Differential relaxant responses of pulmonary arteries and veins in lung explants of guinea pigs

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Shi, Weibin, David H. Eidelman, and René P. Michel. Differential relaxant responses of pulmonary arteries and veins in lung explants of guinea pigs. J. Appl. Physiol. 83(5): 1476–1481, 1997.—The endothelium regulates vascular tone through release of relaxing or contracting factors, with nitric oxide (NO) being a major endothelium-derived relaxing factor. In the present study, we used a lung explant technique to determine the differential abilities and mechanisms of pulmonary arteries and veins of normal guinea pigs to relax after pharmacological interventions. Excised lungs of 15 guinea pigs were filled through the airways with 1% agarose, cut into 1-mm-thick slices, and cultured overnight. Luminal areas of vascular cross sections were measured with an image-analysis system. Vessels were precontracted with U-46619, and responses to histamine, acetylcholine (ACh), sodium nitroprusside, and papaverine were examined. We also determined the effects of Nω-nitro-L-arginine and of indomethacin on ACh-induced responses. We found that histamine relaxed arteries more than veins and that ACh relaxed only arteries. Nω-nitro-L-arginine pretreatment abolished ACh-induced relaxation of arteries and caused ACh-induced contraction of veins, whereas indomethacin markedly augmented ACh-induced relaxation of arteries (maximal relaxation: 48.5 ± 4.7% vs. 19.2 ± 5.1% without it) and induced a dose-dependent relaxation of veins (maximal relaxation: 17.0 ± 4.1%). Sodium nitroprusside induced significantly greater relaxation of arteries than veins, whereas papaverine relaxed them equally. We conclude that in guinea pigs endothelial NO-mediated relaxation is greater in pulmonary arteries than in veins and that ACh-induced NO-mediated relaxation is reduced by the simultaneous release of relaxing or contracting factors, with nitric oxide (NO), a chemically unstable radical, being a major endothelium-derived relaxing factor synthesized from l-arginine (13). Acetylcholine (ACh), histamine, bradykinin, and other agents dilate vascular smooth muscle by stimulating the endothelium to release NO (13, 24). In contrast, nitrogen-containing vasodilators, such as sodium nitroprusside (SNP), act on smooth muscle directly by release of NO (22). In the pulmonary vasculature, the endothelium-derived NO-mediated relaxation responds to these factors and mechanisms, we opted for an in vitro lung-explant technique in guinea pigs. The method was previously used to study airway constriction in the rat (9). 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.

METHODS

Preparation of the lung explants. The procedure was slightly modified from that previously described for airways (9). A total of 15 adult male Hartley strain guinea pigs weighing 556 ± 62 g (mean ± SE) were used for these studies. All the animals were anesthetized with pentobarbital sodium (40 mg/kg ip), heparinized through the dorsal vein of the penis (3,000 U/kg), and intubated through a tracheostomy with sterile polyethylene tubing 9 cm long and 1.9 mm in diameter. The anterior chest wall and upper abdomen were sterilized with 70% ethanol. The abdomen was opened, and the animals were exsanguinated by cutting the abdominal aorta. After removal of the anterior chest wall, the right ventricle was punctured, and a cannula was advanced into the main pulmonary artery. The pulmonary vessels were washed in situ with 10 ml Ringer lactate containing 20 U/ml heparin. The heart and lungs were excised en bloc, and the lungs were inflated to near-total lung capacity with 1% agarose in bicarbonate-buffered culture medium (48 ml/kg body weight) at 37°C, prepared as described previously (9). 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 from which the needle end had been removed, and embedded in 4% agarose in bicarbonate-buffered minimum essential medium at 37°C (9). After 30 min at 4°C, the lung-agarose block was sectioned with a hand-held microtome blade into 0.5- to 1.0-mm-thick transverse slices. These slices were examined with an inverted microscope (IMT-2; Olympus, Tokyo, Japan). 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 bicarbonate-buffered culture medium and incubated overnight at 37°C in 5% CO₂-95% air.

Image acquisition. The culture dish inserts containing the lung explants were transferred to six-well plates containing 2 ml of N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid-buffered culture medium (HCM) (9) and placed on the stage of an inverted microscope (LH50A, Olympus). Arteries and veins were identified and imaged with a video camera (CDS; Sony, Nagano, Japan), and images were recorded with a video disk recorder (TQ2026F; Panasonic, Osaka, J. apam). 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 had a thick media and their inner lining was slightly wrinkled, whereas veins were thinner and wrinkles were inconspicuous.

Experimental protocol. First, in all explants, baseline images of the vessels were generated. Then they were precontracted with 1 or 3 \( \times 10^{-6} \) M 9,11-di-deoxy-11n,9n-epoxymethanoprostaglandin F\(_{2\alpha}\) (U-46619), the thromboxane A\(_2\) analog, added directly to the surface of the lung explants. Images were gathered every 10 s for the first minute, then every minute for another 4 min. Thereafter they were followed for a further 15 min to ensure stable contraction, for a total of 20 min. To test the dilator responses of these precontracted vessels, cumulative dose-response curves were constructed by adding histamine solution in half-log unit intervals from 10\(^{-11}\) to 10\(^{-7}\) M and by adding ACh, SNP, and papaverine solutions in one-log unit intervals from 10\(^{-11}\) to 10\(^{-4}\) M. In addition, to determine the differential roles of the NO and of the prostaglandin pathways, some vessels were preincubated with N\(^\bullet\)nitro-L-arginine benzy1 ester (l-NNA, 10\(^{-4}\) M) or with indomethacin (10\(^{-5}\) M) for 30 min before generating dose-response curves to ACh. In addition, as a control, we tested the effects of the l-NNA and of indomethacin on the vessels in their baseline state without preconstriction. In each explant, for the responses over time, we studied one vessel, whereas for the dose responses, we usually observed one artery and/or one vein and, in a few instances, two veins. We studied a total of 75 arteries and 88 veins from 123 explants. The numbers of animals used in each step of the protocol are indicated in Figs. 1-6.

Image and data analysis. The stored images were digitized by using an 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 (RS5000; IBM, Armonk, NY), and measurements of luminal area were made with Galileo Image Processing Software (Inspiraplex, Montreal, QC, Canada). The contractile responses of arteries or veins to U-46619 were calculated as a percentage of complete vessel closure using the equation:

\[
\text{Response} = \frac{1 - (\text{residual area after drug/baseline area})}{\text{baseline area}} \times 100
\]

Thus a 100% response indicated complete vessel lumenal closure and 0% indicated no effect.

The responses to ACh, histamine, SNP, or papaverine were expressed as a percentage of vessel preconstriction induced by U-46619 by using the equation:

\[
\text{Response} = \frac{(\text{area before dilator} - \text{area after dilator})}{(\text{baseline area} - \text{area before dilator})} \times 100
\]

Here -100% indicates a return to baseline state (i.e., before preconstriction) and 0% indicates full persistence of the precontracted state.

From these responses, time course and dose-response curves of arteries and veins were constructed by plotting the mean values against time and concentrations, respectively. The 50% effective concentration values were determined for each vessel and expressed as negative log molar (pD\(_2\)) values.

Drugs. All drugs were purchased from Sigma Chemical, St. Louis, MO. Histamine (dihydrochloride), ACh (chloride), SNP, papaverine (hydrochloride), and l-NNA (benzyl ester) were prepared as stock solutions in HCM from which dilutions were prepared fresh daily. Indomethacin was dissolved in ethanol and then diluted with HCM. For U-46619, the stock solution at a concentration of 10\(^{-4}\) or 3 \( \times 10^{-4}\) M was used directly. All drugs were added to the explants in the culture wells in 20-μl volumes, and their concentrations were expressed as values after dilution of the 20 μl by the 2 ml of medium (i.e., 100-fold).

Statistical analysis. Data are presented as means ± SE, with n being the number of animals from which the vessels were obtained, and with which all statistical analyses were done. To compare the curves of the dose responses and of the responses over time between arteries and veins or between control and treated groups from the same type of vessels, two-way analysis of variance was used. If the F-value was significant, the Tukey test for unpaired observations or Student’s paired t-test for paired observations was applied to ascertain significance at each concentration or time point. The comparison of maximal responses or pD\(_2\) values was performed by two-way block analysis of variance, with Student’s paired t-test or the Tukey test as post hoc tests. All the analyses were performed by using proprietary software (Systat, Evanston, IL). Differences were considered statistically significant at P < 0.05.

RESULTS

Responses to U-46619. Figure 1 shows the contractile responses to 10\(^{-6}\) M U-46619. The veins contracted rapidly and attained a plateau at 20 s that lasted ~120 s, followed by a slowly increasing contraction.
The arteries reached their peak of contraction at the same time as the veins. However, the artery responses waned substantially up to 120 s and thereafter slowly increased their contraction. Overall, the veins constricted to a greater degree than the arteries (P < 0.01). By 20 min, both arteries and veins reached a plateau of contraction, greater in the latter (P < 0.05).

Responses to histamine. In the arteries, after precontraction with U-46619, histamine (10^{-11} to 10^{-7} M) produced dose-dependent relaxation in arteries and veins, significantly greater in the arteries (Fig. 2). At 10^{-7} M, histamine started to contract the arteries and veins.

Responses to ACh and effects of L-NNA and indomethacin. In precontracted arteries, ACh caused a dose-dependent relaxation (Fig. 3), with maximal responses of 19.2 ± 5.1% and pD_2 values of 8.1 ± 0.7 (Table 1). In the arteries pretreated with L-NNA, ACh caused further constriction instead of relaxation. Indomethacin, however, markedly augmented ACh-induced relaxation (Fig. 3 and Table 1). In precontracted veins, ACh had no significant effect (P > 0.05), although it caused a slight contraction at 10^{-5} and 10^{-4} M (Fig. 4). In veins pretreated with L-NNA, however, ACh induced constriction, whereas after indomethacin, it produced a dose-dependent relaxation, with a maximal relaxation response of −17.0 ± 4.1% (Fig. 4 and Table 1).

Neither L-NNA nor indomethacin alone had significant effects on the baseline luminal areas of arteries (−1.6 ± 0.6 and 3.4 ± 0.8%, respectively) or of veins (−1.3 ± 1.0 and −1.9 ± 0.5%, respectively).

Responses to SNP and papaverine. SNP produced dose-dependent relaxation in both arteries and veins, significantly greater (P < 0.05) in the arteries between 10^{-6} and 10^{-4} M (Fig. 5). The pD_2 values were also significantly greater (P < 0.05) for arteries than veins (Table 1). Papaverine also caused relaxation of both arteries and veins, the extent of which did not differ significantly between either vessel types for the whole curves or in the maximal relaxation responses (P > 0.05; Table 1, Fig. 6).

DISCUSSION

In the present study, we examined the differential responses of intrapulmonary arteries and veins to ACh, histamine, SNP, and papaverine after precontraction with U-46619. We found that 1) ACh relaxed arteries but had no significant effects on veins, and the effect in the former was mediated by NO, not by dilator prostaglandins; 2) histamine and SNP relaxed arteries more than veins; and 3) papaverine relaxed arteries and veins equally.

The lung explant technique has been successfully used to study constriction of the airways in rats (9). Like the airways, the pulmonary arteries and veins in this preparation have a nearly circular cross section within the framework of an intact and supporting

Table 1. Maximal relaxation responses and pD_2 values to ACh, SNP, and papaverine of pulmonary arteries and veins in guinea pigs

<table>
<thead>
<tr>
<th></th>
<th>Maximal Relaxation Responses, %</th>
<th>pD_2</th>
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<tbody>
<tr>
<td></td>
<td>Arteries</td>
<td>Veins</td>
</tr>
<tr>
<td>Histamine</td>
<td>12.3 ± 1.4*</td>
<td>5.7 ± 1.6</td>
</tr>
<tr>
<td>ACh</td>
<td>19.2 ± 5.1</td>
<td>8.1 ± 0.7</td>
</tr>
<tr>
<td>ACh + Indomethacin</td>
<td>48.5 ± 4.7†</td>
<td>17.0 ± 4.1</td>
</tr>
<tr>
<td>ACh + L-NNA</td>
<td>Contraction</td>
<td>Contraction</td>
</tr>
<tr>
<td>SNP</td>
<td>69.7 ± 3.1*</td>
<td>40.2 ± 5.2</td>
</tr>
<tr>
<td>Papaverine</td>
<td>46.9 ± 5.3</td>
<td>33.2 ± 7.7</td>
</tr>
</tbody>
</table>

Values are means ± SE of 4–6 animals. pD_2, negative log molar of dose producing 50% of maximal response; ACh, acetylcholine; L-NNA, N^ω-nitro-L-arginine; SNP, sodium nitroprusside. *P < 0.05 arteries vs. veins; †P < 0.05 compared with ACh alone.
parenchyma, just as they do in vivo. The reasons the pulmonary vessels remain open and nearly circular despite the absence of intraluminal pressure are most likely their low baseline tone and the preload provided by the stretch from the surrounding parenchyma filled through the airways with agarose. In preliminary experiments in rats, we also perfused the vessels with agarose to increase their preload and, although we found an increased contractile response to 5-hydroxytryptamine, qualitative differences between arteries and veins were unchanged. Thus, in the present study, we opted not to perfuse the vessels with agarose because of the possibility that we might impair drug access to the endothelium or that we might damage the endothelium.

In the pulmonary vessels of adult guinea pigs, we found that neither L-NNA nor indomethacin had much effect on baseline vascular areas. This indicates that neither NO nor prostacyclin modulates its baseline vascular tone significantly. In rats and dogs, NO synthase inhibitors have also been found to be without significant effects on pulmonary vascular tone under baseline conditions (12, 20). In mammalian systemic resistance vessels and in adult ovine pulmonary veins, however, inhibition of basal NO production does induce contraction (4, 29). Satoh and Inui (26) first reported that histamine induced endothelium-dependent relaxation in guinea pig pulmonary arteries. Abacioglu et al. (1) found that H₂ receptors on smooth muscle contributed to histamine-induced relaxation, even though their effect was much weaker than that produced by H₁ receptors on the endothelium. A subsequent study indicated that NO was the mediator responsible for histamine-induced endothelium-dependent relaxation in guinea pig pulmonary arteries (24). Our results extend these studies by showing that histamine also relaxed pulmonary veins, although significantly less than arteries. In a separate study in lung explants, we found that L-NNA, but not indomethacin, potentiated the contractile responses of pulmonary arteries and veins to histamine (27). Thus the histamine-induced relaxation in guinea pig pulmonary veins was probably also primarily mediated by NO, not by prostaglandin I₂. Moreover, in the present study, we found that at higher concentrations (>10⁻⁷ M), histamine contracted pulmonary arteries and veins (Fig. 2). This observation is in accordance with the findings of Abacioglu et al. (1) in main pulmonary artery strips of guinea pigs.

Fig. 4. Cumulative dose-responses of pulmonary veins to ACh after precontraction with U-46619; n, no. of animals. ACh failed to induce relaxation. In veins pretreated with indomethacin, ACh induced dose-dependent relaxation, whereas in those pretreated with L-NNA, ACh induced significant contraction. *P < 0.05 vs. ACh only.

Fig. 5. Responses of pulmonary arteries and veins to sodium nitroprusside (SNP) after precontraction with U-46619; n, no. of animals. SNP relaxed arteries more than veins at concentrations from 10⁻⁸ to 10⁻⁴ M. *P < 0.05 vs. veins.

Fig. 6. Responses of pulmonary arteries and veins to papaverine after precontraction with U-46619; n, no. of animals. Papaverine relaxed arteries and veins equally.
It has been suggested that ACh produces endothelium-dependent relaxation of pulmonary arteries in newborn and adult guinea pigs (10, 24, 25). The mediators involved in this relaxation, however, have not been completely elucidated. Sakuma et al. (24) reported that the NO synthase inhibitor Nω-monomethylarginine antagonized only 64% of ACh-induced relaxation. In the present study, however, we found that it was completely abolished by the NO inhibitor L-NNA. One explanation for the discrepancy between the data of Sakuma et al. and ours is that the different analogs of L-arginine could affect endothelium-dependent relaxation differentially (6). Another explanation may be that we used a higher concentration of this inhibitor and/or that responses of smaller intrapulmonary arteries differ from those of larger ones. Our findings that indomethacin potentiated the relaxation of arteries and caused relaxation of the veins with ACh were unexpected, and these results suggest that the relative contribution of vasoconstrictor cyclooxygenase products was greater than that of vasodilator cyclooxygenase products during the response. This contrasts with the results in dogs reported by Miller and Vanhoutte (21), who found that arachidonic acid relaxed pulmonary arteries but contracted veins and that these effects were abolished by inhibitors of the cyclooxygenase pathway and by denudation of the endothelium. Although ACh is a classic agonist of endothelium-dependent relaxation in most blood vessels, it fails to produce relaxation in some blood vessels (for example, in bovine pulmonary veins (16) and in newborn ovine pulmonary arteries (14)), even causing endothelium-dependent constriction. Contraction has also been reported in the pulmonary vessels of rabbits (2) and in coronary arteries of most species (18). Thromboxane A2 is the putative mediator, because constriction could be prevented by cyclooxygenase inhibitors, thromboxane A2 synthase inhibitors, and thromboxane A2 antagonists (2). In addition, our findings seem to exclude an important role for prostacyclin, another endothelium-derived relaxing factor, in the ACh-induced relaxation of guinea pig pulmonary arteries. Compared with the arteries in the present study, the pulmonary veins of guinea pigs showed a weaker relaxation to ACh, and the relaxation occurred only after inhibition of the cyclooxygenase pathway with indomethacin. This relaxation was probably also mediated by NO, because prostacyclin had been inhibited with indomethacin during the response and the NO synthase inhibitor L-NNA enhanced ACh-induced contraction in these veins. The weaker relaxant response of the veins could also be explained by the lower reactivity of their smooth muscle to NO or by a reduced ability of the endothelium to produce NO. We investigated this by using SNP, which acts like exogenous NO (22), and indeed found that the veins responded less to SNP than did the arteries. This finding is in agreement with previous findings in isolated perfused lungs of rats and pigs (7, 23), as well as in systemic vessels (17). The smaller relaxant response of the veins could be due in part to the greater precontraction to U-46619 (28). However, this is unlikely because papaverine, which increases cytosolic guanosine 3′,5′-cyclic monophosphate content by inhibiting the activity of phosphodies- terase independent of the endothelium and the NO pathway (3, 19), relaxed arteries and veins equally. Thus, after inhibition of the cyclooxygenase pathway, the differences between arteries and veins in response to ACh lie in the reduced responsiveness of the venous smooth muscle to NO, with a component of the reduced ability of the venous endothelium to release NO.

In conclusion, our data demonstrate that in guinea pigs, endothelial NO-mediated relaxation is greater in pulmonary arteries than in veins and that ACh-induced relaxation was reduced in the arteries and masked in the veins by constricting factors from the cyclooxygenase pathway. Differences in NO-mediated relaxation of pulmonary arteries and veins may also contribute to their differential contractile responses. Indeed, the data of Bradley et al. (5) in isolated perfused lungs and our own findings in lung explants (27) reveal that, in guinea pigs, pulmonary veins constriction more than arteries in response to histamine and serotonin. Because histamine and 5-hydroxytryptamine stimulate the release of endothelial NO, in addition to contracting vascular smooth muscle (13), the reduced release of NO by the venous endothelium and the diminished responsiveness of the venous smooth muscle to NO may produce a smaller relaxant effect to antagonize the vasoconstriction. Furthermore, the present study, together with others (21, 30), has indicated that endothelium-derived contracting factors contribute to the differential relaxant responses of arteries and veins. Because pulmonary veins are the major site of action of several vasoconstrictors (5, 15, 31), if NO-mediated relaxation in them is decreased and/or if they produce more contracting substances, an exaggerated increase in microvascular pressure could result, potentially contributing, for example, to the formation of pulmonary edema under pathological conditions.

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