution was similar to that of the Krebs solution with the Na^+ replaced with K^+ (124 mM). Once the KPSS contraction had reached a plateau (F_{max}), the tissues were washed and force allowed to return to baseline.

Vasoconstrictor responses Consecutive, cumulative (0.5 log unit) concentration-contraction responses to endothelin-1, 5-HT, noradrenaline (in the presence of propranolol 0.3 μM) and U46619 were obtained in small pulmonary arteries. Vessels were thoroughly washed between each set of contractile responses which were randomized, except for endothelin-1, which was always added last due to its prolonged action.

Vasodilator responses Even though endothelin-1 caused similar contractions in each of the three different sized small arteries, it was difficult to washout and subsequently recontract vessels to similar levels of active force for repeated curve analysis. Therefore, small pulmonary arteries ($\sim$500 μm and $\sim$1000 μm in diameter) were precontracted to approximately 50% F_{max} with a submaximal concentration of 5-HT (0.1–1 μM) in order to assess relaxation responses. Desensitization to 5-HT occurred with repeated additions and thus progressively higher concentrations of 5-HT were required to obtain similar levels of active force. Small arteries, $\sim$200 μm in diameter, were contracted with K^+ (30–50 mM) to approximately 50% F_{max} since 5-HT

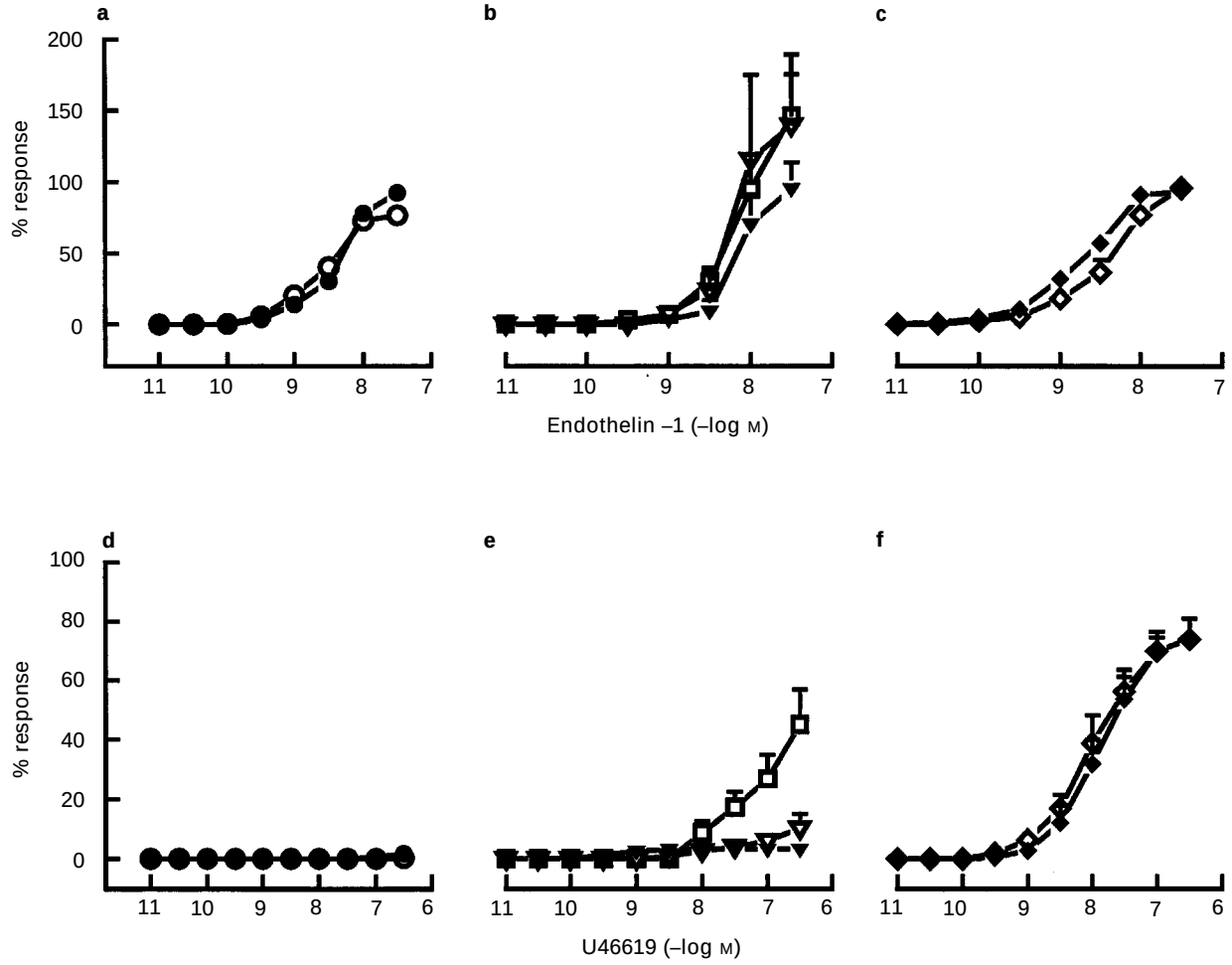

Figure 1 Concentration-contraction curves to endothelin-1 and the thromboxane A_2 mimetic, U46619, in isolated (a and d) large (○, $n=4-9$) and medium-sized (●, $n=4-5$) pulmonary arteries, (b and e) small pulmonary arteries - $\sim$1000 μm in diameter (▽, $n=3-4$), $\sim$500 μm in diameter (▼, $n=5-6$), $\sim$200 μm in diameter (□, $n=3-5$) and (c and f) large (◆, $n=5-7$) and medium-sized (◇, $n=4-5$) pulmonary veins. Responses are expressed as a percentage of the maximum contraction to K^+ (75 mM) in large and medium-sized arteries and veins and of the contraction to KPSS in small arteries. Values are mean and vertical lines show s.e.mean; n = number of rings.

only caused poor contractions in these vessels. For comparison, a subgroup of ~200 μM diameter small arteries were contracted with either endothelin-1 or U46619 to approximately 50% F_{max} and responses to endothelium-dependent and independent vasodilators examined. Cumulative (0.5 log) relaxation responses to ACh, bradykinin and ionomycin were then obtained. Only two dilators were examined per ring of artery and randomized responses to ACh or bradykinin always preceded those to ionomycin. In each case, the maximal relaxation to SNP (10 μM) was obtained following completion of the concentration-response curves. Cumulative relaxation-responses to SNP were obtained in separate rings of artery.

Drugs

Drugs used and their sources were: U46619 ([1,5,5-hydroxy-11α, 9α-(epoxymethano) prosta-5Z, 113E-dienoic acid], Upjohn, Kalamazoo, MI, U.S.A.); (−)-noradrenaline bitartrate, 5-hydroxytryptamine creatinine sulphate, acetylcholine bromide, ionomycin (Sigma, St. Louis, MO, U.S.A.); propranolol HCl (ICI, Villawood, NSW, Australia); endothelin-1 (Auspep, VIC, Australia), sodium nitroprusside dihydrate (Roche, Dee Why, NSW, Australia); bradykinin triacetate (Fluka, Glossop, U.K.). Stock solutions of ionomycin (1 mM) and U46619 (1 mM) were made up in absolute ethanol. All dilutions of stock solutions were in distilled water and all other drugs were made up in distilled water.

Statistical analysis

The individual contraction and relaxation curves were fitted to the sigmoidal logistic equation, $Y = P_1 + P_2/[1 + eP_3(\log X - P_4)]$, where X = agonist concentration, P_1 = lower plateau response, P_2 = range between the lower and the maximal plateau of the concentration-response curve, P_3 = a negative curvature index indicating the slope independently of the range and P_4 = log dose required to produce a half-maximal response (pEC_{50}). From this relationship, computer estimates of the concentrations required to give 50% of the maximum response (pEC_{50}) were determined (see Elghozi & Head, 1990). pEC_{50} values, expressed as −log M, were not determined for contraction and relaxation curves in which the maximum response was <10% F_{max} or maximum relaxation, respectively or where a plateau response was not obtained. However, note that when a plateau response to ionomycin was not obtained at 0.3 μM then the response at this concentration was assumed to be maximal for pEC_{50} determination, as a higher concentration (1 μM) caused contraction.

Comparisons of pEC_{50}, $\%F_{max}$ and maximum relaxation values between different sized arteries and veins were performed by one-way analysis of variance (ANOVA). When the F value exceeded the critical value, Dunnett's modified *t* statistic was used to perform comparisons with large pulmonary arteries. A Student's paired *t* test was used to test differences within the same sized vessel. Results are expressed as mean ± s.e.mean and statistical significance was accepted at the $P < 0.05$ level.

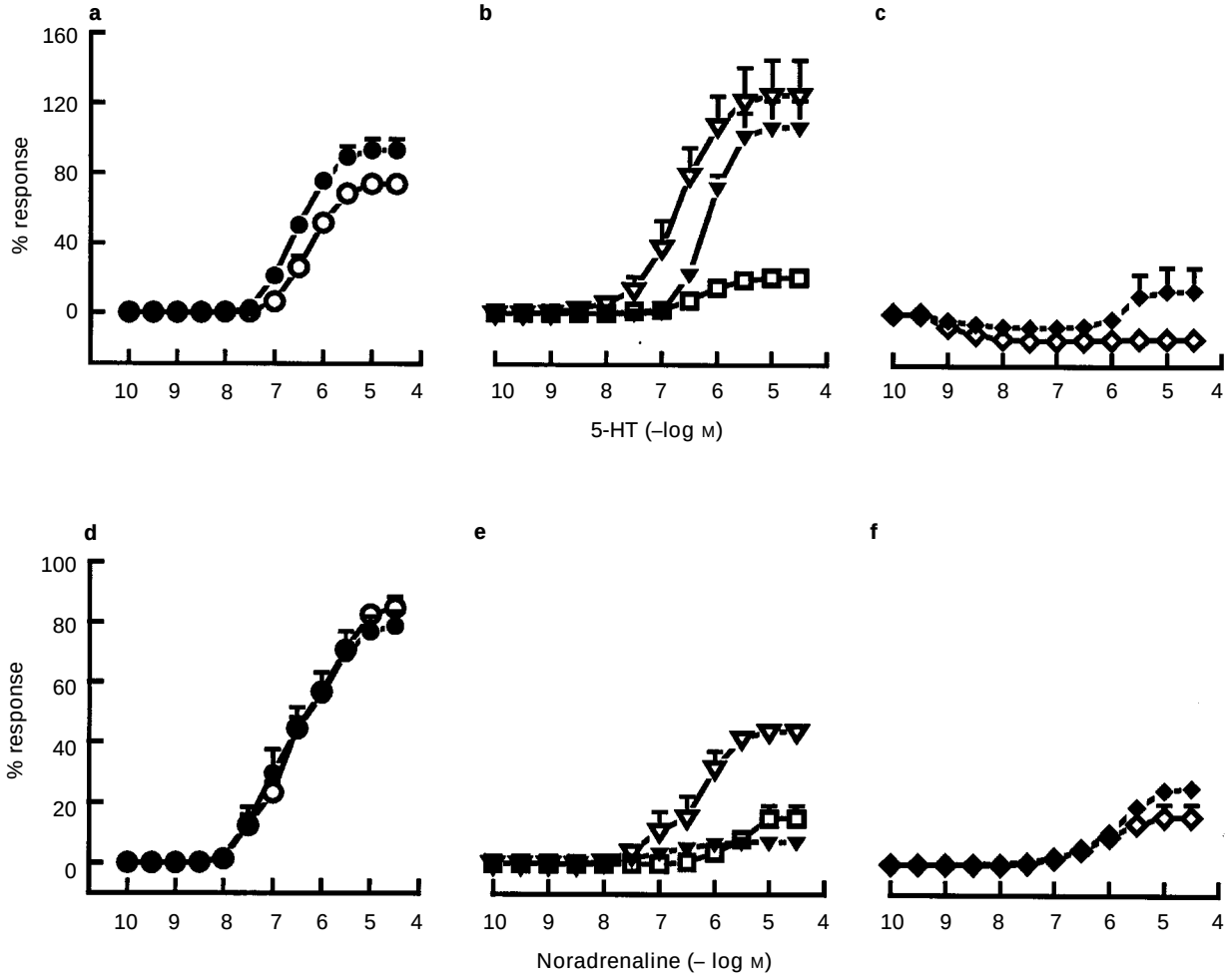

Figure 2 Concentration-contraction curves to serotonin (5-HT) and noradrenaline in isolated (a and d) large (○, $n = 5$) and medium-sized (●, $n = 4$) pulmonary arteries, (b and e) small pulmonary arteries - ~1000 μm in diameter (▽, $n = 4$), ~500 μm in diameter (▼, $n = 5$), ~200 μm in diameter (□, $n = 4–5$) and (c and f) large (◆, $n = 5–6$) and medium-sized (◇, $n = 4–5$) pulmonary veins. Responses are expressed as a percentage of the maximum contraction to K^+ (75 mM) in large and medium-sized arteries and veins and of the contraction to KPSS in small arteries. Values are mean and vertical lines show s.e.mean; n = number of rings.

Results

Resting vessel parameters

A summary of vessel parameters for the different sized arteries and veins is given in Table 1. The maximal contraction to KCl (F_{max}) was greatest in large pulmonary arteries and declined with decreasing vessel diameter (Table 1). However, F_{max} was normalized for vessel diameter, no difference was observed between the groups with the exception of ~ 1000 μm diameter small pulmonary arteries in which the normalized F_{max} value was slightly but significantly ($P<0.05$) lower than in large pulmonary arteries (Table 1).

Vasoconstrictor responses

Endothelin-1 Endothelin-1 caused concentration-dependent contractions in arteries and veins and was the most potent vasoconstrictor tested in all vessels (Figure 1). The maximum response to endothelin-1 was $76.6 \pm 2.6\%$ F_{max} in large arteries and $93.4 \pm 3.9\%$ F_{max} in large veins and did not differ significantly throughout the pulmonary vasculature ($P=0.06$) (Figure 1). The sensitivity to endothelin-1 in large arteries ($pEC_{50}=8.61 \pm 0.09$) was not significantly different from that in medium-sized arteries, large and medium-sized veins and arteries ~ 1000 μm in diameter. However, smaller pulmonary arteries ~ 500 μm ($pEC_{50}=8.13 \pm 0.08$) and ~ 200 μm ($pEC_{50}=8.04 \pm 0.13$) in diameter were significantly ($P<0.01$) less sensitive to endothelin-1 compared with large pulmonary arteries (Figure 1).

U46619 U46619 failed to contract arteries >200 μm in diameter and only contracted arteries ~ 200 μm in diameter to $45.0 \pm 11.8\%$ F_{max} (Figure 1). The greatest response to U46619 was observed in large ($74.3 \pm 6.6\%$ F_{max}, $pEC_{50}=7.85 \pm 0.13$) and medium-sized ($73.9 \pm 3.0\%$ F_{max}, $pEC_{50}=7.95 \pm 0.17$) veins (Figure 1).

5-HT 5-HT caused concentration-dependent contractions in all sized arteries. The maximum contraction to 5-HT was similar in arteries >200 μm in diameter with only the response in ~ 1000 μm vessels ($125.4 \pm 20.2\%$ F_{max}, $P<0.05$) being greater than that in large arteries ($74.2 \pm 2.6\%$ F_{max}) (Figure 2). By contrast, the maximum response to 5-HT was significantly attenuated in the ~ 200 μm diameter small arteries ($20.8 \pm 5.7\%$ F_{max}, $P<0.05$) (Figure 2). In large veins, 5-HT, between concentrations of 1 nM and 100 nM, caused small relaxations which did not attain significance ($-7.5 \pm 3.9\%$ F_{max}, $P>0.1$ compared to baseline, Student's paired t test) and then contractions at higher concentrations (Figure 2). However, in the medium-sized veins only relaxations were observed to 5-HT ($-14.1 \pm 4.1\%$ F_{max}, $P<0.05$ compared to baseline, Student's paired t test). The sensitivity to 5-HT in medium-sized and small arteries was not significantly different from large arteries ($pEC_{50}=6.29 \pm 0.10$).

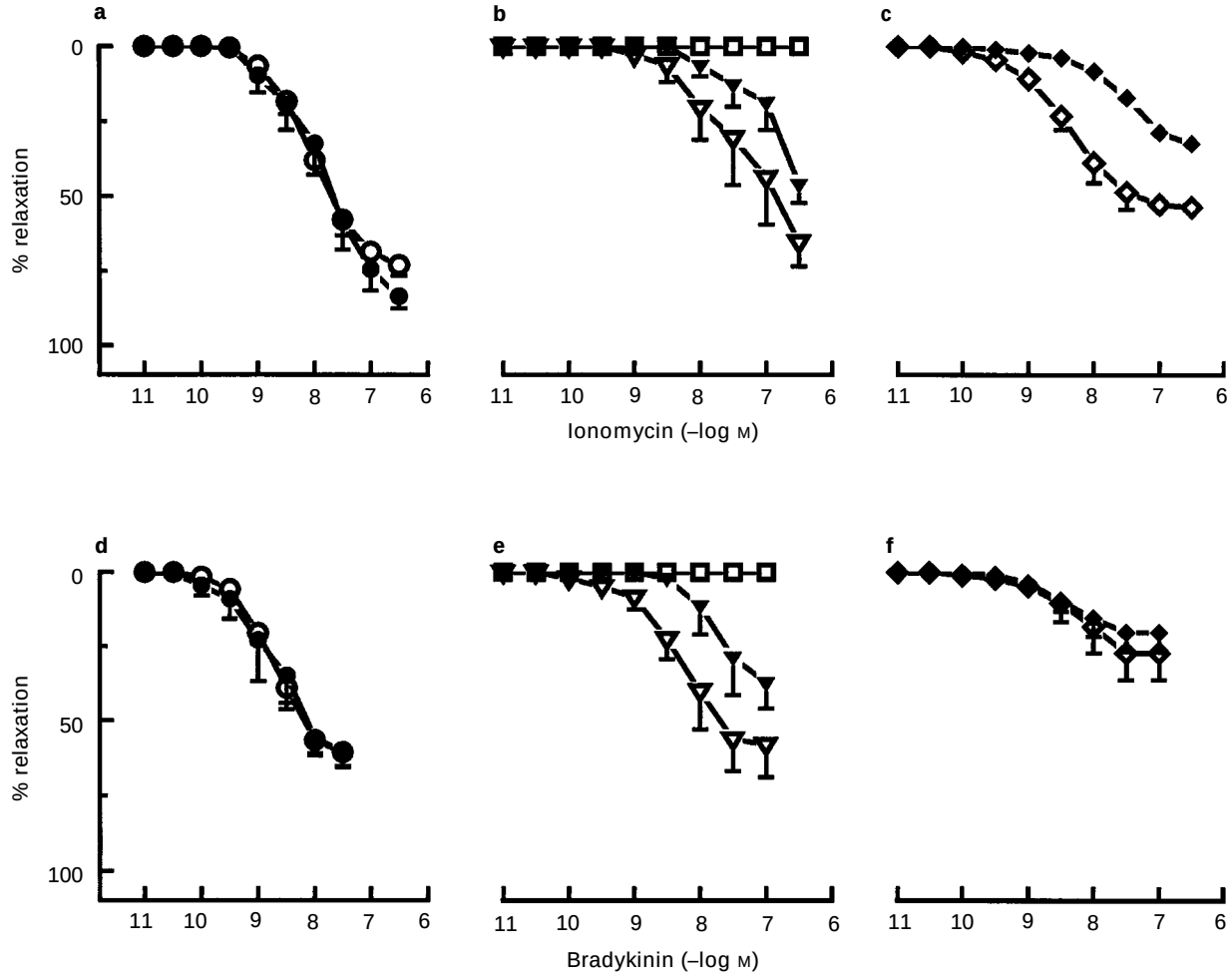

Figure 3 Concentration-relaxation response curves to ionomycin and bradykinin in isolated (a and d) large (○, $n=6-7$) and medium-sized (●, $n=4-5$) pulmonary arteries, (b and e) small pulmonary arteries - ~ 1000 μm in diameter (▽, $n=4-5$), ~ 500 μm in diameter (▼, $n=4$), ~ 200 μm in diameter (□, $n=4-5$) and (c and f) large (◆, $n=5-6$) and medium-sized (◇, $n=4-5$) pulmonary veins. Responses in large, medium-sized and small arteries and veins are expressed as a percentage of the maximum relaxation to SNP (10 mM) from the initial level of precontraction. Values are mean and vertical lines show s.e.mean; n = number of rings. NB: All arteries and veins contracted to ionomycin at concentrations $\geqslant 0.3$ μM.

Noradrenaline Noradrenaline contracted large and medium-sized arteries to 84.7±3.7% and 78.7±5.0% F_{max}, respectively. With decreasing arterial size there was a corresponding significant reduction in this maximum contractile response (Figure 2). In ~1000 μm, ~500 μm and ~200 μm diameter small arteries, noradrenaline caused maximum contractions of 43.9±2.6% ($P<0.01$), 7.4±3.5% ($P<0.01$) and 15.1±4.2% ($P<0.01$) F_{max}, respectively. The maximum contraction to noradrenaline in large (25.5±2.9% F_{max}) and medium-sized (15.9±4.2% F_{max}) veins was also significantly less ($P<0.01$) than in large arteries (Figure 2). The sensitivity to noradrenaline did not differ significantly ($P=0.07$) in the various segments of the pulmonary vasculature (Figure 2).

Vasodilator responses

Relaxation responses to ionomycin, bradykinin and ACh in large and medium-sized pulmonary arteries and veins were abolished upon removal of the endothelium (data not shown).

Ionomycin The maximum relaxation to ionomycin was similar in large (73.0±3.6% SNP), medium-sized (83.5±4.2% SNP) and ~100 μm diameter small (65.3±8.1% SNP) pulmonary arteries but was significantly attenuated in ~500 μm diameter small arteries (45.9±6.3% SNP, $P<0.01$), medium-sized (53.5±2.7% SNP, $P<0.05$) and large (32.3±1.3% SNP, $P<0.01$) veins (Figure 3). Ionomycin failed to relax ~200 μm diameter small arteries contracted with either K^+ ($P<0.01$, Figure 3) or endothelin-1 ($n=2$, data not shown). The pEC_{50} value for ionomycin in medium-sized arteries (8.06±0.21), medium-sized (8.34±0.20) and large veins (7.51±0.04) did not differ in comparison to that obtained in large arteries (8.02±0.16) (Figure 3). An average pEC_{50} value for ionomycin in ~1000 μM and ~500 μm diameter arteries could not be determined as 2 of the 4 relaxation curves in each group were non-sigmoidal.

Bradykinin Bradykinin relaxed large arteries to 60.6±5.2% SNP. The maximum relaxation to bradykinin in ~500 μm diameter arteries (37.3±8.6% SNP) tended to be less than that in large arteries and bradykinin failed to relax ~200 μm diameter arteries contracted with either K^+ ($P<0.01$, Figure 3) or endothelin-1 ($n=2$, data not shown). Bradykinin relaxed medium-sized (27.2±9.1% SNP, $P<0.01$) and large (20.3±6.7% SNP, $P<0.01$) veins to a lesser degree than large arteries (Figure 3). The sensitivity to bradykinin did not differ between large, medium-sized and ~1000 μm arteries and veins ($pEC_{50}=8.74\pm0.11$ large artery vs 8.32±0.26 large vein; $P=0.44$) (Figure 3). Although there was a trend for the ~500 μm arteries to be less sensitive to bradykinin compared with large arteries, an average pEC_{50} value was not determined as 2 of the 4 relaxation response curves were non-sigmoidal.

ACh Maximum relaxations to ACh in large (29.9±6.7% reversal of level of precontraction) and medium-sized

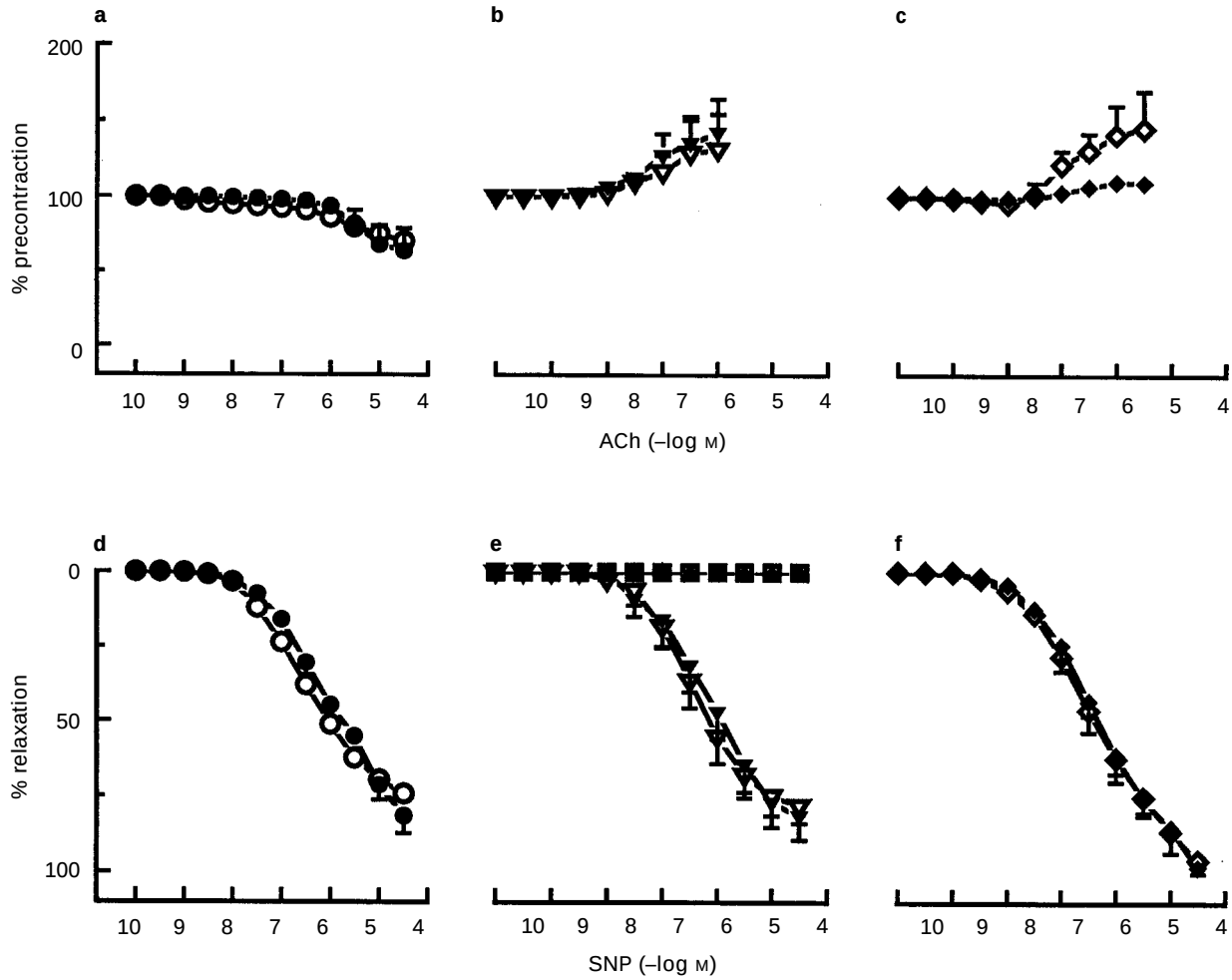

Figure 4 Concentration-relaxation curves to acetylcholine (ACh) and sodium nitroprusside (SNP) in isolated (a and d) large (○, $n=5-6$) and medium-sized (●, $n=4$) pulmonary arteries, (b and e) small pulmonary arteries - ~1000 μm in diameter (▽, $n=3-5$), ~500 μm in diameter (▼, $n=4$), ~200 μm in diameter precontracted either with K^+ (□, $n=6$) or endothelin-1 (▲, $n=4$) and (c and f) large (◆, $n=5$) and medium-sized (◇, $n=5-6$) pulmonary veins. Responses to ACh and SNP are expressed as a percentage of and percentage reversal of the initial level of precontraction, respectively. Values are mean and vertical lines show s.e.mean; n=number of rings.

($36.3 \pm 15.2\%$ reversal of level of precontraction) arteries were not significantly different. pEC_{50} values for ACh in these arteries could not be obtained (Figure 4). Relaxation responses to ACh were not observed in any of the small arteries or medium-sized and large veins. Furthermore, in small arteries (~ 200 μm in diameter) contracted with either U46619 or endothelin-1, ACh failed to cause relaxation ($n=3$, data not shown). Instead, ACh contracted these vessels above the initial level of precontraction (Figure 4). The magnitude of the contraction to ACh ranged from $131.5 \pm 23.4\%$ of the contraction to 5-HT in ~ 500 μm diameter arteries to no response in large veins ($109.7 \pm 6.7\%$ of the contraction to endothelin 1).

SNP The maximum relaxation to SNP in medium-sized and all small arteries (with the exception of ~ 200 μm diameter arteries) did not differ from that in the large artery ($74.3 \pm 2.9\%$ reversal of endothelin-1 contraction). However, the relaxation to SNP in the large ($98.0 \pm 1.6\%$ reversal of endothelin-1 contraction, $P<0.01$) and medium-sized ($95.8 \pm 4.2\%$ reversal of endothelin-1 contraction, $P<0.05$) veins was significantly greater compared with the large artery. SNP failed to relax ~ 200 μm diameter small arteries ($P<0.01$) (Figure 4). This lack of response to SNP was independent of the agonist used to precontract the vessel as SNP failed to relax vessels contracted with either K^+ or endothelin-1 (Figure 4). The pEC_{50} values for SNP did not differ between arteries and veins (Figure 4).

Discussion

This study has demonstrated that the reactivity to many vasoconstrictors and vasodilators is not uniform along the length of the sheep isolated pulmonary vasculature. Furthermore, our study suggests that the pulmonary vasculature displays a distinct, regional pattern of reactivity for vasoconstrictors and vasodilators. These patterns are summarized in Figure 5.

Such differences in longitudinal reactivity to vasoactive agents may arise due to differences in the passive properties and/or pharmacological reactivity of blood vessels. In the present study, arteries and veins were set to passive lengths with pressures which approximated those measured *in vivo* (Brody *et al*., 1968; Kemp *et al*., 1995). Under these conditions passive vessel properties varied along the pulmonary vascular bed such that there was a progressive decrease in the absolute force generated in response to K^+ (F_{max}) with a decrease in arterial size. However, it was unlikely, that the differences in longitudinal contractile and dilator reactivity were due to variations in smooth muscle function along the pulmonary vascular tree, as in general, when F_{max} was normalized for diameter there was no differences between either the arteries or veins. Therefore, differences in receptor types, their location (i.e. endothelium or smooth muscle), numbers, affinities and transducer mechanisms as well as the ability of the endothelium in each vessel to synthesize relaxing factors most likely contributed to the regional variations observed here.

Vasoconstrictor responses

A striking finding from this study was that, in contrast to the other vasoconstrictor agents examined, the response to endothelin-1 was remarkably uniform within both the arterial and venous vasculature (Figure 5). Equivalent maximum responses to endothelin-1 have also been observed in isolated pulmonary arteries and veins from pigs (Zellers *et al*., 1994) and in arterial and venous segments of isolated perfused lungs of the lamb (Toga *et al*., 1991). Previous studies in the systemic (Miller *et al*., 1989; Cocks *et al*., 1989) and pulmonary vasculature (Lippton *et al*., 1991; Toga *et al*., 1992) have shown an increased sensitivity to endothelin-1 in veins compared with arteries. Specifically, Toga *et al*. (1992) obtained a small but significant 1.3 fold increase in sensitivity to endothelin-1 in sheep isolated pulmonary veins compared with arteries. By contrast, in the present study, contractions to endothelin-1 in isolated pulmonary arteries and veins were equipotent. The reason for this discrepancy is unknown.

A distinctly different pattern of contractile responses was observed for the thromboxane A_2 (TXA_2) mimetic, U46619 (Figure 5). Although U46619 is a potent vasoconstrictor of most systemic arteries (Coleman *et al*., 1981), it failed to contract sheep pulmonary arteries greater than 200 μm in diameter yet potently contracted pulmonary veins. These findings suggest that the increase in pulmonary vascular resistance occurring in response to U46619 in sheep *in vivo* (Kuhl *et al*., 1988) is due to a predominant constrictor effect of U46619 on pulmonary veins and perhaps, to a lesser extent, upon small arteries $\leqslant 200$ μm in diameter. This notion is supported by the observation that U46619 increased pulmonary vascular resistance in sheep isolated perfused lungs via

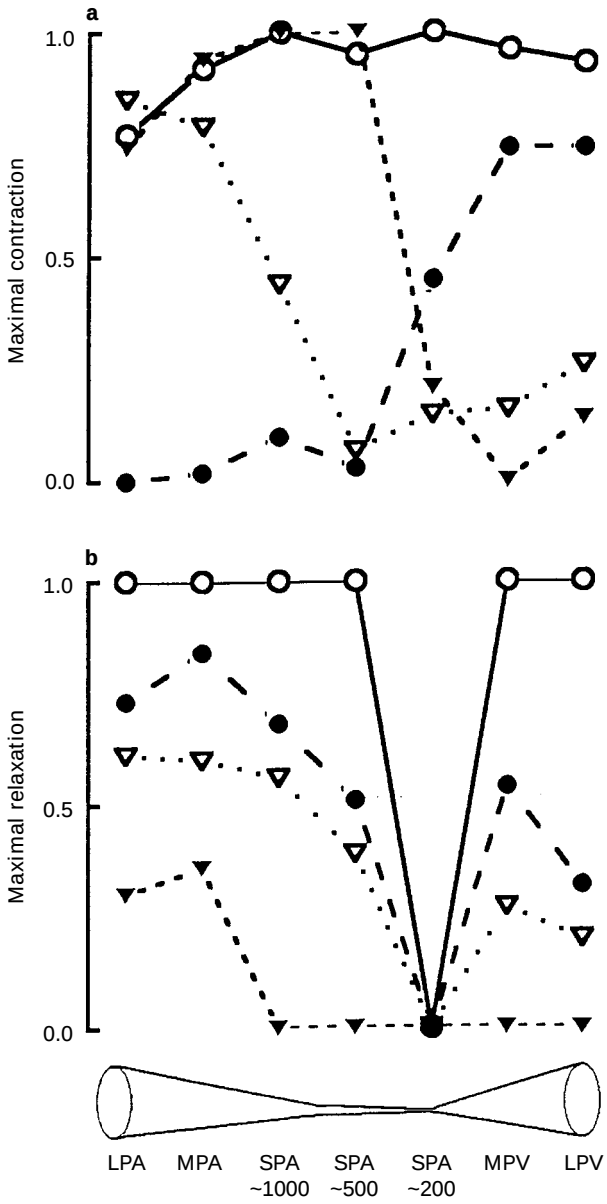

Figure 5 Schema illustrating general trends of maximum responses to (a) vasoconstrictors ((○) endothelin-1, (●), U46619, (▽) noradrenaline and (▼) 5-HT) and (b) vasodilators ((○) SNP, (●) ionomycin, (▽) bradykinin and (▼) ACh) in large (LPA), medium-sized (MPA) and small pulmonary (SPA, ~ 1000, ~ 500, ~ 200 μm in diameter) arteries and medium-sized (MPV) and large pulmonary (LPV) veins. (a) 1 = 100% contraction (F_{max}); (b) 1 = SNP maximum relaxation.

constriction of segments between intrapulmonary veins and small arteries (Kadowitz *et al*., 1985). Also, a greater local production of TXA_2 has been shown in sheep pulmonary veins compared with arteries (Hillyard *et al*., 1992), which may explain the increased responsiveness to U46619 in these vessels. The greater selectivity of U46619 for the pulmonary venous vasculature has also been observed in other species such as the ferret (Raj *et al*., 1992) and dog (Kadowitz & Hyman, 1980).

5-HT and noradrenaline were both predominantly arterial vasoconstrictors in the sheep isolated pulmonary vasculature (Figure 5). Similar findings have been observed for 5-HT in dog isolated pulmonary arteries and veins (Gruetter *et al*., 1981) and isolated perfused lungs (Glazier & Murray, 1971; Hofman 1991). Also, noradrenaline has been found to contract pulmonary arteries from sheep (Joiner *et al*., 1975), rat (Leach *et al*., 1992) and rabbit (Su *et al*., 1978; Kolbeck & Speir, 1987) to a greater extent than pulmonary veins. An interesting finding from this study was that although the actions of both 5-HT and noradrenaline were confined predominantly to the pulmonary arterial vasculature, their responses in different sized arteries were markedly different. Thus, 5-HT contracted pulmonary arteries >200 μm in diameter to a similar magnitude yet the response to noradrenaline declined progressively with decreasing arterial diameter (Figure 5).

These heterogeneous responses to 5-HT and noradrenaline both between arteries and veins and within the arterial segment may be related to differences in the distribution of receptor subtypes. Thus, the absence of a constrictor response to 5-HT in the pulmonary veins may have been due to 5-HT activating smooth muscle 5-HT_4 receptors which mediate relaxation (Cocks & Arnold, 1992). Similar 5-HT_4 receptor-mediated relaxation to 5-HT occurs in small ($\sim$200 μm in diameter) but not in larger pulmonary arteries (Kemp & Cocks, unpublished results) and this most likely contributed to the poor contractile response to 5-HT observed in the small arteries. Similarly for noradrenaline, the longitudinal variability in adrenoceptor densities in the pulmonary vascular bed (Su *et al*., 1978; Altiere *et al*., 1983) may have contributed to the attenuated contraction to this agonist along the sheep pulmonary vascular tree. Thus, Laher & Bevan (1985), demonstrated an excess of α-adrenoceptors in rabbit pulmonary arteries (800–1000 μm in diameter) compared with smaller, non-elastic, pulmonary arteries (650–800 μm in diameter).

Finally, a greater release of endothelium-derived relaxing factors in response to 5-HT or noradrenaline (Cocks & Angus, 1983; Miller & Vanhoutte, 1985; Hofman *et al*., 1991) in small pulmonary arteries and veins could contribute to reduce contractile responses in these vessels. For example, α_2-adrenoceptor-mediated endothelium-dependent relaxation of dog isolated pulmonary arteries is more difficult to demonstrate than in veins and it has been speculated that a greater number of smooth muscle α_2-receptors are present in arteries than veins thus masking the endothelium-dependent relaxation response (Miller & Vanhoutte, 1985).

Vasodilator responses

The observation that ACh relaxed sheep large pulmonary arteries, albeit poorly, but contracted small arteries and veins (Figure 5), correlates well with previous studies in isolated pulmonary vessels from juvenile sheep (Steinhorn *et al*., 1993), rabbit (Loiacono & Story, 1984; Altiere *et al*., 1986), dog (Franklin, 1932; Chang & Altura, 1981; De Mey & Vanhoutte, 1982), cow (Gruetter & Lemke, 1986) and rat (McCormack *et al*., 1988). Furthermore, the present finding that both bradykinin and ionomycin were more effective relaxing agents in sheep pulmonary arteries than veins (Figure 5) is similar to studies in the dog isolated perfused dog lung, where bradykinin induced a greater decrease in pulmonary arterial than pulmonary venous pressure (Levine *et al*., 1973), and in the bovine isolated pulmonary vasculature where the maximum relaxation to the calcium ionophore, A23187, has been shown to be greater in arteries than veins (Gruetter & Lemke, 1986).

The decreased efficacy of endothelium-dependent vasodilators in the sheep pulmonary venous vasculature may be due to a decreased ability of venous endothelial cells to synthesize and/or release nitric oxide (NO), as has been demonstrated for systemic veins (Seidel & LaRochelle, 1987). Indeed, pulmonary veins appear to have a decreased ability to synthesize NO as basal levels of guanosine 3′,5′-cyclic monophosphate (cyclic GMP), the transducer for NO (Ignarro, 1991), are lower in bovine isolated pulmonary veins compared with arteries (Ignarro *et al*., 1986; 1987). It is unlikely that the decreased endothelium-dependent relaxation in sheep pulmonary veins was due to a diminished ability of the venous smooth muscle to relax to NO as the NO-donor, SNP, was an equipotent and effective vasodilator of all pulmonary arteries and veins with the exception of the $\sim$200 μm diameter arteries. These findings concur with those in dog (Kadowitz *et al*., 1981) and rat (Eichinger & Walker, 1994) isolated perfused lungs in which SNP has been found to cause similar dilatations in the arterial and venous segments.

The finding here that $\sim$200 μm diameter arteries failed to relax to ionomycin, bradykinin ACh and SNP (Figure 5) confirms an earlier finding in rat isolated pulmonary arteries (100–300 μmin diameter) where endothelium-dependent and -independent nitrovasodilators also failed to cause any relaxation (Leach *et al*., 1990). The reason for this apparent general lack of relaxation to vasodilators in the $\sim$200 μm diameter arteries is unclear. However it was unlikely that precontraction of these vessels with KCl masked the responses to bradykinin, ionomycin, ACh and SNP, particularly if they were mediated by hyperpolarization (Taylor & Weston, 1988; Feletou & Vanhoutte, 1988; Garland *et al*., 1995), since these vasodilators also failed to relax arteries contracted with either endothelin-1 or U46619. In addition, it is unlikely that damage to the endothelium accounted for the absence of endothelium-dependent vasodilator responses for, although the presence of an intact endothelium was not confirmed histologically, we have previously demonstrated endothelium-dependent relaxation in similar-sized arteries mounted in the myograph (Kemp & Cocks, 1997). Rather, the inability of the smooth muscle to relax in response to SNP and thus NO appears to contribute to the lack of endothelium-dependent relaxation in these small pulmonary arteries. However, it is not known if the synthesis and/or release of endothelium-derived relaxing factors is also decreased in these vessels compared with larger arteries and veins. Interestingly, the inability of these small pulmonary arteries to relax does not appear to be specific for nitrovasodilators since isoprenaline also failed to relax arteries contracted with either KCl or endothelin-1 (Kemp & Cocks, unpublished observations). Thus, in small pulmonary arteries NO- and β-adrenoceptor-mediated relaxation of the smooth muscle is impaired and further investigation is required to determine if these vessels can relax in response to any other vasodilator agents.

Conclusion

This study has demonstrated that isolated pulmonary arteries and veins displayed substantial specialization in their responsiveness to vasoconstrictor and vasodilator agents (see Figure 5). The physiological relevance of such regional specialization is yet to be fully elucidated. However, if similar specialization occurs in man it may provide useful site-specific targets for therapeutic agents in the treatment of primary and secondary pulmonary hypertension.

This work was supported by the National Health and Medical Research Council of Australia. The authors thank Dr M. Walker for his useful comments.

References

ALTIERE, R.J., DOUGLAS, J.S. & GILLIS, C.N. (1983). Pharmacological analysis of norepinephrine responses in rabbit pulmonary blood vessels. *J. Pharmacol. Exp. Ther.*, **224,** 579–589.

ALTIERE, R.J., KIRITSY-ROY, J.A. & CATRAVAS, J.D. (1986). Acetylcholine-induced contractions in isolated rabbit pulmonary arteries: role of thromboxane A_2. *J. Pharmacol. Exp. Ther.*, **236,** 535–541.

ANGUS, J.A., BROUGHTON, A. & MULVANY, M.J. (1988). Role of α-adrenoceptors in constrictor responses in rat, guinea-pig and rabbit small arteries to neural activation. *J. Physiol.*, **403,** 495–510.

ANGUS, J.A., COCKS, T.M. & SATOH, K. (1986). α_2-Adrenoceptors and endothelium-dependent relaxation in canine large arteries. *Br. J. Pharmacol.*, **88,** 767–777.

BARER, G.R. (1976). The physiology of the pulmonary circulation and methods of study. *Pharmacol. Ther. B.*, **2,** 247–273.

BARMAN, S.A., SENTENO, E., SMITH, S. & TAYLOR, A. (1989). Acetylcholine's effect on vascular resistance and compliance in the pulmonary circulation. *J. Appl. Physiol.*, **67,** 1495–1503.

BERGOFSKY, E.H. (1980). Humoral control of the pulmonary circulation. *Ann. Rev. Physiol.*, **42,** 221–233.

BRADLEY, J.D., ZANABONI, P.B. & DAHMS, T.E. (1993). Species differences in pulmonary vasoactive responses to histamine, 5-hydroxytryptamine, and KCl. *J. Appl. Physiol.*, **74,** 139–146.

BRODY, J.S., STEMMLER, E.J. & DUBOIS, A.B. (1968). Longitudinal distribution of vascular resistance in the pulmonary arteries, capillaries, and veins. *J. Clin. Invest.*, **47,** 783–808.

CHAND, N. & ALTURA, B.M. (1981). Acetylcholine and bradykinin relax intrapulmonary arteries by acting on endothelial cells: role in lung vascular diseases. *Science*, **213,** 1376–1379.

COCKS, T.M. & ANGUS, J.A. (1983). Endothelium dependent relaxation of coronary arteries by noradrenaline and serotonin. *Nature*, **305,** 627–630.

COCKS, T.M. & ARNOLD, P.J. (1992). 5-Hydroxytryptamine (5-HT) mediates potent relaxation in the sheep isolated pulmonary vein via activation of 5-HT_4 receptors. *Br. J. Pharmacol.*, **107,** 591–596.

COCKS, T.M., FAULKNER, N.L., SUDHIR, K. & ANGUS, J.A. (1989). Reactivity of endothelin-1 on human canine large veins compared with large arteries *in vitro*. *Eur. J. Pharmacol.*, **171,** 17–24.

COLEMAN, R.A., HUMPHREY, P.P.A., KENNEDY, I., LEVY, G.P. & LUMLEY, P. (1981). Comparison of the actions of U-46619, a prostaglandin H_2-analogue, with those of prostaglandin H_2 and thromboxane A_2 on some isolated smooth muscle preparations. *Br. J. Pharmacol.*, **73,** 773–778.

DE MEY, J.G. & VANHOUTTE, P.M. (1982). Heterogeneous behaviour of the canine arterial and venous wall. *Circ. Res.*, **51,** 439–447.

EICHINGER, M.R. & WALKER, B.R. (1994). Segmental heterogeneity of NO-mediated pulmonary vasodilation in rats. *Am. J. Physiol.*, **267,** H484–H499.

ELGHOZI, J. & HEAD, G.A. (1990). Spinal noradrenergic pathways and pressor responses to central angiotensin II. *Am. J. Physiol.*, **258,** H240–H246.

FELETOU, M. & VANHOUTTE, P.M. (1988). Endothelium-dependent hyperpolarization of canine coronary smooth muscle. *Br. J. Pharmacol.*, **93,** 515–524.

FRANKLIN, K.J. (1932). The actions of adrenaline and of acetylcholine on the isolated pulmonary vessels and azygos vein of the dog. *J. Physiol.*, **75,** 471–479.

GARLAND, C.J., PLANE, F., KEMP, B.K. & COCKS, T.M. (1995). Endothelium-dependent hyperpolarization: a role in the control of vascular tone. *Trends Pharmacol. Sci.*, **16,** 23–30.

GLAZIER, J.B. & MURRAY, J.F. (1971). Site of pulmonary vasomotor reactivity in the dog during alveolar hypoxia and serotonin and histamine infusion. *J. Clin. Invest.*, **50,** 2550–2558.

GRUETTER, C.A., IGNARRO, L.J., HYMAN, A.L. & KADOWITZ, P.J. (1981). Contractile effects of 5-hydroxytryptamine in isolated intrapulmonary arteries and vein. *Can. J. Physiol. Pharmacol.*, **59,** 157–162.

GRUETTER, C.A. & LEMKE, S.M. (1986). Comparison of endothelium-dependent relaxation in bovine intrapulmonary artery and vein by acetylcholine and A23187. *J. Pharmacol. Exp. Ther.*, **238,** 1055–1062.

HILLYARD, R., IBE, B.O. & RAH, J.U. (1992). Developmental differences in endogenous production of eicosanoids by ovine-intrapulmonary vessels. *Ped. Res.*, **31,** 310A.

HOFMAN, W.F., JACKSON, W.F., EL-KASHEF, H. & EHRHART, I.C. (1991). Modulation of vascular reactivity to serotonin in the dog lung. *J. Appl. Physiol.*, **71,** 217–222.

IGNARRO, L.J. (1991). Signal transduction mechanisms involving nitric oxide. *Biochem. Pharmacol.*, **41,** 485–490.

IGNARRO, L.J., BYRNS, R.E. & WOOD, K.S. (1987). Endothelium-dependent modulation of cGMP levels and intrinsic smooth muscle tone in isolated bovine intrapulmonary artery and vein. *Circ. Res.*, **60,** 82–92.

IGNARRO, L.J., HARBISON, R.G., WOOD, K.S. & KADOWITZ, P.J. (1986). Activation of purified soluble guanylate cyclase by endothelium-derived relaxing factor from intrapulmonary artery and vein: stimulation by acetylcholine, bradykinin and arachidonic acid. *J. Pharmacol. Exp. Ther.*, **237,** 893–900.

JOINER, P.D., KADOWITZ, P.J., HUGHES, J.P. & HYMAN, A.L. (1975). NE and ACh responses of intrapulmonary vessels from dog, swine, sheep and man. *Am. J. Physiol.*, **226,** 1821–1827.

KADOWITZ, P.J. & HYMAN, A.L. (1980). Comparative effects of thromboxane B_2 on the canine and feline pulmonary vascular bed. *J. Pharmacol. Exp. Ther.*, **213,** 300–305.

KADOWITZ, P.J., MCNAMARA, D.B. & HYMAN, A.L. (1985). Pulmonary venous responses to leukotrienes. In *Leukotrienes in Cardiovascular and Pulmonary Function*, ed. Lefer, A.M. & Gee, M.H. pp. 247–257. Alan R. Liss, Inc.

KADOWITZ, P.J., NANDIWADA, P. & GRUETTER, C.A. (1981). Pulmonary vasodilator responses to nitroprusside and nitroglycerin in the dog. *J. Clin. Invest.*, **67,** 893–902.

KEMP, B.K., SMOLICH, J.J., RITCHIE, B.C. & COCKS, T.M. (1995). Endothelium-dependent relaxations in sheep pulmonary arteries and veins: resistance to block by N^G-nitro-L-arginine in pulmonary hypertension. *Br. J. Pharmacol.*, **116,** 2457–2467.

KEMP, B.K. & COCKS, T.M. (1997). Evidence that mechanisms dependent and independent of nitric oxide mediate endothelium-dependent relaxation to bradykinin in human small resistance-like coronary arteries. *Br. J. Pharmacol.*, **120,** 757–762.

KOLBECK, R.C. & SPEIR, W.A. (1987). Regional contractile responses in pulmonary artery to α- and β-adrenoceptor agonists. *Can. J. Physiol. Pharmacol.*, **65,** 1165–1170.

KUHL, P.G., BOLDS, J.M., LOYD, J.E., SNAPPER, J.R. & FITZGERALD, G.A. (1988). Thromboxane receptor-mediated bronchial and haemodynamic responses in ovine endotoxemia. *Am. J. Physiol.*, **254,** R310–R319.

LAHER, I. & BEVAN, J.A. (1985). Alpha adrenoceptor number limits response of some rabbit arteries to norepinephrine. *J. Pharmacol. Exp. Ther.*, **233,** 290–297.

LEACH, R.M., TWORT, C.H.C., CAMERON, I.R. & WARD, J.P.T. (1990). The mechanism of action of endothelin-1 on small pulmonary arterial vessels. *Pulm. Pharmacol.*, **3,** 103–109.

LEACH, R.M., TWORT, C.H.C., MERON, I.R.C. & WARD, J.P.T. (1992). A comparison of the pharmacological and mechanical properties *in vitro* of large and small pulmonary arteries of the rat. *Clin. Sci.*, **82,** 55–62.

LEVINE, B.W., TALAMO, R.C. & KAZEMI, H. (1973). Action and metabolism of bradykinin in dog lung. *J. Appl. Physiol.*, **34,** 821–826.

LINEHAN, J.H. & DAWSON, C.A. (1983). A three-compartment model of the pulmonary vasculature: effects of vasoconstriction. *J. Appl. Physiol.*, **55,** 923–928.

LIPPTON, H.L., OHLSTEIN, E.H., SUMMER, W.R. & HYMAN, A.L. (1991). Analysis of responses to endothelins in the rabbit pulmonary and systemic vascular beds. *J. Appl. Physiol.*, **70,** 331–341.

LOIACONO, R.E. & STORY, D.F. (1984). Acetylcholine-induced inhibition of responses to field stimulation in rabbit pulmonary artery is unaffected by endothelium removal. *J. Pharm. Pharmacol.*, **36,** 262–264.

MCCORMACK, D.D., MAK, J.C., MINETTE, P. & BARNES, P.J. (1988). Muscarinic receptor subtypes mediating vasodilation in the pulmonary artery. *Eur. J. Pharmacol.*, **158,** 293–297.

MILLER, V.M., KOMORI, K., BURNETT, J.C. & VANHOUTTE, P.M. (1989). Differential sensitivity to endothelin in canine arteries and veins. *Am. J. Physiol.*, **257,** H1127–H1131.

MILLER, V.M. & VANHOUTTE, P.M. (1985). Endothelial α_2-adrenoceptors in canine pulmonary and systemic blood vessels. *Eur. J. Pharmacol.*, **118,** 123–129.

MULVANY, M.J. & HALPERN, W. (1977). Contractile responses of small resistance vessels in spontaneously hypertensive and normotensive rats. *Circ. Res.*, **41,** 19–26.

RAJ, J.U., TOGA, H., IBE, B.O. & ANDERSON, J. (1992). Effects of endothelin, platelet activating factor and thromboxane A_2 in ferret lungs. *Resp. Physiol.*, **88,** 129–140.

SAID, S.I. (1982). Metabolic functions of the pulmonary circulation. *Circ. Res.*, **50,** 325–333.

SEIDEL, C.L. & LAROCHELLE, J. (1987). Venous and arterial endothelia: different dilator abilities in dog vessels. *Circ. Res.*, **60,** 626–630.

STEINHORN, R.H., MORIN, R.D., GUGINO, S.F., GIESE, E.C. & RUSSELL, J.A. (1993). Developmental differences in endothelium-dependent responses in isolated ovine pulmonary arteries and veins. *Am. J. Physiol.*, **264,** H2162–H2167.

SU, C., BEVAN, R.D., DUCKLES, S.P. & BEVAN, J.A. (1978). Functional studies of the small pulmonary arteries. *Microvasc. Res.*, **15,** 37–44.

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

TOGA, H., IBE, B.O. & USHA, R.A.J. (1992). *In vitro* responses of ovine intrapulmonary arteries and veins to endothelin-1. *Am. J. Physiol.*, **263,** L15–L21.

TOGA, H., RAJ, J.U., HILLYARD, R., KU, B. & ANDERSON, J. (1991). Endothelin effects in isolated, perfused lamb lungs: role of cycloxygenase inhibition and vasomotor tone. *Am. J. Physiol.*, **261,** H443–H450.

ZELLERS, T.M., MCCORMICK, J. & YIQUN, W. (1994). Interaction among ET-1, endothelium-derived nitric oxide, and prostacyclin in pulmonary arteries and veins. *Am. J. Physiol.*, **267,** H139–H147.

(Received August 27, 1996
Revised November 18, 1996
Accepted January 8, 1997)