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$PGF_{2\alpha}$ Causes Bronchoconstriction and Pulmonary Vasoconstriction Via Thromboxane Receptors in Rat Lung

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We determined the vascular and airway effects of $PGF_{2\alpha}$ and its mechanism of action on isolated-perfused lungs of rats were isolated and perfused at 50 ml/kg/min with Krebs-Henseleit bicarbonate buffer solution containing 3% bovine serum albumin. The lungs were ventilated with 21% O2 and 5% CO_2 at a tidal volume of 2 ml, frequency of 60 per minute and positive end expiratory pressure of 3 cmH_2O. Following injection of 50 μg $PGF_{2\alpha}$ into the afferent pulmonary catheter, there was a marked rise in pulmonary arterial pressure (Ppa) and in resistance to airflow across the lung (R_L) and a fall in dynamic lung compliance (C_{dyn}). Double vascular occlusion technique revealed that 29% of the rise in Ppa was due to an increase in upstream and 71% to downstream resistance. N^{ω}-nitro-L-arginine, 100 μm, a NO synthase inhibitor potentiated the Ppa response two-fold with significant change in airway mechanics. Rat atrial natriuretic factor (r-ANF), 40 μg, quickly reversed the changes in Ppa, R_L and C_{dyn}. Infusion of r-ANF prior to $PGF_{2\alpha}$ attenuated the Ppa response by 38%, R_L by 44% and C_{dyn} by 12%. SQ 29548, a thromboxane receptor blocker and CI, a protein kinase C (PKC) inhibitor, fully blocked both the vascular and airway responses to $PGF_{2\alpha}$. $PGF_{2\alpha}$ is a constrictor of pulmonary vessels and airways in rat lungs via thromboxane SQ 29548 receptors, thansduced by intracellular PKC.

Key Words : *Pulmonary Circulation, Prostaglandins, Vasoconstriction, Bronchoconstriction*

INTRODUCTION

Prostanoids exert a variety of actions in the lung and have been implicated in the pathogenesis of a number of pulmonary diseases. Of the biological actions of prostanoids, the best documented are their effects on smooth muscles. $PGF_{2\alpha}$ is one of the most frequently studied cyclooxygenase-derived prostanoid for its contractile action on the smooth muscle. Lung is one of the major sites of prostanoid synthesis and degradation. $PGF_{2\alpha}$ causes bronchoconstriction and pulmonary vasoconstriction in vivo in all species of animals studied[1]. However, in vitro, the action of $PGF_{2\alpha}$ is not uniform among different species of animals. $PGF_{2\alpha}$ contracts trachea in man, both the trachea and parenchymal strips in the guinea pig, parenchymal strips only in dogs, with no effect on trachea. Data in rats, one of the most widely utilized species of laboratory animals, are limted to vascular effects of $PGF_{2\alpha}$[2-4], al-

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though the site of vasoconstriction is unknown. Little is known about the effect of $PGF_{2\alpha}$ on airway mechanics in rats.

The purpose of this study was to determine both the vascular and airway effects of $PGF_{2\alpha}$ and its mechanism of action in isolated rat lungs.

MATERIAL AND METHODS

1. Animal Preparation

Sprague-Dawley rats weighing 250-400g were used. Rats were anesthetized by intraperitoneal injection of thiamylal sodium (150 mg/kg). After tracheostomy and the insertion of a tracheal cannula, the animals were ventilated with a Harvard respirator (Model 683). The respirator was set at a tidal volume (V_T) of 2 ml and a breathing rate of 60 per minute. The lungs were hyperinflated at 20 cmH_2O PEEP by a water seal to prevent atelectasis. Ventilatory gas concentrations were 21% O2 and 5% CO_2, and 74% N_2. The chest was opened by midline incision, followed by an intracardiac injection of heparin (1,000 IU/kg). The lungs were initially perfused in an open, non-recirculating manner for 10 minutes and the residual pulmonary blood was slowly washed out with 50 ml of Krebs-Henseleit bicarbonate buffer solution containing 3% bovine serum albumin at 37.5 ℃. The pulmonary artery was cannulated through an incision in the right ventricle and the cannula containing a bubble trap ws secured in place with a ligature around both the pulmonary artery and aorta. A cannula was also placed in the left ventricle and secured in place with a silk ligature. The heart and lungs were then removed from the thoracic cavity and suspended in a humidified, water-jacketed chamber at 39℃.

Pulmonary arterial and left ventricular catheters were connected to Validyne pressure transducers to continuously record inflow and outflow pressure on a strip chart recorder (Astro-Med Model MT 9500). The perfusion chamber (reservoir) had a volume of 50 ml and was maintained at 37-39℃. The height of the reservoir was adjusted to -4 cmH_2O relative to the left atrium. Perfusion was then changed to a recirculating mode. The cannulas were manipulated until resistance to flow was less than 1 mm Hg/ml per minute.

Perfusion rate was advanced in small steps to 0.05 ml/gm per minute and then held constant throughout the experiment using a Masterflex peristaltic pump[5]. During studies, samples of effluent perfusate were collected from the left ventricular cannula for determination of blood gases and pH. Partial pressure of CO_2 was maintained in the range of 30-35 mmHg and pH at 7.3-7.4.

Proximal airway opening pressure (Pao) was measured via a side port in the tracheal cannula. Tidal airflow was continuously monitored by measuring the pressure difference across a heated pneumotachograph (Hans Rudolph, Model 8300) connected to the tracheal cannula. All pressure signals were measured with Validyne differential pressure transducers. Airflow was calibrated by using a rotameter. V_T was obtained by electrical integration of the flow signal by using a Buxco Pulmonary Mechanics Computer (Model 6). The latter also computed on a breath-by-breath basis the dynamic lung compliance (C_{dyn})and the resistance to airflow across the lung (R_L) from the input signals of volume (V_T), pressure (Pao) and flow. C_{dyn} was calculated as V_T divided by Pao at ponits of zero flow and expressed as milliliters per centimeter of water. R_L was calculated as the difference between the inspiratory and expiratory Pao at mid-V_T divided by th difference between the inspiratory and expiratory flow at mid-V_T[6]. R_L was expressed as centimeters of water per milliliter per second. R_L measures total pulmonary resistance, of which approximately 80% is airway resistance and 20% is tissue viscous resistance. Pao, flow, V_T, C_{dyn} and R_L were all recorded continuously on the strip chart recorder.

Before experiments were started, a 30-minute equilibration perfusion period was allowed to establish stable values of pulmonary arterial pressure, dynamic lung compliance and lung resistance to airflow. Based on our previous experiments[7], a single dose

of 50 μg of $PGF_{2\alpha}$ was used in all experiments. This dose of $PGF_{2\alpha}$ injected into the afferent pulmonary arterial catheter produced maximal vascular and airway responses in the isolated-perfused lungs of rats. The following procedures were carried out with a minimum interval of 10 minutes between the procedures or until the vascular and airway responses returned to the baseline values following a particular procedure.

2. Effect of $PGF_{2\alpha}$ on Ppa and Microvascular Pressure

To obtain an estimate of the microvascular pressure of the experimental animals, a double vascular occlusion technique[8, 9] was applied in 15 rat lungs at the end of exhalation by simultaneously occluding the pulmonary artery and the left atrial catheters and turning off the ventilatory pump. The vascular occlusion technique was applied first under stable baseline conditions and then at the peak Ppa response to $PGF_{2\alpha}$

3. Effects of Different Vasoactive and Blocking Agents on Vascular and Airway Responses to $PGF_{2\alpha}$

Atrial Natriuretic Factor (ANF). ANF is a potent short-lived peptide pulmonary vasodilator that we used to determine the reversibility of the contractile response to $PGF_{2\alpha}$[10, 11]. Two sets of experiments were performed with ANF, with each set containing 6 rat lungs. In one set, 40 μg of r-ANF (r-ANF, 28 amino acids; Peninsula Laboratories, Belmont, CA) were added when the Ppa reached its peak after the injection of 50 μg of $PGF_{2\alpha}$. In the second set, 40 μg of rat ANF were injected into the pulmonary artery chatheter 2 minutes before the injection of 50 μg of $PGF_{2\alpha}$. The dose of ANF chosen was based on our previous experiments for maximal response[15].

N^{ω}-nitro-L-arginine(NNLA). In 12 rat lungs, following two consecutive doses of 50 μg of $PGF_{2\alpha}$, NNLA, a nitric oxide synthase inhibitor was added to the perfusate at a final concentration of 100 μM[12, 13]. Three minutes later, another dose of 50 μg of $PGF_{2\alpha}$ was injected into the pulmonary artery catherter.

Thromboxane (TX) Receptor Inhibitor, SQ 29548. This compound was provided by Squibb. 50 μg of $PGF_{2\alpha}$ was injected into the pulmonary arterial catheter of 4 rat lungs before and after the addition of SQ 29548 to the perfusate at a final concentration of 40 μM[7, 14].

1-(5-isoquinolinesulfonyl)Piperazine (CI). 50 μg of $PGF_{2\alpha}$ were injected into the pulmonary arterial catheter of 4 rat lungs before and after the addition in the perfusate of 0.2 μM of CI, an inhibitor of protein kinase C[15, 16].

4. Statistical Analyses

Means and stantard errors of mean are presented for values obtained in each series of experiments. The statistical significance of diffenrences among the means was analyzed by Student's t-test and paired t-test where applicable.

RESULTS

1. Effects of $PGF_{2\alpha}$ on Microvascular Pressure

$PGF_{2\alpha}$ caused significant vascular and airway responses in isolated rat lungs. The results of double occlusion studies are shown in Fig. 1. Ppa rose from 8.6±0.3 to 14.2±0.6 cmH_2O after 50 μg of $PGF_{2\alpha}$ ($p<0.01$) and microvascular pressure (Pmv) increased from 2.7 ± 0.3 to 6.7 ± 0.5 cmH_2O ($p<0.01$).

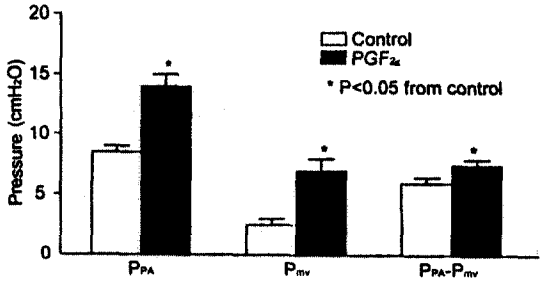

Fig. 1. Change in longitudinal vascular segment pressure to a bolus dose of 50 μg $PGF_{2\alpha}$ in 15 isolated-perfused rat lungs, measured by double occlusion. P_{PA}, pulmonary arterial pressure; P_{mv}, microvascular pressure; P_{PA}-P_{mv}, difference. Most of the pressure change was in the downstream segment.

Table 1. ANF Caused a More Rapid Decay of Pulmonary Arterial Pressure Following $PGF_{2\alpha}$

	CONTROL	ANF
Basal P_{PA}(cm H_2O)	8.7 ± 0.3	8.3±0.3
Peak P_{PA}(cm H_2O)	14.0± 0.9	14.0± 1.0
Time to Baseline(min)	8.2 ± 0.6	1.2 ±0.1*
Time to Half of Peak(min)	4.2 ± 0.4	0.3 ±0.1*

Values are means ±SE of 6 experiments. ANF, atrial natriuretic factor. *$p < 0.05$

Table 2. ANF at Peak Response to $PGF_{2\alpha}$ Caused a Partial Recovery of Lung Resistance (R_L) and Dynamic Compliance (C_{dyn}) Values

	R_L (cm H_2O/ml/sec)	C_{dyn}(ml/cmH_2O)
Baseline	0.21 ± 0.02	0.29 ± 0.02
Peak Response	0.32 ± 0.04	0.22 ± 0.01
After ANF	0.24 ± 0.03	0.25 ± 0.02
% Recovery(1min)	78.33± 4.87	47.67± 1.96

Values are means ± SE of 6 experiments

Of the rise in Ppa, 29% was due to an increase in upstream and 71% due to an increase in downstream resistance. Venous resistance could not be calculated, because the lungs were under zone II conditions (outfolw pressure less than alveolar pressure). Yet, following the application of the double vascular occlusion technique, pulmonary arterial pressure dropped and left atrial pressure rose to the identical value, giving an estimate of the microvascular pressure. Following 50 μg $PGF_{2\alpha}$, R_L increased from baseline value of 0.21±0.02 to 0.32±0.04 cmH_2O/ml/sec ($p<0.01$) and C_{dyn} declined from 0.29±0.02 to 0.22±0.01 ml/cmH_2O ($p<0.01$).

2. Effects of ANF on Vascular and Airway Responses

Rat atrial natriuretic factor quickly reserved the changes in Ppa, C_{dyn} and R_L induced by $PGF_{2\alpha}$. When 40 μg of r-ANF were injected at the peak Ppa rise after 50 μg of $PGF_{2\alpha}$ in 6 rat lungs, the half time for the decline of Ppa to the baseline was 0.3 min as opposed to 4 min when no r-ANF was added (Table 1). The return time to baseline Ppa was shortened by 73%. Injection of r-ANF at peak response caused a 78% recovery in R_L and 48% recovery in C_{dyn} within one minute (Table 2). Injection of 40 μg or r-ANF in another set of 6 rat lungs before the injection of 50 μg $PGF_{2\alpha}$ caused a 38% reduction in peak Ppa rise, a 44% reduction in the rise in R_L and a 12% reduction in the decline in C_{dyn}, compared with the pre-ANF injection response to 50 μg $PGF_{2\alpha}$ in the same rat lung (Fig. 2). The ANF effect was greater on R_L than on C_{dyn}.

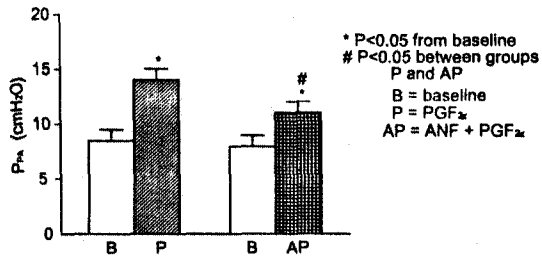

Fig. 2. Pulmonary arterial pressure response to $PGF_{2\alpha}$ without and with pre-treatment with atrial natriuretic factor(ANF). Values are means±SE of 6 experiments.

3. Mechanism of Ppa Response to $PGF_{2\alpha}$

NNLA increased baseline Ppa only at doses)100 μM. Subsequent injection of $PGF_{2\alpha}$ caused pulmonary edema associated with a pronounced increase ()60 mmH_2O) in Ppa. The dose of NNLA we chose did not affect baseline Ppa, nor was there any change in airway mechanics after the addition of NNLA

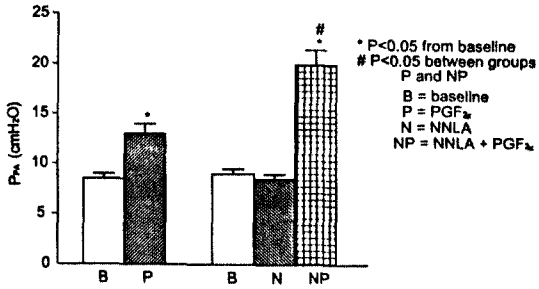

Fig. 3. Pulmonary arterial pressure response to $PGF_{2\alpha}$ without and with pretreatment with N^{w}-nitro-L-arginine(NNLA). Values are means±SE of 12 experiments.

in the perfusate. 100 μM of NNLA potentiated the peak Ppa response to 50 μg $PGF_{2\alpha}$ with no statistically significant change in R_L and C_{dyn} (Fig. 3).

Before and after addition of NNLA, the response to 50 μg $PGF_{2\alpha}$ were as follows : Ppa values increased from 8.7±0.3 to 13.1±0.5 cmH_2O without NNLA and from 9.0±0.3 to 20.0±1.1 cmH_2O with NNLA. The difference in the rise in Ppa with and without NNLA was significant at $p<0.01$. Similarly, $PGF_{2\alpha}$ increased R_L values from 0.20±0.01 to 0.31±0.01 cmH_2O/ml/sec without NNLA and from 0.22±0.01 to 0.31±0.01 cmH_2O/ml/sec with NNLA. C_{dyn} decreased from 0.28 ±0.02 to 0.21±0.01 ml/cmH_2O without NNLA and from 0.26±0.01 to 0.20±0.01 ml/cmH_2O with NNLA. The addition of SQ 29548 and CI fully blocked the pulmonary vascular and airway effects of $PGF_{2\alpha}$ (data not shown).

DISCUSSION

$PGF_{2\alpha}$ caused both pulmonary vasoconstriction and bronchoconstriction in rat lungs which were isolated and perfused with Krebs-Henseleit bicarbonate buffer solution. The vascualr effects are similar to those reported by others[1]. It has been shown that $PGF_{2\alpha}$ increases Ppa with an increase in pulmonary vascular resistance but has no effect on pulmonary vascular permeability either in vitro or in vivo in dogs and sheep. In addition, $PGF_{2\alpha}$ causes bronchoconstriction both in vitro and in vivo in guinea pigs, dogs and humans. Although we wanted a maximal pulmonary response following $PGF_{2\alpha}$ injection into rat lungs, we limited the $PGF_{2\alpha}$ dose to 50 μg, because severe pulmonary hypertension and pulmonary edema developed at higher doses. Only a small part (29%) of the pulmonary arterial pressure rise was due to an increase in arterial resistance. With an F_2-isoprostane, 8-epi-$PGF_{2\alpha}$, we also found that only 28% of the rise in Ppa in rat lungs was due to an increase in arterial resistance[7]. On the other hand, in rabbits, 78% of the 8-epi-$PGF_{2\alpha}$-induced increase in Ppa was contributed by the increase in arterial resistance[11]. Ducharme et al[17] found that the primary site of activity of $PGF_{2\alpha}$ in dogs was the smooth muscle of the small veins[2]. In contrast, taylor and his co-workers pavititioned the effect of $PGF_{2\alpha}$ on vascalar resistance of isolated rat lungs into segments of large and small arteries and veins and found that the primary site of action was the small arteries[2]. In rat lungs, $PGF_{2\alpha}$ caused contraction of the airways, as observed with other species of animals[1]. However, $PGF_{2\alpha}$ caused a larger change in lung resistance than in dynamic compliance, which suggests that effects of $PGF_{2\alpha}$ is greater on central than on peripheral airways of rats[18]. This needs to be confirmed with tissue bath experiments containing different segments of the airways.

In our experiments, NNLA, an NO synthase inhibitor, potentiated the pulmonary vascular response to $PGF_{2\alpha}$ with no change in airways mechanics. This suggests that the effect of $PGF_{2\alpha}$ on pulmonary vasculature can be modulated by the release of NO. It is somewhat controversial whether NO contributes to normal low pulmonary arterial tone[19]. In isolated perfused lungs of pigs, sheep and humans, Cremona et al[20] showed that release of NO regulates basal pulmonary vascular tone. On the other hand, Hasunuma et al[21], like us, found that NO inhibitors did not increase the baseline perfusion pressure in isolated rat lungs. Also, Nishiwaki et al[22] showed in conscious dogs that NNLA had no effect on the baseline pulmonary vascular

pressure-flow relationship but did potentiate the pulmonary vasoconstrictor response to the TX mimetic, U-46619. However, Thomas et al[23] showed in dogs, in vivo, an increase in pulmonary vascular tone in both normoxic and hypoxic lung after the administration of N-methyl arginine, another NO blocker. The use of methyl arginine, as an adequate probe to study the role of endogenous EDRF has recently been challenged by Hyman and his co-workers[24]. These authors failed to demonstrate the inhibition of NO in adult feline pulmonary vascular bed in vivo, by this enzyme which contains a methyl group at the quanidino-nitrogen position. The same methyl group, containing enzyme, has recently been found to paradoxically relax preconstricted canine intrapulmonary arteries and a role of an intermediate dilator-prostaglandin has been suggested for its action[25]. We conclude that NO has variable effects on resting tone, depending on species and baseline conditions, but that NO synthase inhibition by NNLA is associated with augmented vasoconstrictor responses.

Both the airway hemodynamic effects of $PGF_{2\alpha}$ were fully prevented by SQ 29548, an endoperoxide-TX receptor blocker. This strongly suggests that the mechanism of $PGF_{2\alpha}$-induced pulmonary vasoconstriction and bronchoconstriction is due to TX receptor activation. We showed earlier, in sheep, that TX receptor blockade antagonized the effect of $PGD_{2\alpha}$ and $PGF_{2\alpha}$ on the pulmonary vasculature, with no effect on either the systemic vasculature or the airways[26]. Evidence for TX receptor-mediated contraction by $PGD_{2\alpha}$ $PGE_{2\alpha}$ and $PGF_{2\alpha}$ has been shown in guinea pig and human airways in vitro by several investigators[20, 27-29]. Finally, at the cellular level, the muscular contraction of pulmonary vasculature and the airways may be due to the involvement of protein kinase C (PKC), since the use of CI, a PKC inhibitor completely blocked the pulmonary response to $PGF_{2\alpha}$. There is evidence for the participation of both intracellular calcium and PKC in $PGF_{2\alpha}$ -induced vasoconstriction[4, 30]. Involvement of PKC in the tonic phase of smooth muscle contraction has also been suggested by other investigators[31-33]. However, the pulmonary response of isolated-perfused lungs of rats to $PGF_{2\alpha}$ was more of a phasic rather than a tonic nature. Thus, we cannot assert fully that these data are sufficient to prove the definitive role of PKC in the observed response.

In summary, $PGF_{2\alpha}$ is both pulmonary vasoconstrictor, primarily a venoscostrictor, and a bronchoconstrictor in isolated perfused lungs of rats. The effect of $PGF_{2\alpha}$ on rat lungs appears to be greater on central than on peripheral airways. The vaso-, but not the bronchoconstrictor effect, is potentiated by the use of NNLA, a nitric oxide (EDRF) synthase inhibitor. However, both the vaso and bronchoconstrictor effects are partially reversed by the use of atrial natriuretic peptide. The mechanism of action of $PGF_{2\alpha}$ on the pulmonary vasculature and the airways appears to be due to the activation of SQ 29548-responsive thromboxane receptors. Also, it is likely that protein kinase C may play a role in the smooth muscle response of pulmonary vessels and airways to $PGF_{2\alpha}$ intracellularly.

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