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Differential responses of pulmonary arteries and veins to histamine and 5-HT in lung explants of guinea-pigs

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1 The mechanisms by which histamine and 5-HT differentially contract pulmonary arteries and veins are unclear. In lung explants from 26 guinea-pigs, we compared responses of pulmonary arteries and vein to histamine, 5-HT and KCl, and examined potential determinants for the differential responses. Lungs were filled with agarose, sectioned into ~1 mm thick slices, and vascular luminal areas measured by image analysis.

2 Histamine and 5-HT produced a concentration-dependent constriction in arteries and veins, greater in the latter. KCl constricted arteries and veins equally.

3 The histamine H_1 antagonist chlorpheniramine (10^{-4} M) abolished contractions to histamine; the H_2 antagonist cimetidine enhanced maximal responses and sensitivity of arteries and veins to histamine, and diminished the differences between their maximal responses; the NO synthase inhibitor N^{ω}-nitro-L-arginine (L-NOARG) increased the maximal responses of arteries and veins, and the differences between their responses; indomethacin had no effect.

4 Contractions to 5-HT were abolished in arteries and markedly reduced in veins by the 5-HT_2 antagonist ketanserin (10^{-4} M); L-NOARG potentiated the maximal responses of arteries but not of veins; indomethacin increased the maximal responses of arteries but reduced them in veins.

5 By morphometry, arteries had a greater medial thickness and luminal diameter than veins.

6 The data suggest that in guinea-pigs, H_2 receptors are responsible for the differential contractile responses of pulmonary arteries and veins to histamine, whereas endothelium-derived vasoactive substances are responsible for their differential contractile responses to 5-HT.

Keywords: Vasoconstriction; pulmonary artery; pulmonary vein; histamine; 5-HT; nitric oxide; lung culture; morphometry

Introduction

Differential alterations in the reactivity of pulmonary arteries and veins to pharmacological agents can influence perfusion, ventilation-perfusion relationships and vascular resistance in the lung, all of which may affect fluid exchange and raise right ventricular afterload. The biogenic amines histamine and 5-hydroxytryptamine (5-HT) are known to constrict differentially pulmonary arteries and veins in several species: for example, in dogs, histamine contracts pulmonary veins, and 5-HT contracts pulmonary arteries (Hakim *et al*., 1982; Michel *et al*., 1990; Bradley *et al*., 1993); in rabbits, both amines primarily contract pulmonary arteries (Albert *et al*., 1989; Bradley *et al*., 1993), whereas in guinea-pigs, they predominantly contract pulmonary veins (Bradley *et al*., 1993).

The determinants for the differential responses of pulmonary arteries and veins to histamine and to 5-HT are not known. Putative explanations include differences in receptor subtypes and density (van Nueten *et al*., 1984), in the endothelium that produces several vasoactive substances affecting vascular tone (Furchgott & Vanhoutte, 1989; Gao *et al*., 1995; Feletou *et al*., 1995), and difference in structure (Bradley *et al*., 1993). Indeed, not only do histamine and 5-HT contract vascular smooth muscle but they also relax it by activation of specific receptors on smooth muscle and endothelial cells (Toda, 1990; Cushing & Cohen, 1992; Neely *et al*., 1993). For example, histamine constricts vessels by activating H_1 receptors on smooth muscle and relaxes them directly through H_2 receptors on smooth muscle, and indirectly via H_1 receptors on endothelial cells (Toda, 1990) that release relaxing factors such as nitric oxide (NO) and prostacyclin (PGI_2) (Sakuma *et al*., 1988; Furchgott & Vanhoutte, 1989). Arteries and veins differ in their dilator responses (Furchgott & Vanhoutte, 1989; Gao *et al*., 1995), and this difference could contribute to their differential contractile responses to these two amines. Structural differences of pulmonary arteries and veins could also contribute to their differential contractile responses to histamine and 5-HT (Ferencz, 1969).

Recently, the lung explant technique was applied to study airway constriction by Dandurand *et al*. (1993). In this preparation, small airways and vessels are readily and directly visualized by light microscopy and the structural relationships between vessels, airways and parenchyma are preserved; moreover, the explants can be fixed and morphometric measurements made on the same vessels stimulated with pharmacological agents.

Therefore the principal aims of the present study were, by use of lung explants, to (1) test the differential reactivity of intrapulmonary arteries and veins of guinea-pigs to histamine and 5-HT, (2) explore potential mechanisms for the differential responses, specifically endothelial modulation, receptor subtypes and vascular structure.

Methods

Preparation of the lung explants

The procedure was slightly modified from that previously described for airways (Dandurand *et al*., 1993). A total of 26 male adult Hartley strain guinea-pigs weighing 474±11 g (mean±s.e.) were used for these studies.

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All the animals were anaesthetized with pentobarbitone (40 mg kg^{-1}, i.p.), administered heparin through the dorsal vein of the penis (3000 u kg^{-1}) and intubated through a tracheostomy with sterile polyethylene tubing 1.9 mm in diameter. Their anterior chest wall and upper abdomen were sterilized with 70% ethanol, the abdomen was opened, and they were exsanguinated by cutting the abdominal aorta. After removal of the anterior chest wall, the right ventricle was punctured and a cannula advanced into the main pulmonary artery and the pulmonary vessels washed *in situ* with 10 ml Ringer's lactate containing 20 u ml^{-1} heparin. Thereafter, the heart and lungs were excised *en bloc* and the lungs inflated to near total lung capacity with 1% agarose in bicarbonate-buffered culture medium (BCM, 48 ml kg^{-1} body weight) (Dandurand *et al*., 1993). The preparation was left to cool for 20 min at 4°C. Then the lungs were separated from the heart, placed in a sterile 50 ml syringe, the needle end of which had been removed, and embedded in 4% agarose in bicarbonate buffered minimum essential medium at 37°C (Dandurand *et al*., 1993). After 30 min at 4°C, the lung-agarose block was sectioned with a hand-held microtome blade into 0.5–1.0 mm thick transverse slices. These were examined with an inverted microscope (IMT-2, Olympus, Tokyo, Japan) and those that contained at least one cross-section of a vessel were placed in a 30 mm culture well insert within a six-well plate containing 2 ml of BCM and incubated overnight at 37°C in 5% CO_2–95% air.

Image acquisition

The culture dish inserts containing the lung explants were transferred to six-well plates containing 2 ml of HEPES-buffered culture medium (HCM) (Dandurand *et al*., 1993), and placed on the stage of an inverted microscope (LH50A, Olympus, Tokyo, Japan). Arteries and veins were identified and imaged with a video camera (CDS, Sony, Nagano, Japan) and images recorded with a video disk recorder (TQ2026F, Panasonic, Osaka, Japan). To distinguish arteries from veins, we used the following criteria: (1) the arteries usually accompanied airways, whereas veins were at a distance from them, and (2) arterial walls were thick and their inner lining was slightly wrinkled, whereas veins were thinner and wrinkles were not conspicuous. In addition, we confirmed the identities of the vessels by histological examination (see below).

Experimental protocol

First, in 13 guinea-pigs, we compared the concentration-responses of arteries and veins to histamine and 5-HT. To do this, drugs were added in a cumulative manner: after generation of baseline images of the vessels, 10^{-11} M histamine or 5-HT were added to the explants. Twenty seconds later (preliminary experiments showed that 20 s was the time to peak responses for most concentrations), images of the vessels were taken. Then 10^{-10} M of the appropriate drug was added and images again taken. This procedure was repeated until the final concentrations of 10^{-3} M for histamine and 10^{-4} M for 5-HT were reached.

Second, in four guinea-pigs, concentration-responses were generated to KCl by use of a similar protocol, except that after the baseline images had been generated, 4 mM KCl was added to the explants and images were taken at 10 min, corresponding to the time of peak contraction. The sequential addition of incremental concentrations was stopped at a final concentration of 60 mM KCl.

Third, we examined the effects of the NO synthase inhibitor N^{ω}-nitro-L-arginine benzyl ester (L-NOARG, 10^{-4} M), of the cyclo-oxygenase inhibitor indomethacin (10^{-5} M), of the H_1 receptor antagonist chlorpheniramine (10^{-4} M), and of the H_2 receptor antagonist cimetidine (10^{-4} M) on the concentration-responses to histamine, in seven guinea-pigs (including the four guinea-pigs that were used also for the KCl study). We also studied the effects of L-NOARG (10^{-4} M), of indomethacin (10^{-5} M), and of the 5-HT_2 receptor antagonist ketanserin (10^{-4} M) on the concentration-responses to 5-HT, in another six guinea-pigs. The lung explants were preincubated with the drugs for 30 min.

In each animal, we usually used 24 explant slices. In each explant, we usually observed one artery and/or one vein, and in a few instances two veins. Each vessel was studied only once. On average, two to three arteries and two to three veins from each animal were used for each treatment.

Image and data analysis

The stored images were digitized with a 80386 Intel-based microcomputer equipped with a frame-grabber board (PIP1024B, Matrox, Montreal, QC, Canada). The digitized images were then transferred to a scientific work station (RS6000, IBM, Armonk, NY), and measurements of luminal area were made with Galileo Image Processing Software (Inspiraplex, Montreal, QC, Canada). The responses of arteries or veins to histamine, 5-HT and KCl were calculated as a percentage change in luminal areas over baseline. Thus a 100% response indicated complete vessel luminal closure and 0% no effect.

From these responses, concentration-response curves of arteries and veins were constructed by plotting the mean values against concentrations. The maximum responses and EC_{50} values were determined for each individual vessel, and the latter were expressed as negative log molar (pD_2) values.

Drugs

All drugs were purchased from Sigma Chemical (St. Louis, MO) except ketanserin tartrate, which was purchased from Research Biochemicals (Natick, MA). Histamine (dihydrochloride), 5-HT (hydrochloride), chlorpheniramine (maleate), cimetidine, KCl, ketanserin, and N^{ω}-nitro-L-arginine benzyl ester were prepared as stock solutions in HCM, from which dilutions were prepared fresh daily. Indomethacin was initially dissolved in ethanol and then diluted with HCM. Each of the drugs was added in 20 μl volumes to 2 ml of the medium, and their concentrations are expressed as values after dilution by the 2 ml of medium.

Histology and morphometry

At the end of the experiments, the explants from the first 13 guinea-pigs were fixed by immersion in 10% buffered formalin, processed by use of standard histological techniques and embedded in paraffin. Five micrometre thick sections were cut and stained with haematoxylin-eosin and, in selected ones, with Van Gieson's elastic stain. The arteries and veins which had been used to study responses to histamine and 5-HT were identified, based on maps drawn at the time of image acquisition. Morphometric measurements were then made on those vessels that had an intact wall, by use of previously described methods (Michel, 1982; Michel & Hakim, 1991): with an ocular micrometer on a optical microscope (Leitz, Wetzlar, Germany), we measured the inside diameter at a

magnification of $\times 100$, and the medial muscle thickness of both walls at the same position, at a magnification of $\times 250$ to $\times 400$ (for greater precision); the sum of the inside diameter and of the medial muscle thickness of both walls equalled the outside diameter. The few veins that contained cardiac muscle in their wall were excluded. Morphometric measurements were made on a total of 68 arteries and 75 veins.

In addition, in selected sections of vessels from five guinea-pigs, we ascertained that the endothelial cells were intact by staining for the von Willebrand factor with a rabbit polyclonal antibody as previously described (Doornekamp *et al*., 1996).

Statistical analysis

Data are presented as means ± s.e. These means were obtained by averaging data from each animal, and only this average was used for statistical analyses, with *n* thus being the number of animals from which the vessels were obtained. Because these means were reasonably normally distributed, parametric tests were used for statistical analyses. To compare the curves of the concentration-responses between arteries and veins, or between groups from the same type of vessels that received histamine or 5-HT alone, and those that were pretreated with the modulator agents, we used two-way analysis of variance (ANOVA). If the *F* value was significant, the Tukey test was applied to ascertain significance at each concentration. The comparisons of maximal responses and pD_2 values were also performed by two-way ANOVA. When only two means were compared, Student's paired or unpaired *t* test was used. All the analyses were performed with proprietary software (Systat, Evanston, IL). Differences were considered statistically significant at $P<0.05$.

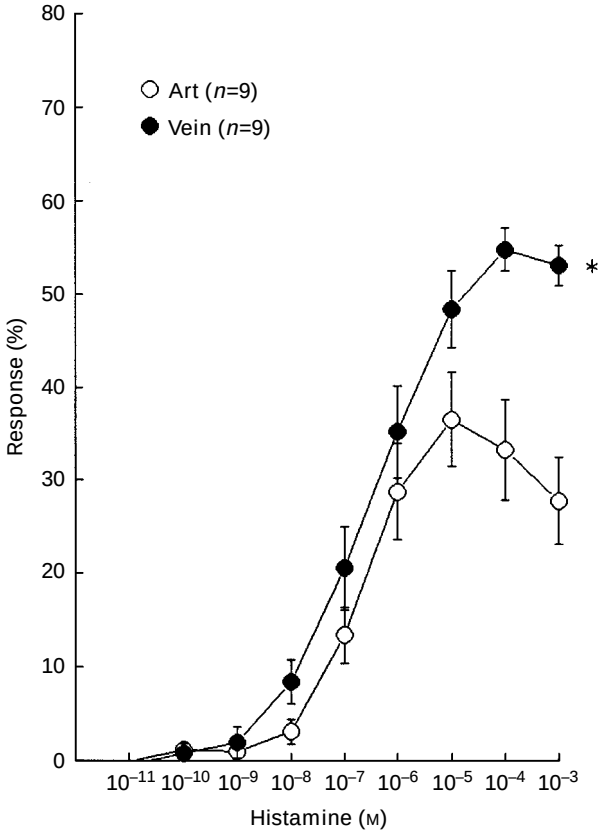

Figure 1 Cumulative concentration-responses of pulmonary arteries and veins to histamine. In arteries (Art), histamine (Hist) caused concentration-dependent constriction at concentrations up to 10^{-5} M and dose-dependent relaxation 10^{-5} M to 10^{-3} M. Veins constricted more than arteries for the whole curve and at 10^{-5} to 10^{-3} M. $*P<0.05$ compared with arteries. *n*, number of animals.

Results

Cumulative concentration-responses to histamine, 5-HT and KCl

These results are plotted in Figures 1, 2 and 3. Histamine and 5-HT produced concentration-dependent contraction of arteries and veins. Although the shapes of the curves for the responses to histamine and 5-HT were similar, 5-HT was approximately 100 times more potent than histamine. The most important observation was that for both drugs, the veins constricted significantly more ($P<0.01$) than the arteries. The second observation was that contraction of the arteries were reversed at the higher concentrations, a finding essentially absent or very inconspicuous in the veins. This occurred at concentrations above 10^{-5} M for histamine and 10^{-7} M for 5-HT. In contrast, KCl (4 to 60 mM) caused a concentration-dependent contraction of similar degree in arteries and veins (Figure 3).

Effects of chlorpheniramine, cimetidine, L-NOARG and indomethacin on responses to histamine

The effects of these four drugs on the concentration-responses to histamine are shown in Figure 4. At 10^{-4} M, the H_1 receptor antagonist chlorpheniramine abolished histamine-induced contraction of both arteries and veins. Cimetidine, the histamine H_2 receptor antagonist, not only significantly enhanced the sensitivity (pD_2 values) and the maximal responses of arteries and veins ($P<0.05$), but also abolished the differences between their maximal responses (Table 1). L-NOARG, the NO inhibitor, significantly increased the maximal responses of the arteries and veins to histamine without affecting the sensitivity. However, the increase in the maximal response was greater in the veins than in the arteries (Table 1). In contrast, indomethacin, a cyclo-oxygenase inhibitor, did not affect either the sensitivity or the maximal response to histamine.

Effects of ketanserin, L-NOARG and indomethacin on responses to 5-HT

The effects of ketanserin, L-NOARG and indomethacin on the concentration-responses to 5-HT are shown in Figure 5. At 10^{-4} M, ketanserin prevented contraction of the arteries and markedly reduced it in veins. L-NOARG significantly potentiated the maximal responses of the arteries but not of the veins, whereas indomethacin increased the maximal responses of the arteries but reduced them in the veins significantly ($P<0.05$, Table 1). Thus, both L-NOARG and indomethacin abolished the differences in the maximal responses to 5-HT between arteries and veins, and in addition, indomethacin diminished the differences between their pD_2 values (Table 1).

Histology and morphometry

Representative images of an artery and of a vein from the explants and the corresponding light photomicrographs from the histology are shown in Figure 6. Light microscopy revealed that the intrapulmonary arteries were muscular in type

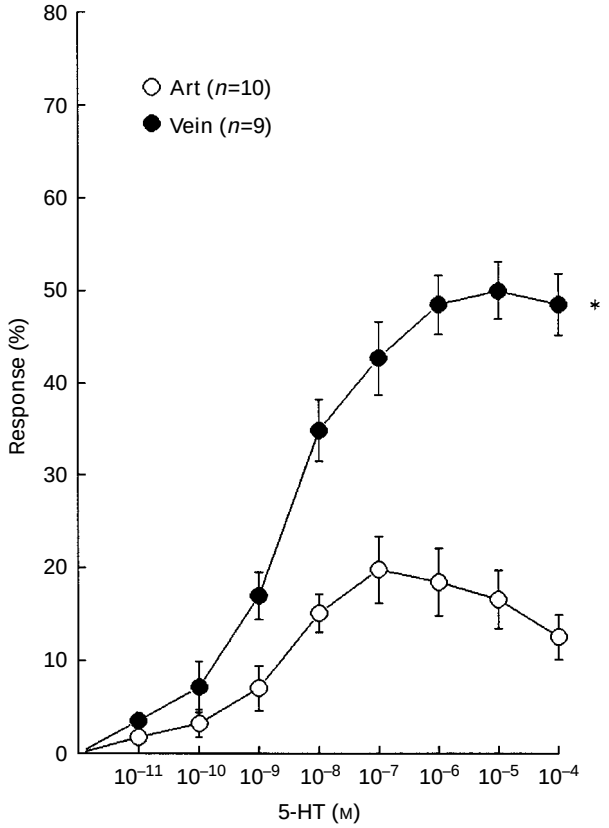

Figure 2 Cumulative concentration-responses of pulmonary arteries and veins to 5-HT. In arteries (Art), 5-HT induced a concentration-dependent constriction up to 10^{-7} M, and relaxation from 10^{-6} M to 10^{-4} M. Veins constricted more than arteries from 10^{-9} to 10^{-4} M and did not relax. *$P<0.05$ compared with arteries.

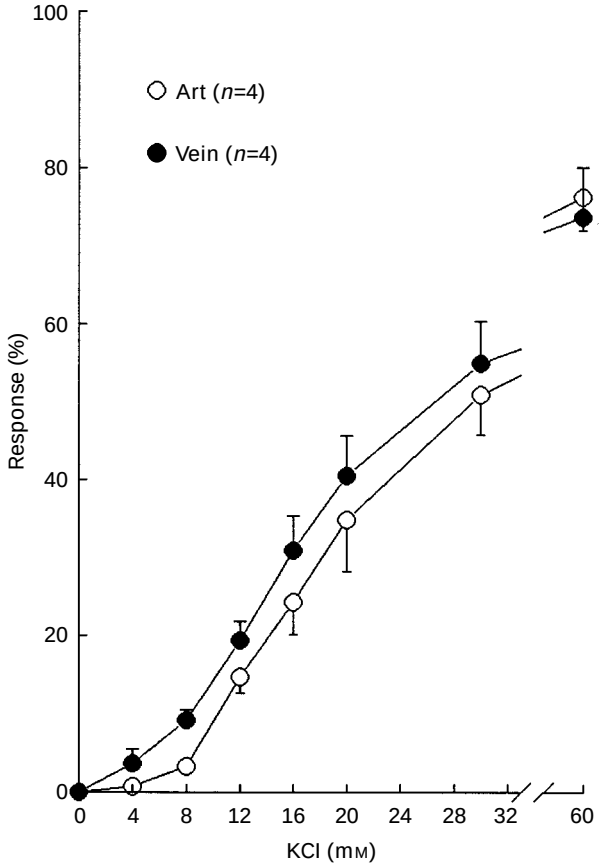

Figure 3 Cumulative concentration-responses of pulmonary arteries (Art) and veins to KCl. In both, KCl caused a similar concentration-dependent constriction ($P>0.05$).

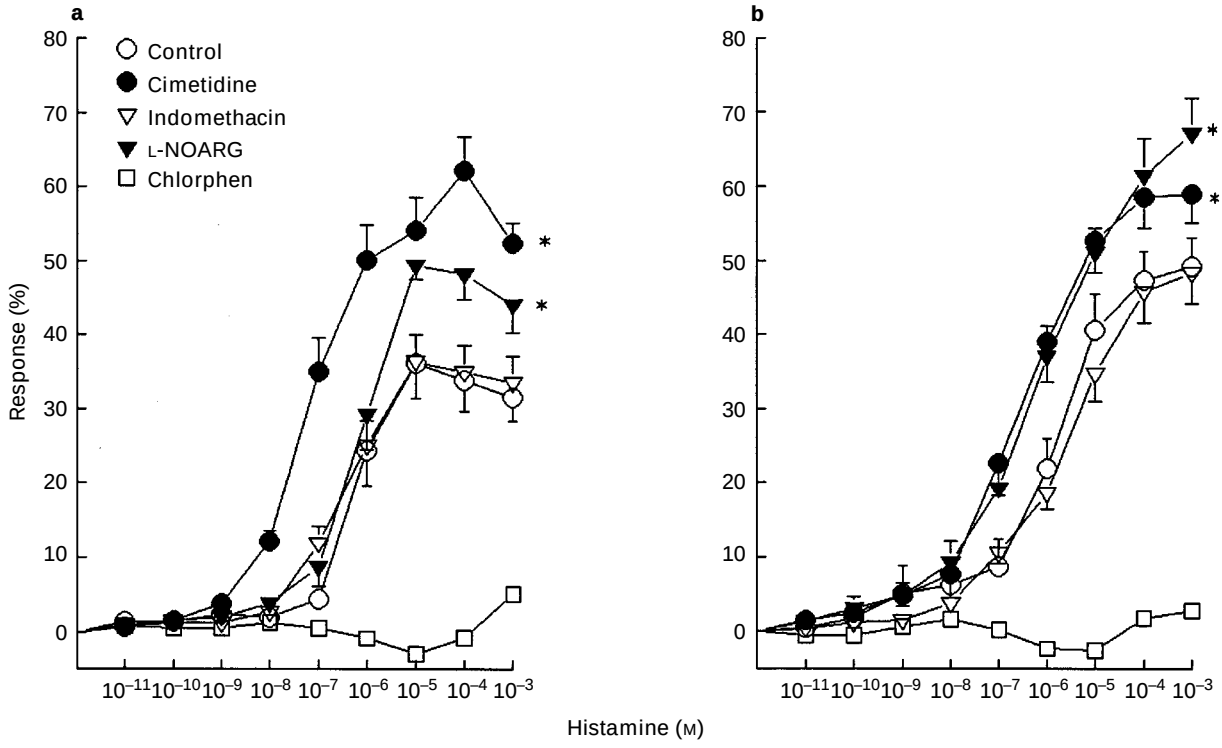

Figure 4 Cumulative concentration-responses of pulmonary arteries (a) and veins (b) to histamine after pretreatment with chlorpheniramine, cimetidine, indomethacin or L-NOARG. Results are means of seven guinea-pigs; vertical lines show s.e.mean. *$P<0.05$ compared with histamine alone. Chlorphen, chlorpheniramine.

Table 1 Effects of L-NOARG, indomethacin and cimetidine on maximal responses (R_{max}) and pD_2 values of pulmonary arteries and veins to histamine and 5-HT

	Arteries		*Veins*	
	R_{max} (%)	pD_2	R_{max} (%)	pD_2
Histamine alone	39.3±4.5	6.1±0.2	52.1±3.0^{+}	5.8±0.3
Cimetidine	65.6±6.3*	7.0±0.2*	60.1±5.8*	6.4±0.2*$^{+}$
L-NOARG	49.6±3.8*	6.2±0.1	67.2±4.8*$^{+}$	6.0±0.2
Indomethacin	39.5±6.7	6.1±0.2	48.7±4.2^{+}	5.8±0.1
5-HT alone	27.1±5.4	8.0±0.2	43.4±4.8^{+}	7.3±0.2^{+}
L-NOARG	37.9±4.9*	7.8±0.3	44.1±4.4	7.0±0.2^{+}
Indomethacin	34.6±5.6*	7.7±0.3	34.1±4.4*	7.4±0.2

Values are means±s.e. of six or seven guinea-pigs. *$P<0.005$ vs histamine or 5-HT alone, and $^{+}P<0.05$ arteries vs veins within the same treatment group.

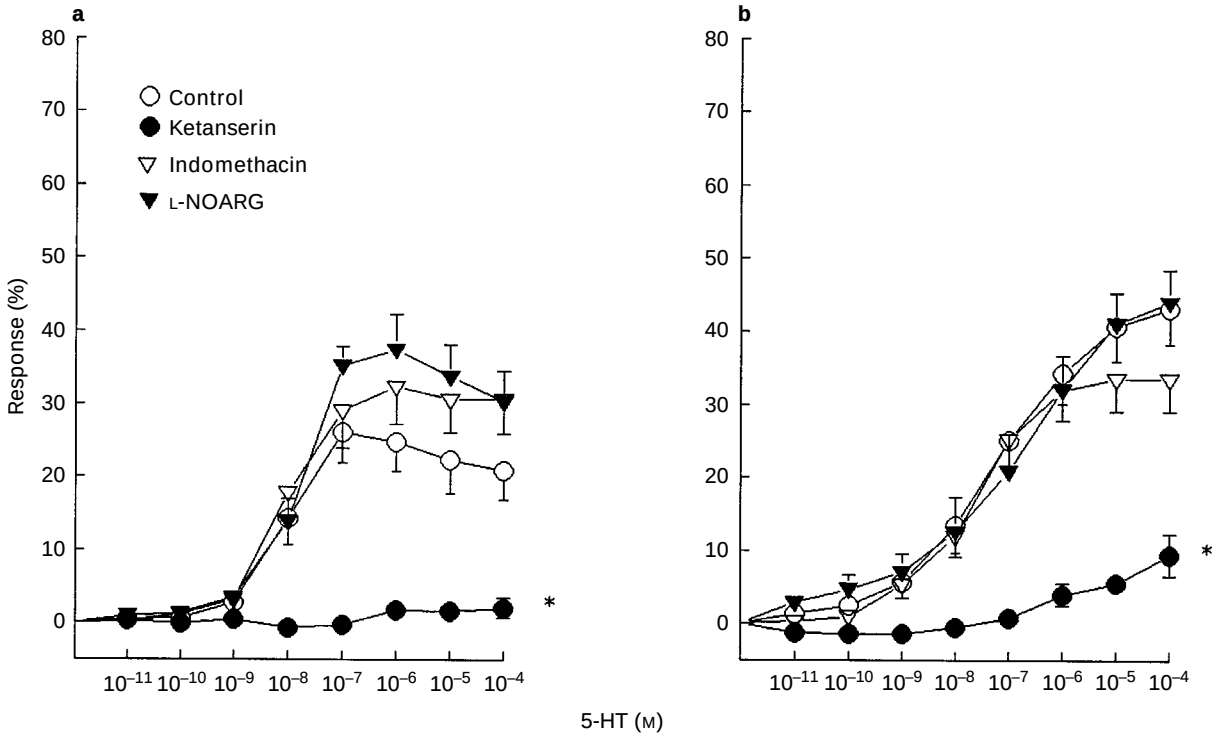

Figure 5 Cumulative concentration-responses of pulmonary arteries (a) and veins (b) to 5-HT after pretreatment with indomethacin, ketanserin or L-NOARG. Results are means of six guinea-pigs; vertical lines show s.e.mean. *$P<0.05$ compared with 5-HT alone.

(Figure 6c), and that they had a thick and complete inner elastic lamina, a media composed of compact smooth muscle cells with irregular elastic fibres in the larger arteries, and a thin attenuated external elastic lamina often seen only with Van Gieson's elastic stain. The veins were also muscular in type but differed from the arteries since their media was thinner, and their internal elastic lamina was also much thinner or frequently absent; in addition, there was no external elastic lamina (Figure 6f). The two veins that had cardiac muscle in their walls were excluded from the analyses. The morphometric measurements revealed that the inside and outside diameters and medial thickness of arteries were greater than those of veins (Table 2, $P<0.05$). The immunostaining for the von Willebrand factor confirmed that the endothelium was preserved in arteries and veins.

Discussion

In the present study, we used lung explants to examine *in vitro* the responses of intrapulmonary arteries and veins to histamine and 5-HT, and to test potential determinants for the differential responses. The principal findings were: (1) histamine and 5-HT both produced greater constriction of the pulmonary veins than of the arteries; (2) both cimetidine and L-NOARG enhanced responses of arteries and veins to histamine, but only cimetidine abolished the differences between their responses; (3) L-NOARG potentiated the maximal responses of arteries but not of veins to 5-HT, and indomethacin increased the maximal responses of arteries and reduced them in veins; (4) responses of arteries and veins to KCl were similar; (5) by morphometric analysis, the diameters and medial thickness of the arteries were significantly greater than those of the veins.

Lung explants provide a convenient means to assess directly intraparenchymal vascular constriction or dilatation, and permit the generation of concentration-response curves. In this preparation, pulmonary arteries and veins are within the framework of an intact and supporting parenchyma as *in vivo*; their lumina remain open and are nearly circular, most likely due to their low baseline tone and to the preload provided by the stretch from the surrounding parenchyma that is filled

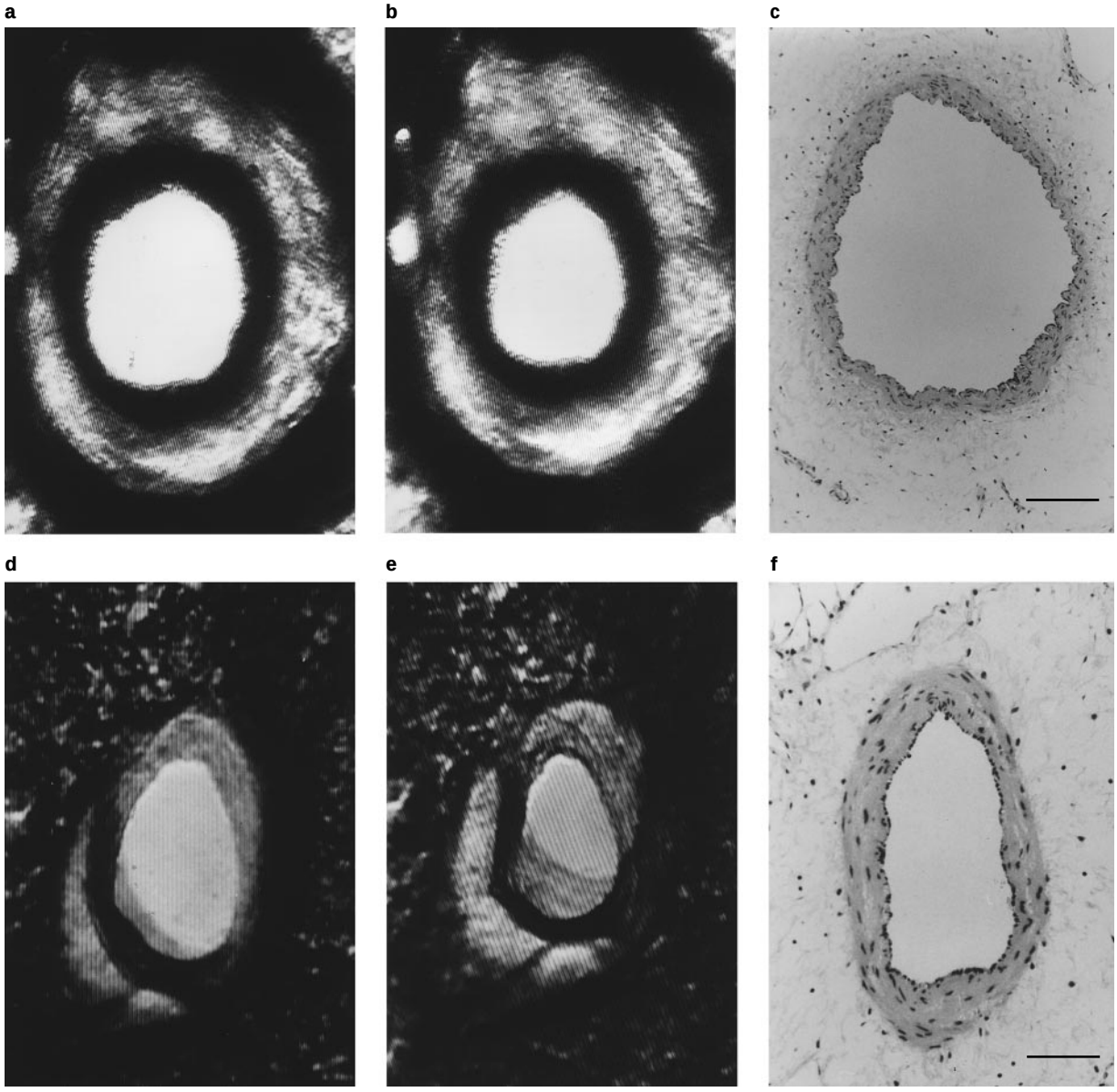

Figure 6 Video images and light microscopy of an artery (a–c) and a vein (d–f). Video images of an artery at baseline (a) and after addition of 10^{-7} M 5-HT (b). Light photomicrograph of the same artery (c). Video images of a vein at baseline (d) and after addition of 10^{-7} M histamine (e). Light photomicrograph of the same vein (f). Scales bars = (c and f) Haematoxylin and eosin staining; scale bar = 0.15 mm.

Table 2 Baseline video image diameter and histological measurements in pulmonary vessels of guinea-pigs

	Baseline video image i.d.	*i.d.*	*o.d.*	*m.t.*
Arteries	427±23*	406±21*	522±23*	56±3*
Veins	288±21	228±17	323±18	47±3

Values, in μm, are means±s.e. of 13 guinea-pigs. *$P<0.05$ versus veins. Only those vessels used to study responses to histamine and 5-HT and amenable to histological measurements were included. i.d.: inside diameter, o.d.: outside diameter; m.t.: medial thickness, o.d. = i.d. + 2 m.t.

through the airways with agarose. Although the control of vascular tension and preload are perhaps not as precise as in vascular strip or ring preparations, we believe this is offset by the provision of the aforementioned stretch of the parenchymal distension that is closer to the *in vivo* situation. The afterload to the vessels is also presumably provided by the surrounding parenchyma. Because of the likelihood that the load from the parenchyma remains constant, the contraction of the vessels in the lung explants probably resembles most closely isotonic rather than isometric contraction *in vitro* (Paton, 1975) and is supported by the fact we are measuring a reduction in vessel size.

In the explants, airways, arteries and veins were readily visualized and differentiated (Dandurand *et al*., 1993): airways were identified by their thin walls and beating cilia, whereas arteries were distinguished from veins by their wall structure and position within the acinus. Their identities could be confirmed by histological examination.

The major difference that we observed between arteries and veins was a significantly greater constriction of the veins to histamine and to 5-HT in the concentration-responses (Figures 1 and 2). In isolated perfused lungs of the guinea-pig, Bradley *et al*. (1993) also found that veins responded more than arteries to histamine and 5-HT, and they attributed this partly to the greater amount of smooth muscle in the veins, based on

responses to KCl. Indeed, since KCl contracts vessels by depolarizing the smooth muscle cell membrane independent of specific receptors (Karaki & Weiss, 1988) or of the endothelium (Kimura *et al*., 1992; Holecyova *et al*, 1993), the degree of contraction it produces may well depend on smooth muscle mass. However, our findings (Table 2) that the mean smooth muscle thickness of the arteries was greater than that of the veins, and that the arteries and veins contracted equally in response to KCl (Figure 3), appear to contradict the conclusions of Bradley *et al*. (1993); part of the difference may be due to the variable structure of the arterial media with its alternating thick and thin areas, compared with the venous media with nearly even thickness, which may contract more effectively (McLaughlin *et al*., 1966; Ferencz, 1969). An even more plausible explanation for the apparent discrepancy between the data of Bradley *et al*. (1993) and ours is that they measured changes in resistance that, in accordance with Poiseuille's equation, varies with the inverse fourth power of radius. We found that the veins that we studied had a similar diameter than the arteries (Table 2), so that the resistance of the former would increase more with the same % muscle contraction. Thus, our findings that the mean smooth muscle thickness of the arteries was greater than that of the veins, and the similar magnitude of contraction with KCl seem to exclude a significant role of smooth muscle mass in the greater venous responses to histamine and 5-HT.

Our findings that arteries and veins differed in their responses to histamine and 5-HT but not to KCl suggest that differences in receptor densities or in the production of endothelium-derived vasoactive substances are responsible for their differential contractile responses to these two amines (Karaki & Weiss, 1988; Kimura *et al*., 1992). For histamine, there is considerable evidence for a dual receptor mechanism in both pulmonary and systemic vessels (Turker, 1973; Abacioglu *et al*., 1987; Toda, 1990). Histamine contracts vessels via H_1 receptors on smooth muscle and relaxes them via H_1 receptors on endothelial cells and H_2 receptors on smooth muscle (Toda, 1990). We found this dual effect of histamine clearly demonstrated in guinea-pig pulmonary vessels, since the H_1 receptor antagonist blocked the contraction and the H_2 receptor antagonist significantly augmented it. In isolated perfused lungs of guinea-pigs, Turker (1973) also showed that H_2 receptor antagonists increased the histamine-induced pressor response. Our results extend these studies by suggesting that the role of H_2 receptors was more prominent in arteries than in veins since cimetidine diminished the differences in their maximal contractile responses to histamine (Table 1).

Our data also indicate that NO synthase inhibition significantly enhanced the contractile responses of pulmonary arteries and veins to histamine, but that cyclo-oxygenase inhibition had no effect, suggesting that NO rather than PGI_2 was released to modulate the responses to histamine in these tissues. These results agree with previous findings in guinea-pig pulmonary arteries that histamine-induced relaxation is abolished by NO synthase inhibitors but not by indomethacin (Sakuma *et al*., 1988) and extend them by showing that inhibition by NO of the contractile response to histamine was more prominent in veins than arteries, since the concentration-response curve was shifted about one log interval leftward only in the veins, and that the difference in the maximal contraction of arteries and veins was increased.

Although multiple 5-HT receptor subtypes exist (Hoyer *et al*., 1994), our results suggest that the contraction of pulmonary vessels of guinea-pigs with 5-HT is primarily mediated by 5-HT_2 receptors, since ketanserin abolished contraction in arteries and markedly reduced it in veins. This finding is consistent with previous observations in perfused lungs of guinea-pigs and cats, and in pulmonary arterial rings of rabbits (Selig *et al*., 1988; Neely *et al*., 1993; el-Kashef, 1996). The partial return of the contraction of the veins at the high concentrations of 5-HT, despite the presence of ketanserin (Figure 5), could be due to the production of contractile cyclo-oxygenase substances. Supporting evidence for this argument is that indomethacin suppressed 5-HT-induced maximal contractions (Table 1); furthermore, in a separate study, we found that acetylcholine also induced the release of contractile cyclo-oxygenase products in the pulmonary vessels of guinea-pigs (Shi *et al*, 1997).

Unlike their effects on the responses to histamine, L-NOARG and indomethacin increased the maximal responses of arteries to 5-HT, whereas they either had no effect on the veins or even reduced their maximal responses. These findings suggest that in arteries, NO and vasodilator cyclo-oxygenase substances were responsible for their reduced responses to 5-HT, and that these mediators account for the differences in contractility to this amine between arteries and veins. Thus in the latter, NO did not modulate the contractile response to 5-HT and vasoconstrictor cyclo-oxygenase products appeared to contribute partially to their greater contraction. An increase in contraction to 5-HT after inhibition of NO synthase and cyclo-oxygenase activity has been demonstrated in pulmonary arteries of dogs and rabbits (Hofman *et al*., 1991; el-Kashef, 1996). However, the involvement of NO and the cyclo-oxygenase pathways in differential responses to 5-HT between pulmonary arteries and veins has not been demonstrated, and may account for their differential contractile responses, since both L-NOARG and indomethacin abolished differences in the maximal response and the latter also abolished differences in the pD_2 values.

The reduced contraction of the arteries to histamine and to 5-HT compared with the veins may also be partly explained by the waning of the response at higher concentrations, because the contraction of the veins was maintained. The mechanisms for this phenomenon are unclear. Cushing and Cohen (1992) also showed that high concentrations of 5-HT (above 10^{-7} M) produced concentration-dependent relaxation of canine coronary arteries devoid of endothelium, although the receptors responsible have not been characterized.

Our histological studies enabled us to confirm the identities of the vessels that we had studied pharmacologically and to measure them. There are few descriptions of guinea-pig pulmonary vessels in the literature (McLaughlin *et al*., 1966; Ferencz, 1969) and these indicate that they are similar to those of rats. The principal characteristics are that the arteries have a variable muscle thickness (Ferencz, 1969) (Figure 6), nevertheless thicker than the veins (Table 2). Although this property should confer upon them the ability to contract more, we observed the opposite. The veins have a thinner but more regular media and the large veins also have cardiac muscle in their walls (Ferencz, 1969), although it does not extend as far distally as in the rat (Best & Heath, 1961); the veins containing cardiac muscle were excluded from our pharmacological or morphological data. The effect of cardiac muscle on reactivity is not clear. Cheung (1981) found in guinea-pigs that pulmonary venous smooth muscle was electrically quiescent, and that electrical stimulation elicited action potentials in cardiac muscle, but not in smooth muscle. Therefore, it is likely that the two types of muscle respond differently to pharmacological stimuli as well, and that a study of intrapulmonary veins should not *a priori* include those with cardiac muscle in their wall.

In conclusion, we have demonstrated in lung explants of guinea-pig that pulmonary veins contract more than arteries to

histamine and 5-HT, and that receptors mediating vasodilatation and endothelial modulation appear to contribute more to their differential contractile responses to these amines than structure. Furthermore, the differences are drug-specific: for histamine, the differences between arteries and veins are related to H_2 receptors on smooth muscle, whereas for 5-HT, they are due to NO and dilator prostaglandins.

Pulmonary veins have long been considered as conduit vessels with little reactivity. In fact, they contribute significantly to total pulmonary vascular resistance (Hakim *et al*., 1982). Moreover, recent studies indicate that in several species, pulmonary veins exhibit equal or even greater reactivity than arteries in response to a variety of stimuli: for example, the pulmonary veins of rats contract more than arteries during hypoxia (Zhao *et al*., 1993), ferret pulmonary veins react more to platelet-activating factor (Gao *et al*., 1995), and in most species, pulmonary veins contract more to endothelin (Levin, 1995). These studies, together with our data, suggest that pulmonary veins respond prominently to a number of constrictors, which could lead to an increase in microvascular pressure, contributing to the formation of pulmonary oedema under pathological conditions.

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