Effects of Prostaglandin Cyclic Endoperoxides on the Lung Circulation of Unanesthetized Sheep

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A B S T R A C T   Although prostaglandins E_2 and $F_{2\alpha}$ have been suggested as mediators of the pulmonary hypertension seen after endotoxin infusion or during alveolar hypoxia, their precursors, the endoperoxides (prostaglandins G_2 and H_2) are much more potent vasoconstrictors in vitro. In this study we compared the effects of prostaglandin (PG)H_2, a stable 9-methylene ether analogue of PGH_2 (PGH_2-A), PGE_2, and $PGF_{2\alpha}$ on pulmonary hemodynamics in awake sheep. The animals were prepared to allow for measurement of (*a*) lung lymph flow; (*b*) plasma and lymph protein concentration; (*c*) systemic and pulmonary vascular pressures; and (*d*) cardiac output. We also determined the effect of prolonged PGH_2-A infusions on lung fluid balance and vascular permeability by indicator dilution methods, and by assessing the response of lung lymph. Both PGH_2 and PGH_2-A caused a dose-related increase in pulmonary artery pressure: 0.25 μg/kg × min tripled pulmonary vascular resistance without substantially affecting systemic pressures. Both were 100 times more potent than PGE_2 or $PGF_{2\alpha}$ in this preparation. PGH_2-A, as our analysis of lung lymph and indicator dilution measurements show, does not increase the permeability of exchanging vessels in the lung to fluid and protein. It does, however, augment lung fluid transport by increasing hydrostatic pressure in the pulmonary circulation. We conclude: (*a*) that PGH_2 is likely to be an important mediator of pulmonary vasoconstriction; (*b*) its effects are probably not a result of its metabolites PGE_2 or $PGF_{2\alpha}$.

INTRODUCTION

The agents mediating the pulmonary hypertension seen with alveolar hypoxia and gram-negative endotoxin infusion have not been identified. Because prostaglandins (PG)[1] E_2 and $F_{2\alpha}$ are released from the lungs during these reactions, they have been implicated as the mediators, but neither is an impressive vasoconstrictor in vitro (1, 2). In contrast, their precursors, the endoperoxides PGG_2 and PGH_2, are powerful vasoconstrictors, $\cong 100$ times more potent in constricting isolated smooth muscle (3).

Because the endoperoxides are unstable and difficult to synthesize in the large amounts needed for in vivo studies, we first used a stable 9-methylene ether analogue of PGH_2 (PGH_2-A; [15S] hydroxy-11α, 9α-[epoxymethano] prosta-5Z, 13E-dienoic acid, [4]) to assess the effects of endoperoxides on pulmonary vascular pressures, permeability of the lung microcirculation, and lung fluid balance in awake sheep. We then prepared PGH_2, itself, in amounts large enough for brief steady-state infusions, and compared its effects to those of the analogue, PGE_2 and $PGF_{2\alpha}$.

PGH_2 and PGH_2-A were potent vasoconstrictors, 100 times more potent than PGE_2 and $PGF_{2\alpha}$. PGH_2-A, as our studies of lung lymph and indicator dilution measurements showed, did not increase the permeability of exchanging vessels in the lung to fluid and protein, although it did augment fluid transport by increasing hydrostatic pressure in these vessels.

METHODS

Materials. We prepared PGH_2 by incubating arachidonic acid with sheep seminal vesicle microsomes. After isolating PGH_2 by silicic acid chromatography, we confirmed the structure by mass spectrometry (5, 6). We stored PGH_2 in an

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[1] *Abbreviations used in this paper:* PG, prostaglandin; PGH_2-A, stable 9-methylene ether analogue of PGH_2.

acetone solution at −60°C and infused it with normal saline so that the amount of acetone delivered was <0.1 ml over 15 min. This amount of acetone infused alone had no effect on vascular pressures or lymph flow.

PGH₂ analogue, which was supplied by Upjohn Co., Kalamazoo, Mich. was synthesized as described by Bundy (4). We stored PGH₂-A in absolute ethanol solutions at 4°C, then diluted it with normal saline shortly before an experiment.

PGE₂ and PGF₂ₐ were also supplied by Upjohn Co. We dissolved them in normal saline and adjusted pH to between 6 and 7 with bicarbonate buffer before infusion.

Experimental preparation. We prepared sheep by a series of thoracotomies as described (7–9). Through a left thoracotomy, we put catheters in the pulmonary artery and left atrium and a Doppler ultrasonic flow cuff (Park Electronics Laboratory, Beaverton, Oreg.) around the main pulmonary artery. In some sheep, we put a Foley balloon catheter in the left atrium. Several days later, through a right thoracotomy, we cannulated the efferent lung lymph vessel emerging from the caudal mediastinal lymph node, resected the tail of the node, and passed catheters into the thoracic aorta and vena cava through neck vessels. Because lymph flow from sheep prepared this way responds to changes in pulmonary venous pressure, but not to changes in systemic venous pressure, it comes primarily from the lungs (7).

After the last operation, we waited until the lung lymph flow, vascular pressures, and cardiac output were stable and the lymph was free of blood before doing experiments. In several sheep, lymph flow stopped spontaneously. We used these animals in experiments measuring only vascular pressures and cardiac output or for indicator dilution studies.

Protein analysis. We measured total protein concentration in lymph and blood plasma with an automated system (AutoAnalyzer, Technicon Instruments, Corp., Tarrytown, N. Y.) using a modified biuret method (10); duplicate samples differed <5%.

Assessment of vascular permeability. We used total protein concentrations to calculate protein osmotic pressure (Π) from the regression equation of Landis and Pappenheimer (11). Then we calculated the difference between plasma and lymph protein osmotic pressure ($\Delta\Pi$): $\Delta\Pi = \Pi$ plasma − Π lymph. After normalizing lymph flow and $\Delta\Pi$ to base line to compensate for variations among sheep, we compared graphically the relationship between $\Delta\Pi$ and lymph flow for these experiments to two relationships established by previous studies: (*a*) increased pulmonary venous pressure and (*b*) histamine infusion (3). The first relationship is an example of increased lymph flow caused by high pressure in pulmonary exchanging vessels of normal permeability, whereas the second is an example of high lung lymph flow due to high vascular permeability.

Indicator dilution studies. To obtain in vivo measurements of extravascular lung water, cardiac output, and pulmonary capillary permeability, we injected a bolus of ⁵¹Cr-tagged erythrocytes, ¹²⁵I-labeled albumin, [¹⁴C]urea, and [³H]water into the right atrium and sampled blood from the aorta as discussed in several earlier publications (12–16).

First we ran curves under base-line conditions, then infused PGH₂-A at 0.01, 0.10, or 0.25 μg/kg × min into the superior vena cava and repeated curves during the steady-state response period. Later, we counted samples of blood for gamma and beta activity, plotted normalized time-concentration curves, calculated flow as the inverse of the area under the intravascular tracer curves, and calculated extravascular water volume and [¹⁴C]urea permeability surface area product using a Krogh-convolution circulatory model, the details of which have been published (16).

Experimental protocols

During all experiments the sheep were awake and standing in a cage. In each experiment we measured vascular pressures using calibrated miniature strain gauges (Micron Instruments, Inc., Los Angeles, Calif.) positioned at the level of the left atrium. With the pulmonary artery diameter (measured when we installed the flow cuff), and a signal proportional to the blood velocity from a Doppler flow meter (Parks Electronics model 802), we calibrated an electronic recorder (Hewlett-Packard 770 series Hewlett-Packard Co., Palo Alto, Calif.) and directly recorded blood flow in the pulmonary artery (17). We also recorded pulmonary and systemic vascular pressures continuously during each experiment. We measured lymph flow at 15-min intervals by recording the volume drained into graduated tubes, and we measured total protein concentration in plasma from blood drawn each hour and lymph pooled at 30-min intervals.

INCREASED PRESSURE STUDIES

Once in each of nine sheep we measured responses to increases in left atrial pressure. After at least 1 h of base-line observation we inflated the left atrial balloon enough to increase left atrial pressure by 15–20 cm H₂O and kept pressure stable for 4 h. A steady-state lymph flow rate was reached ≅2 h after the balloon was inflated (18).

PROSTAGLANDIN STUDIES

Effects on pulmonary hemodynamics. (*a*) PGH₂-A: We infused PGH₂-A at 0.01 μg/kg × min (10 experiments in 8 sheep), 0.1 μg/kg × min (13 experiments in 8 sheep), or 0.25 μg/kg × min (14 experiments in 10 sheep) through the superior vena cava catheter. We measured base-line and steady-state cardiac output and vascular pressures. (*b*) PGH₂: Three times in three sheep we infused PGH₂ at 0.1 or 0.25 μg/kg × min into the superior vena cava catheter and measured base-line and steady-state cardiac output and vascular pressures. In one sheep we infused 0.1 μg/kg × min into the left atrium. (*c*) Three times in three sheep we infused PGE₂ and PGF₂ₐ at rates from 0.1 to 30 μg/kg × min and measured steady-state base line and experimental cardiac output and vascular pressure.

Effects on lung lymph flow and protein concentration. (*a*) We infused PGH₂-A at either 0.01 μg/kg × min (4 times in 4 sheep), 0.10 μg/kg × min (8 times in 8 sheep), or 0.25 μg/kg × min (10 times in 10 sheep) while measuring lung lymph flow, vascular pressures, and blood and lymph protein concentrations.

Indicator dilution studies. Nine times in seven sheep we infused 0.01, 0.10, and 0.25 μg/kg × min of PGH₂-A into the superior vena cava, measuring steady-state base line and experimental cardiac output, extravascular lung water volume and [¹⁴C]urea permeability surface area product by quadruple indicator dilution methods before injection of PGH₂-A and during the plateau of the pulmonary artery pressure response.

PGH₂-A, INDOMETHACIN STUDIES

Twice in two sheep we infused PGH₂-A at 0.1 and 0.25 μg/kg × min, while giving 5 mg/kg × h of indomethacin intravenously beginning 1 h before PGH₂-A infusion.

Statistics

Where the summary data are given, we show the average ±SE of measurement. We compared the significance of data

TABLE I

Effects of Prostaglandin Endoperoxide (PGH₂) on Steady-State Pulmonary Hemodynamics

	Continuous infusion into the superior vena cava				
	Mean pressure				
Infusion rate	Pulmonary artery	Left atrium	Cardiac output	Pulmonary vascular resistance	Pulmonary vascular resistance
	cm H₂O		ml/s	(cm H₂O·s)/ml	experimental/base line
Base line (n = 3)	21±4*	1±3	88±8	0.25±0.07	1.00
0.10 μg/kg × min (n = 3)	42±6	5±1	96±9	0.50±0.10	2.00±1
0.25 μg/kg × min (n = 1)	53	5	80	0.73	2.90

* Mean±SEM.

by the Wilcoxon rank sum test or Student's t test, accepting $P < 0.05$ as significant. Where appropriate, we tested data for normal distribution using the Kolmogorov-Smirnov test at 80% confidence level (19).

RESULTS

Increased left atrial pressure. We evaluated the effects of elevated pulmonary venous pressure once in each of nine sheep. Lymph flow and lymph to plasma protein concentration ratios were like the results reported (18, 6, 7): for an average increase in left atrial pressure of 14 cm H₂O, lymph flow doubled, and lymph protein concentration always fell absolutely and relative to plasma.

Infusion of PGH₂ and its analogue: hemodynamics. PGH₂ and its analogue PGH₂-A had identical hemodynamic effects. The data are summarized in Tables I and II and illustrated in Fig. 1. Both substances caused a dose-related increase in pulmonary vascular resistance leading to a threefold increase in pulmonary artery pressure at the 0.25 μg/kg × min dose. There was a small, dose-related drop in cardiac output. Infusion of PGH₂ into the left atrium caused no change in pulmonary or systemic pressures or cardiac output, but left atrial PGH₂-A infusions caused pulmonary hemodynamic changes similar to intravenous infusion of the same substance.

PGE₂ and PGF₂α caused little change in pulmonary vascular resistance until the dose reached 10 μg/kg × min, almost 100 times the effective PGH₂ dose (Fig. 2). At higher doses, pulmonary arterial and left atrial pressure rose as cardiac output fell. At the higher doses the animals developed respiratory distress and often collapsed.

TABLE II

Effects of the Endoperoxide Analogue (PGH₂A) on Steady-State Pulmonary Hemodynamics

	Continuous infusions into the superior vena cava					
	Mean pressure		Systolic diastolic pressure aorta			
Infusion rate	Pulmonary artery	Left atrium		Cardiac output	Pulmonary vascular resistance	Pulmonary vascular resistance
	cm H₂O		Torr	ml/s	(cm H₂O·s)/ml	experimental/base line
Base line (n = 17)	21±1	1±1	$\frac{107±2}{74±2}$	99±5	0.22±0.02	1.0
0.01 μg/kg × min (n = 10)	25±1*	2±1	$\frac{109±3}{75±3}$	96±7*	0.28±0.02*	1.2±105*
0.1 μg/kg × min (n = 13)	37±4*	2±1	$\frac{108±2}{70±6}$	90±6*	0.42±0.04*	2.0±0.20*
0.25 μg/kg × min (n = 14)	52±2*	1±1	$\frac{112±2}{80±4}$	86±5*	0.62±0.05*	2.9±0.14*

Average weight of the sheep, 41.5 kg (mean±SEM).
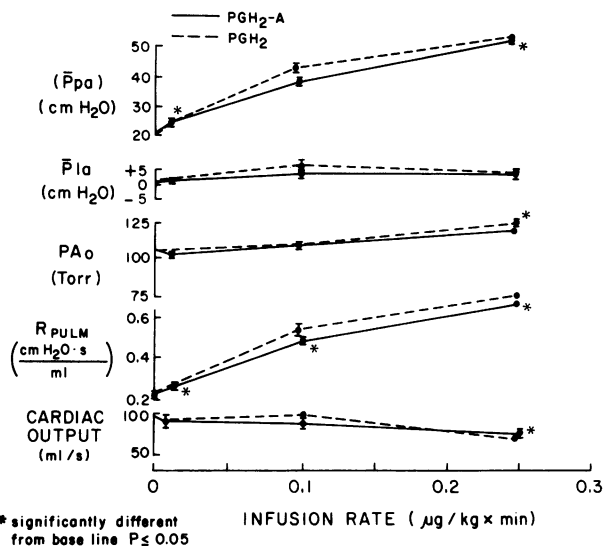* Significantly different from base line (P < 0.05).

FIGURE 1 Hemodynamic effects of PGH_2-A and PGH_2 in the pulmonary circulation. $\bar{P}pa$, mean pulmonary artery pressure; $\bar{P}la$, mean left atrial pressure; $\dot{P}Ao$, systolic pressure in the aorta; R pulm, pulmonary vascular resistance.

significantly different from base line $P \leq 0.05$

PGH₂-A: effects on lung lymph flow and protein concentrations. As shown in Fig. 3, infusion of PGH_2-A caused a dose-related increase in lung lymph flow, corresponding to increases in pulmonary artery pressure. Although the lymph flow increased substantially at higher doses, the lymph protein concentration fell. These data are summarized in Table III. The fall in lymph protein concentration as lymph flow increases is a characteristic effect of high pressure (8). In fact, the effect of PGH_2-A and high left atrial pressure on $\Delta\Pi$ are indistinguishable (Fig. 4). The $\Delta\Pi$ increases linearly with lymph flow after both interventions as a result of the fall in lymph protein concentration relative to
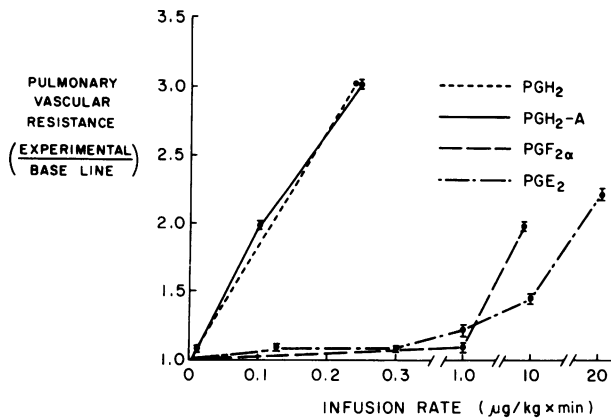

FIGURE 2 Comparison of the effects of PGH_2, PGH_2-A, PGE_2, and $PGF_{2\alpha}$ on pulmonary vascular resistance normalized to base line.
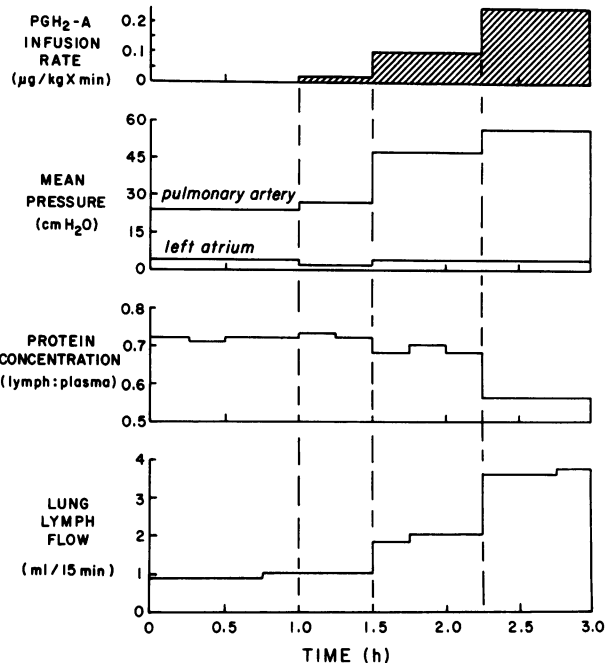

FIGURE 3 Typical response of pulmonary vascular pressures, lymph flow and lymph:plasma protein concentration ratio to increasing intravenous infusion rates of PGH_2-A. Data are averaged over 15-min intervals.

plasma protein concentration (Table III). The two regression lines (of $\Delta\Pi$ vs. lymph flow) in Fig. 4, one derived from high left atrial pressure experiments, the other from endoperoxide studies, are not significantly different ($P > 0.3$), and are in clear contrast to the line derived from histamine studies where permeability was increased.

PGH₂-A infusions after treatment with indomethacin. Pretreatment with 5 mg/kg × h of indomethacin had no effect on the response of pulmonary pressures or lymph flow rate to PGH_2-A infusion.

Indicator dilution studies. There was no significant change from base line in the steady-state extravascular lung water or [^{14}C]urea permeability surface area product during PGH_2-A infusions. The data are summarized in Table IV.

DISCUSSION

This study shows that PGH_2 and a 9-methylene cyclic ether analogue are potent and specific pulmonary vasoconstrictors in doses which have little effect on the systemic circulation. A 0.25-μg/kg × min i.v. dose tripled pulmonary vascular resistance, whereas as little as 0.01 μg/kg × min had an effect. Although studies in vitro (3, 20) suggest this potency of the endoperoxide, its specificity for the pulmonary circulation in vivo is unexpected. Few, if any, known endogenous sub-

TABLE III

TABLE III
Effects of the Endoperoxide Analogue on Steady-State Lung Lymph Flow
and Protein Concentration

| | Continuous infusions into the superior vena cava | | | |
| | | Total protein concentration | | |
Infusion rate	Lymph flow	Plasma	Lymph	Protein concentration
	experimental/base line	g/dl		lymph/plasma
Base line ($n = 10$)	1.00	6.4±0.2	4.3±0.2	0.67±0.01
0.01 μg/kg × min ($n = 4$)	1.13±0.1*‡	6.4±0.5	4.1±5*	0.64±0.03
0.10 μg/kg × min ($n = 8$)	1.65±0.1*	6.7±0.3	4.0±0.2*	0.59±0.02*
0.25 μg/kg × min ($n = 10$)	2.50±0.3*	6.6±0.2	3.4±0.2*	0.51±0.03*

* Significantly different from base line ($P < 0.05$).
‡ Mean±SEM.

stances are such powerful, yet specific pulmonary vaso-constrictors.

Could an endoperoxide be the pressor agent mediating endotoxin-induced pulmonary hypertension? In calfs, Anderson et al. (21) blocked this effect of endotoxin with indomethacin and implicated prostaglandins as mediators. Anderson thought $PGF_{2\alpha}$ was probably the agent, because the concentration of this prostaglandin rose in pulmonary venous blood after endotoxin infusion. Our study shows that the precursor endoperoxide PGH_2 is a 100 times more potent pulmonary vasoconstrictor than $PGF_{2\alpha}$. Since Anderson et al. (21) found that the lung releases considerable amounts of $PGF_{2\alpha}$ in response to endotoxin infusion, PGH_2 (an obligatory intermediate) must have been formed in substantial amounts too. If so, PGH_2 is more likely to have caused pulmonary hypertension than $PGF_{2\alpha}$.

Do PGH_2 and PGH_2-A act by aggregating platelets?

FIGURE 4 Normalized net osmotic pressure difference ($\Delta\Pi$ = Π plasma-Π lymph) as a function of normalized lymph flow. The null hypothesis that lines (1) and (2) are derived from the same population cannot be rejected ($P > 0.30$). Line (3) derived from studies of histamine infusion shows the characteristic failure of $\Delta\Pi$ to increase with lymph flow when vascular permeability is high.

Both will aggregate platelets in vitro (3, 5), but their effects on pulmonary vascular resistance probably are not a result of intravascular platelet aggregation because: (a) the high pulmonary artery pressure produced by infusion of PGH_2 and PGH_2-A returned to normal within 5 min after the infusion was stopped; and (b) infusion of PGH_2 into the left atrium had no apparent physiological effects. If platelet aggregation and vascular plugging were involved the effect should persist for longer than 5 min.

From our data, we cannot determine whether the effects of PGH_2 are a direct result of its action on smooth muscle of the resistance vessels of the lung, or whether conversion to its metabolite thromboxane A_2 is necessary first. Thromboxane A_2 is more potent than PGH_2 in contracting large arterial smooth muscle and in inducing aggregation of platelets in vitro (22, 23). Because it is formed from PGH_2 in the lung (24), it is possible that the hemodynamic effects of PGH_2 are mediated through this more active metabolite. Our finding that PGH_2-A, the analogue (which is not metabolized to thromboxane A_2), had effects similar to those of biosynthesized PGH_2 suggests that PGH_2 itself may cause vasoconstriction. However, if the analogue mimics thromboxane A_2 physiologically, our conclusion that the identical vasoconstriction PGH_2 and PGH_2-A cause is an innate effect of both may be wrong. Instead, PGH_2's conversion to, and PGH_2-A's imitation of, thromboxane A_2 could explain their common effect.

The response to infusion of exogenous PGH_2 into the pulmonary circulation does not prove that PGH_2, when synthesized in the lung, has an identical effect. This could occur if PGH_2 is synthesized in amounts comparable to those infused and if PGH_2 has access to the smooth muscle of the resistance vessels. The exquisite sensitivity of the pulmonary circulation to PGH_2 suggests that amounts synthesized in the lung could be physiologically significant. Lung vessels would also
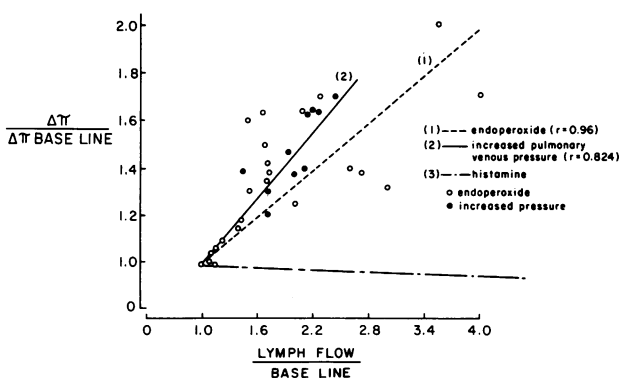
TABLE IV
The Effect of Endoperoxide Analogue Infusion on Indicator Dilution Lung Water
and [^{14}C]Urea Permeability Surface Area Products*

	Base line	Infusion rate, PGH$_2$-A		
		0.01	0.10	0.25
		μg/kg × min		
Extravascular lung water, ml	229±35‡	243±40	255±32	230±36
Number of studies	9	5	9	9
[^{14}C]Urea permeability				
Surface area product, ml/s	10±1	11.5±1.6	10±1	11.9±1.3
Number of studies	8	7	8	7

* Average sheep body weight, 41.1±2 kg.
‡ Mean±SEM.

constrict in response to PGH$_2$ released into venous blood by another organ. This effect might be particularly important if the ability of the lung to metabolize PGH$_2$ were impaired by disease.

The results of this study agree with those of Kadowitz et al. (25) who found PGH$_2$-A to be a potent pulmonary vasoconstrictor in dogs. But, our findings on the effects of PGH$_2$ itself differ from those of Kadowitz et al. He found PGH$_2$ to be weaker than PGH$_2$-A, while we find it to be equivalent in strength. This difference may have occurred because we gave PGH$_2$ by continuous infusion and Kadowitz et al. used a 2-μg bolus. It is interesting that Kadowitz et al. found that PGH$_2$ contracted isolated pulmonary veins more powerfully than PGH$_2$-A.

Vascular permeability. The equivalent effects of high pulmonary venous pressures and PGH$_2$-A infusion on lung lymph protein concentration and osmotic pressures (Fig. 4) indicate that PGH$_2$-A causes pulmonary hypertension without increasing vascular permeability. Others have found that prostaglandins alter permeability in some vascular beds (26, 27) but we found no evidence that lung vessel integrity changed. This effect is like that of serotonin but unlike the response to histamine in this preparation: histamine causes increased flow of lymph with a relatively high protein concentration (8), whereas serotonin causes an increased flow of lymph of low protein content (9). With histamine, the osmotic pressure difference at high lymph flow falls, but with serotonin, mechanically increased pulmonary venous pressure, and, as this study shows, PGH$_2$-A infusion, the oncotic pressure difference between lymph and plasma increases as lymph flow increases (Fig. 4). Thus, the increase in transvascular fluid filtration caused by the prostaglandins is apparently due solely to their effects on pressure in exchanging vessels.

Inactivation of PGH$_2$ in vivo. PGH$_2$ was apparently rapidly inactivated in the sheep circulation because injections into the left atrium did not change systemic or pulmonary pressures. This indicates that PGH$_2$ was inactivated during one passage from the left atrium to the pulmonary circulation.

Site of action of PGH$_2$-A. Although we cannot state with certainty the site of action of PGH$_2$-A, at least some of the vasoconstriction must have been downstream from exchanging vessels. If the vasoconstriction had all been upstream to the exchanging site, lymph flow should not have increased. The latter phenomenon, pulmonary hypertension without increased lymph flow, is characteristic of the response of alveolar hypoxia in this preparation, as Bland et al. (28) showed. This difference between the effects of PGH$_2$-A infusions and alveolar hypoxia suggests that prostaglandin endoperoxides do not mediate hypoxic pulmonary vasoconstriction.

CONCLUSIONS

(a) PGH$_2$ and PGH$_2$-A are potent vasoconstrictors in the pulmonary circulation in doses that have little effect on the systemic circulation. (b) PGH$_2$-A does not increase lung microvascular permeability, but increases filtration from lung exchanging vessels by increasing vascular pressures. (c) Because it is much more potent, PGH$_2$ (or an active metabolite) is more likely to be an important mediator of pulmonary vasoconstriction than either PGE$_2$ or PGF$_{2\alpha}$. (d) PGH$_2$ is rapidly inactivated in the sheep circulation. (e) PGH$_2$-A causes some vasoconstriction downstream to exchanging vessels in the lung.

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