Release of Vasodilator Prostaglandin, PGI\(_2\), from Isolated Rat Lung during Vasoconstriction

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SUMMARY Prostaglandins are generated by the lungs and released into the circulation. Since inhibitors of prostaglandin synthesis enhance hypoxic pulmonary vasoconstriction, we wondered whether vasodilator prostacyclin was synthesized by the lungs in response to vasoconstriction. To test this hypothesis, we measured vasoconstriction induced by angiotensin II in isolated rat lungs before and after inhibition of prostaglandin synthetase. We found that sodium meclofenamate enhanced and prostacyclin and its precursor arachidonate abolished pulmonary vasoconstriction. In lungs labeled with \(^{14}\)C-arachidonate, effluent radioactivity increased after angiotensin II-induced vasoconstriction. Hypoxic vasoconstriction, but not hypoxia per se, caused an increase in lung effluent radioactivity. Chromatographic analysis of lung effluent showed that 6-keto-prostaglandin F\(_\alpha\), was the major arachidonic acid metabolite released during pulmonary vasoconstriction. We concluded that prostacyclin is produced by the lungs in response to vasoconstriction. Hypoxia per se seems not to be the adequate stimulus for enhanced lung prostacyclin formation. Lung prostacyclin may protect the pulmonary circulation against excessive vasoconstriction. Circ Res 48: 207-213, 1981

THE LUNG has a large capacity for prostaglandin production (Gryglewski et al., 1978a, 1978b), but the biological role of the lung prostaglandins is not clear. One possible role is that the lung produces vasodilator prostaglandins to assist in maintaining a low resistance pulmonary circulation by opposing vasoconstrictor stimuli (Vane et al., 1974; Vaage et al., 1975; Weir et al., 1976).

In agreement with this concept are data showing that inhibition of prostaglandin synthesis augments pressor responses to hypoxia and other pulmonary vasoconstrictors (Weir et al., 1974; Vaage et al., 1975; Weir et al., 1976), including those released in canine experimental asthma (Cohn et al., 1978). Also, stimulation of prostaglandin synthesis in dogs given small amounts of endotoxin or of the prostaglandin precursor arachidonic acid causes pulmonary vasodilation (Reeves and Grover, 1974; Gerber et al., 1980). However, arachidonic metabolism can produce both constrictor and dilator prostaglandins, and in some species endotoxin and arachidonic acid cause pulmonary vasoconstriction (Reeves et al., 1972; Kadowitz et al., 1977). A recent, preliminary study (Gryglewski et al., 1980) indicated that angiotensin II released a substance from isolated guinea pig lungs that was characterized as prostacyclin by bioassay. However, one could not say if release of prostacyclin was specific for angiotensin II or was a phenomenon related to pulmonary vasoconstriction in general.

In this study we wished to examine whether pulmonary vasoconstriction per se stimulated prostaglandin synthesis in the lung and, if so, to determine which prostaglandins were synthesized.

Our results indicate that prostacyclin produced by the lung acts on lung vessels to maintain a low resistance in the pulmonary circulation and to oppose pulmonary vasoconstrictor stimuli.

Methods

Male Sprague-Dawley rats with an average weight of 350 ± 20 g (n = 42) were anesthetized with pentobarbital sodium (Nembutal) 30 mg/kg, ip. The lungs were isolated as described previously (Hauge, 1968; McMurry et al., 1976) and perfused with either rat blood, physiological salt solution, or red blood cells suspended in physiological salt solution. The lungs were ventilated with a gas mixture of either 21% O\(_2\), 5% CO\(_2\), and 74% N\(_2\) or, for the hypoxic challenges, 3% O\(_2\), 5% CO\(_2\), and 92% N\(_2\). The pulmonary arterial pressure was measured with a Statham transducer (P23AA) and recorded on a Gilson recorder.

Effects of Sodium Meclofenamate, Arachidonic Acid, and Prostaglandin I\(_2\) (PGI\(_2\)) on Pressor Responses in Blood-Perfused Lungs

Six lungs were perfused with homologous blood at a constant flow rate (0.03 ml/g body weight)
using a recirculating system. Following an equilibration period of 30 minutes, angiotensin II (Ciba or Sigma, 1 μg in 0.1 ml normal saline) was injected into the pulmonary artery and the pressor response (maximum increase in perfusion pressure minus baseline perfusion pressure) was measured. After addition of sodium meclofenamate 50 μg/ml (Parke-Davis) to the blood reservoir and an equilibration period of 20 minutes, the pulmonary pressor response to 1 μg angiotensin II was evaluated again.

Six different lungs served as time controls, and normal saline was added to the blood reservoir instead of meclofenamate. Because of the avid binding of meclofenamate by plasma protein, the relatively large meclofenamate concentration of 50 μg/ml was found in preliminary experiments to be necessary to inhibit the pulmonary vasodilation produced by an infusion of sodium arachidonate. In the absence of protein binding, e.g., in protein-free perfusate, meclofenamate is active in concentrations of 1 μg/ml.

In a second experiment, arachidonic acid (Nu-check) in a dose of 5 μg/ml was added to the perfusate of 10 different rat lungs during the 6th minute of a 10-minute hypoxic pressor response, and pulmonary artery pressure was monitored.

In four different blood-perfused lungs, prostaglandin I₂ was injected into the perfusion line to confirm that the reported vasodilation (Hyman and Kadowitz, 1979) was observed in our preparation. In two of the four lungs, 300 and 500 ng of PGI₂ were administered during the 6th minute of the 4th hypoxic pressor response (Pao₂ 33 and 35 mm Hg). In the other two lungs we gave 300 and 500 ng PGI₂, respectively, immediately after the peak of the 4th angiotensin II vasoconstriction.

**Effects of Angiotensin II-Induced Vasoconstriction on Release of Arachidonic Acid Metabolites from Lungs Perfused with Physiological Salt Solution**

To study prostaglandin production by the lung tissue, lungs were isolated from six rats and initially perfused with blood as described above. After responsiveness of the preparation to intraarterial angiotensin II injection and to a hypoxic challenge had been established, the blood perfusate was exchanged for a physiological salt solution (NaCl, 119 mM; KCl, 4.7 mM; MgSO₄, 1.17 mM; NaHCO₃, 17 mM; KH₂PO₄, 1.18 mM; dextrose, 5.5 mM; sucrose, 50 mM; CaCl₂, 1.16 mM) to which 4% Ficoll had been added. The perfusate was changed to a salt solution to allow binding of the prostaglandin precursor arachidonic acid to the lung tissue and avoid binding to plasma proteins and blood cells. This solution was gassed with 95% O₂, 5% CO₂ to give a pH of 7.4 at 37°C.

The perfusate recirculated for 10 minutes, after which the effluent was wasted to achieve a non-recirculating system for the remainder of the experiment. Then, over 15 minutes, 2 μCi of ¹⁴C-sodium arachidonate were infused into the pulmonary artery to label the endogenous arachidonic acid pool. Uptake of arachidonate was 96% as measured by arteriovenous difference (counts/min per ml) in the perfusate radioactivity. For the next 15 minutes, the perfusate contained 1% albumin to remove nonspecifically bound arachidonate (Isakson et al., 1976). Perfusion was then continued with albumin-free physiological salt solution. Three 5-ml samples of the lung effluent were collected at 10-minute intervals prior to injection of angiotensin for estimation of baseline radioactivity. After bolus injection of 1 μg of angiotensin II, the effluent was collected in 1-minute intervals during the first 3 minutes and again at the 5th minute.

**Measurement of Lung Effluent Radioactivity**

The effluent was acidified to pH 3.0 with 0.1 N HCl and immediately extracted into five volumes of ethyl acetate. The ethyl acetate extract was evaporated to dryness and resuspended in 500 μl of methanol. One-half of the extract was counted in a liquid scintillation counter (Nuclear-Chicago) to determine the total amount of radioactivity released from the lung during each sampling period. Because in these samples there was no measurable quenching, the results were expressed as counts/min per ml of sample. The other half of the sample was plated on a 250-μm silica gel G thin layer plate for separation of the prostaglandins using the organic phase of the solvent system ethyl acetate:isoctane:acetic acid:water (11:5:2:10) (vol/vol). Standards of 6-keto-PGF₁α, PGF₂α, PGE₂, thromboxane B₂, PGD₂, 15-keto-13,14-dihydro PGE₂, and arachidonic acid were run with each separation. Sample spots corresponding to the prostaglandin standards were scraped from each plate, extracted into methanol, and counted for radioactivity.

After labeling of the lungs with radioactive arachidonic and in a similar fashion, meclofenamate (1 μg/ml) was added to the perfusate of two lungs. Effluent was sampled for radioactivity 15 minutes after beginning meclofenamate administration, and angiotensin II was injected 30 minutes later. Thin layer chromatographic identification of arachidonate metabolites was performed for one of these lungs.

**Effects of Hypoxic-Induced Vasoconstriction on Release of Arachidonic Acid Metabolites from Lungs Perfused with Erythrocytes in Salt Solution**

Hypoxia causes only small vasopressor responses in lungs perfused with physiological salt solution, but good hypoxic responses are found if erythrocytes are added (McMurtry et al., 1978). To obtain erythrocytes for each experiment, blood from six donor rats was centrifuged at 3000 rpm for 15 minutes, and the plasma and buffy coat were discarded.
The red cells were washed twice in normal saline and suspended in physiological salt solution to give a hematocrit of approximately 30%. In this perfusate, the platelet counts ranged from 8 to 15 \times 10^6; (normal in our rats were 150-225 \times 10^3 per ml). After a 30-minute equilibration period, we challenged the lung with a 6-minute exposure to hypoxia and with an injection of angiotensin II. Then, for the remainder of the experiment, the effluent was wasted to achieve a nonrecirculating system. First, using the physiological salt solution, we labeled the lung with radioactive arachidonate as described above. Then, as above, the perfusate containing 1% albumin was used for 15 minutes to wash out non-specifically bound arachidonic. For the rest of the experiment the lungs were perfused in a nonrecirculating fashion with the original erythrocyte salt solution. In six lungs, three 5-ml samples of the effluent were collected at 1-minute intervals prior to the hypoxic challenge for estimation of baseline radioactivity. Immediately after collection of the last baseline sample, the lung was challenged with the hypoxic gas mixture and the effluent was collected during the 1st, 2nd, and 6th minute of the hypoxic challenge. In two additional lungs, verapamil (10/tg/ml) (Isoptin, Knoll) was added to the perfusate reservoir 1 minute before collection of the first effluent sample to abolish the subsequent hypoxic pressor response. These experiments were done to see whether hypoxia per se or the vasoconstriction produced by hypoxia was responsible for an enhanced prostaglandin production. To evaluate the lungs' response to vasoconstriction substances after the addition of verapamil, the lungs were challenged with a bolus injection of 1 mg of prostaglandin I_2, and effluent was collected in two samples for measurement of radioactivity. These results were expressed as dpm/ml because of some quenching in the samples. Effluent radioactivity was measured in all eight of these hypoxia experiments. Thin layer chromatography was performed for two lungs from pooled samples during baseline perfusion and during hypoxic vasoconstriction, but not for the lungs that were pretreated with verapamil.

**Statistical Analysis**

Data are expressed as mean ± SD. Comparison of the angiotensin II responses before and after meclofenamate and evaluation of the arachidonic acid effect on hypoxia-induced vasoconstriction were done with the t-test for paired data. Analysis of the effluent radioactivity before and after angiotensin or hypoxia, respectively, was performed with the two-way analysis of variance, using the Student-Newman-Keuls test for multiple comparisons.

**Results**

**Effects of Meclofenamate, Arachidonate, and Prostaglandin I_2 on Pressor Responses in Blood-Perfused Lungs**

Intraarterial injections of angiotensin II increased the pressure by 17 ± 1 mm Hg (n = 6). Twenty minutes after the addition of meclofenamate to the blood reservoir, the baseline perfusion pressure had risen by 3 mm Hg and the pressor response to angiotensin II increased to 27 ± 2 mm Hg (P < 0.05).

The vasodilation time following the pressor response slowed from 30 ± 3 seconds before meclofenamate to 62 ± 5 seconds after meclofenamate addition (Fig. 1). In time-matched controls (Fig. 1), the addition of normal saline instead of meclofenamate did not affect vasodilation time. Intraarterial arachidonate given as a bolus during hypoxia caused in each of 10 rat lungs a brief pressor response followed by vasodilation which markedly attenuated the ongoing hypoxic pressor response (Fig. 2). Prostaglandin I_2 given as a bolus into the pulmonary artery abolished an ongoing hypoxic pulmonary pressor response in two experiments and markedly attenuated the pressor effect of an ongoing angiotensin response in two additional experiments.

![Figure 1](http://circres.ahajournals.org/)

**Figure 1** Pulmonary arterial pressor responses to 1 \mu g angiotensin II given by close arterial injection in isolated rat lungs perfused with blood at constant flow. Left: In six rat lungs the peak pressor responses to angiotensin II were greater and the times required for the pressure to return halfway to baseline (vasodilation times) were longer after, than they were before, the administration of meclofenamate dissolved in normal saline. Right: In six other rat lungs, peak pressor responses to angiotensin II and vasodilation times were not altered by the administration of normal saline without meclofenamate.
Effects of Angiotensin II-Induced Vasoconstriction on Release of Arachidonic Acid Metabolites from Lungs Perfused with Physiological Salt Solution

To eliminate prostaglandin synthesis by the formed elements of the blood, six rat lungs were perfused with physiological salt solution and the endogenous arachidonic pool was labeled as described. In the 1st minute after a bolus injection of angiotensin, the pulmonary arterial pressure rose 15 ± 1 mm Hg and the effluent radioactivity increased from 68 ± 8 to 145 ± 10 counts/min per ml (P < 0.05). Subsequently, as the pulmonary arterial pressure fell, the effluent radioactivity also returned to baseline values (Fig. 3). In two experiments, thin layer chromatography of the effluent identified 6-keto-PGF\(_1\alpha\), the major metabolic product of arachidonate. During angiotensin II-induced vasoconstriction, 6-keto-PGF\(_1\alpha\) increased more than the other metabolites (Fig. 3).

Two additional experiments were performed in lungs prelabeled with \(^{3}C\)arachidonate where meclofenamate had been added to the perfusate. The
radioactivity in the effluent was low (35 and 36 counts/min per ml) during baseling conditions. The subsequent angiotensin-induced pressor responses were high (17 and 18 mm Hg) and the radioactivity in the concomitant effluent did not change (Fig. 3). For one of these lungs, thin layer chromatography showed almost no 6-keto-PGF₁₀ and no increase during angiotensin II-induced vasoconstriction; the only peak found was arachidonic acid.

**Effects of Hypoxia-Induced Vasoconstriction on the Release of Arachidonic Acid Metabolites from Lungs Perfused with Erythrocytes in Salt Solution**

The mean pressor response to hypoxia in six lungs was 14 ± 2 mm Hg. Effluent radioactivity increased from 168 ± 15 dpm/ml during baseling conditions to 240 ± 18 dpm/ml (P < 0.05) during hypoxia-induced vasoconstriction. Thin layer chromatography of the effluent sampled before and during hypoxia-induced vasoconstriction (n = 2) showed that 6-keto-PGF₁₀ was the major arachi-

donic acid product (Fig. 4), and that it increased during hypoxia.

In two lungs where the pressor response to hypoxia had been abolished by pretreatment with verapamil (Fig. 5), during hypoxic ventilation no alteration of the effluent radioactivity occurred. However, a subsequent 10 mm Hg pressor response to angiotensin II in one of the lungs caused a 55% increase in effluent radioactivity (not shown), demonstrating that verapamil did not abolish vasoactivity and did not impair arachidonic acid metabolism.

**Discussion**

Although the pharmacological effects of various naturally occurring prostaglandins and newly synthesized prostaglandin analogues on the pulmonary circulation are well described (Hyman et al., 1977), we have lacked information as to the physiological role of endogenous lung prostaglandins. The results of the present study indicate that the perfused rat lung continuously produced the vasodilator, prostacyclin, even under baseline conditions and that vasoconstriction resulted in an increased production of pulmonary prostacyclin which could modulate pulmonary pressor responses.

In the initial experiments with the isolated, blood-perfused rat lung, we showed that inhibition of prostaglandin synthesis enhanced the vasoconstrictor effect of angiotensin II. These findings suggested the participation of a vasodilator prostaglandin in the response. Moreover, addition of the prostaglandin percursor, arachidonate, promoted prolonged vasodilation, and prostacyclin was also a potent pulmonary vasodilator in the blood-perfused rat lung. These results confirmed and extended the findings of Gryglewski et al. (1978a) and Wiberg et al. (1978) and suggested that the rat lung was an
appropriate model for the study of prostaglandin metabolism as it relates to pulmonary vasoconstriction.

We then used the isolated rat lung perfused with a salt solution so that prostaglandin production by white blood cells and platelets could be avoided and the plasma binding of fatty acids would be eliminated. We confirmed that angiotensin II was a pressor agent in this system and that the effects were potentiated by sodium meclofenamate, an inhibitor of prostaglandin synthesis. In these lungs perfused with salt solution, radioactive sodium arachidonate was infused at a rate that did not alter pulmonary hemodynamics. The arachidonate was nearly entirely extracted from the perfusate during passage through the lung, thereby labeling the endogenous arachidonate pool. After the labeling and removal of loosely bound arachidonate with albumin, the lungs continuously released radioactivity, which predominately reflected the breakdown product of prostacyclin, 6-keto-PGF\(_{1\alpha}\) (Moncada et al., 1976, 1977; Dusting et al., 1978), although small amounts of arachidonate and other metabolites also were seen.

With angiotensin II administration, the pulmonary arterial pressure and effluent radioactivity from the lung increased concomitantly and then subsided, indicating increased release of \(^{14}\)C-arachidonate and/or its metabolites. The pattern of radioactive products suggested that the major prostaglandin released was prostacyclin, consistent with our physiological observations that had suggested the release of a vasodilator prostaglandin from the perfused lung which modulated the vasoconstriction produced by angiotensin II. Because the thin layer chromatographic analysis of the lung effluent arachidonate metabolites is a semiquantitative method that does not account for all of the radioactivity, the increase of prostacyclin production with angiotensin vasoconstriction could not be determined exactly. However, since the total radioactivity increased significantly and the ratio of 6-keto-PGF\(_{1\alpha}\) to arachidonic acid did not change, part of the increase in radioactivity during angiotensin-induced vasoconstriction must be accounted for by an increase in 6-keto-PGF\(_{1\alpha}\) production by the lung.

In the meclofenamate-treated lungs, that had an enhanced vasoconstrictor response to angiotensin II, the low baseline radioactivity and the lack of any increase in prostaglandins following angiotensin II confirmed that prostaglandin synthesis had been inhibited. Not only was the magnitude of the angiotensin II pressor response enhanced by meclofenamate treatment, but the duration of the response was prolonged. It is of interest that impairment of vasodilation under certain circumstances (Dusting et al., 1977; Raz et al., 1977; Roberts et al., 1979) has been attributed to an impairment of prostaglandin production. Our physiological data suggest that pulmonary vasodilation following agonist-induced vasoconstriction to some degree depends on the production of vasodilator prostaglandins.

Ventilation hypoxia, like angiotensin II, induces a pressor response that is enhanced by inhibitors of prostaglandin synthesis, again suggesting the production of a pulmonary vasodilator prostaglandin (Weir et al., 1974; Vaage et al., 1975; Hales et al., 1978). In our radiolabeled, isolated lung model perfused with a salt solution containing red cells, alveolar hypoxia resulted in a significant increase in effluent radioactivity concomitant with the pulmonary vasoconstriction. The pattern of the radioactive products again suggested that the vasodilator, prostacyclin, was the major prostaglandin released, confirming the hypothesis based on the physiological observations.

Published reports of experiments on isolated coronary arteries showed an interrelationship between oxygen tension, vascular tone, and prostaglandin production, but it was not clear whether vascular tone or oxygen lack stimulated the prostaglandin production. In the present study we abolished the hypoxic vasoconstriction in the isolated lung with verapamil, an inhibitor of calcium influx (McMurtry et al., 1976). The effluent radioactivity from these lungs did not increase although oxygen tensions were as low as 35 torr. Therefore, hypoxia, per se, was not sufficient to cause release of arachidonate or its metabolites, implying that vasoconstriction was the necessary stimulus to enhanced lung prostaglandin synthesis.

Our data are consistent with the hypothesis that both angiotensin II and alveolar hypoxia produce pulmonary vasoconstriction that stimulates the production of a vasodilator, probably prostacyclin, which opposes the vasoconstriction. One wonders how vasoconstriction triggers prostaglandin synthesis. Possibilities include deformation of endothelial and smooth muscle cells either because of vasoconstriction, or because of increased endothelial shear stress secondary to augmented velocity of blood flow. Possibly an increase in free cytoplasmic calcium causing constriction of the vascular muscle cells could cause activation of the phospholipase A (Haseh and Needleman, 1978) and thereby initiate prostacyclin production.

Whatever the mechanism regulating prostacyclin production, we have considered that it may be of physiological importance for the lung circulation. First, by its constant production and release into the pulmonary microcirculation, prostacyclin may act as a local hormone that helps to maintain a low pulmonary arterial pressure. Second, prostacyclin appears to be important as a defense against excessive pulmonary vasoconstriction.

References
Dusting GJ, Moncada S, Vane JR (1977) Prostacyclin (PGX) is the endogenous metabolite responsible for relaxation of coronary arteries induced by arachidonic acid. Prostaglandins 13: 3-16


Hsueh W, Needleman P (1978) Sites of lipase activation and prostaglandin synthesis in isolated perfused rabbit hearts and hydreneprotic kidneys. Prostaglandin 16: 661-681


Isaakson PC, Raz A, Needleman P (1976) Selective incorporation of 14-C-arachidonic acid into the phospholipids of intact tissue and subsequent metabolism to 14-C-prostaglandins. Prostaglandins 12: 739-748


Reeves JT, Daoud FS, Estridge M (1972) Pulmonary hypertension caused by minute amounts of endotoxin in calves. J Appl Physiol 33: 739-743


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