The Effects of Angiotensin II and Indomethacin on the Pulmonary Arterial Prostaglandin E Levels and Pulmonary Circulation in Dogs

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SUMMARY

We studied whether pulmonary arterial levels of plasma prostaglandin E rose following pressor effects of angiotensin II in the intact dogs. Furthermore, the modulation by indomethacin of the effect of angiotensin II, $\text{PGF}_{2\alpha}$, and PGE_1 on pulmonary circulation was evaluated. Pulmonary arterial plasma PGE levels rose significantly from 0.52 ± 0.10 (SE) ng/ml to 0.85 ± 0.07 by the infusion of angiotensin II. This increase was not observed when indomethacin was given. The rise in pulmonary arterial pressure and total pulmonary resistance by angiotensin II was augmented significantly with pretreatment of indomethacin. These results showed that there was an augmentation of the pulmonary vasoconstrictor action of angiotensin II as indomethacin blocked an increase of pulmonary arterial PGE level. Thus, it was suggested that prostaglandins (especially PGE) modulated the pulmonary vasoconstrictor effects of angiotensin II.

Additional Indexing Words:

Radioimmunoassay Prostaglandin E_1 Prostaglandin $\text{F}_{2\alpha}$ Total
pulmonary resistance Pulmonary arterial blood pressure Cardiac
output Max RV dp/dt

THE prostaglandins are a group of unsaturated hydroxy-carboxylic acid that are widely distributed in normal tissues and have diverse biological activity. The study on the cardiovascular actions of these compounds in man and experimental animals showed that prostaglandins of E- and A-type were potent vasodilators, but prostaglandin $\text{F}_{2\alpha}$ ($\text{PGF}_{2\alpha}$) was a weak vasoconstrictor agent in most peripheral vascular beds.^{1),2)} In the pulmonary vascular bed, $\text{PGF}_{2\alpha}$ is a potent vasoconstrictor, whereas PGE_1 and PGA_1 are moderately active vasodilator agents.^{3),4)} In addition to their direct effect on vascular smooth muscle, the prostaglandins modulate effects of pressor hormones in a number of vascular beds.⁵⁾⁻¹¹⁾ It has been reported that angiotensin II, a vasoconstrictor agent, stimulates releases of prostaglandins from the kidney,^{5),6)}

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cultured vascular endothelium⁷⁾ and vascular wall.^{9),10)} Although there are reports showed that angiotensin II strongly stimulated a release and synthesis of prostaglandin, there is no report showing the effect of angiotensin II on pulmonary arterial blood levels of prostaglandin E.

We studied whether pulmonary arterial levels of plasma prostaglandin E rose following pressor effects of angiotensin II in the intact dogs. Furthermore, the modulation by indomethacin, an inhibitor of prostaglandin synthesis, of the effects of angiotensin II, PGF_{2α} and PGE₁ on pulmonary circulation was evaluated in the intact dogs.

MATERIALS AND METHODS

Experimental procedure:

Forty-eight mongrel dogs of either sex weighing 7.5–19 Kg were used for this experiment and anesthetized with pentobarbital sodium. A 7F catheter was positioned in the peripheral pulmonary arterial branch of the right lower pulmonary lobe under fluoroscopic guidance and pulmonary arterial blood pressure was recorded on a polygraph connected to a transducer. Aortic and right ventricular pressures and max dp/dt of right ventricle (RV) were measured continuously with transducers connected to catheters placed both in the aorta and in the right ventricle simultaneously. Cardiac output was measured by the thermal dilution method with a 5F thermodilution catheter placed in the pulmonary artery in 14 dogs using the KIMRY Model 3500 cardiac output computer. Total pulmonary resistance (TPR) was obtained by the following formula:

$$\text{TPR} = \frac{\text{mean pulmonary artery pressure} \times 1332 \times 60}{\text{cardiac output (ml/min)}} \text{ dyne/sec/cm}^{-5}$$

All infusions were made through a catheter in the right ventricle. The angiotensin II, PGF_{2α}, and PGE₁ freshly solved daily in saline solution were infused for 10 min into the right ventricle; angiotensin II at a rate of 0.5 μg/Kg/min, PGF_{2α}, and PGE₁ at a rate of 1.0 μg/Kg/min with an infusion pump. Indomethacin (5 mg/Kg) was mixed with equimolar amounts of sodium carbonate, and the resulting solution was diluted in 20 ml physiologic saline and injected into the right ventricle 20 min before the infusion of these substances. The responses of pulmonary arterial, aortic, and right ventricular pressures, cardiac output, total pulmonary resistance, and max dp/dt of RV in dogs treated with indomethacin following intra-ventricular infusion of these substances were compared with those in control dogs. Two ml of peripheral pulmonary blood sample was obtained before and after the continuous infusion of 0.5 μg/Kg/min of angiotensin II for 10 min and centrifuged at 3,000 rpm and plasma obtained was freezed in a refrigerator at –20°C. Extreme care was taken to remove all red blood cells and platelets from the supernatant fluid before freezing.

PGE assay:

Plasma PGE was assayed by a radioimmunoassay of Inagawa.¹²⁾ As shown

in Fig. 1, plasma sample (1 ml) was extracted with Folch solution. Sephadex G-25 column was used to separate the non-lipid contaminants. After the evaporation of the eluted solution, the residue was dissolved in carbon tetrachloride and prostaglandins were extracted with phosphate buffer. The aqueous phase was acidified with hydrochloric acid to pH 3.0. After the extraction of prostaglandins with ethyl acetate, the ethyl acetate layer was neutralized with ammonium hydroxide. After the evaporation of the solvent, thin layer chromatography of silica gel was used to separate three major groups of prostaglandins. After the separation, PGE was assayed using anti-PGE₁ antibody. Dextran coated charcoal in phosphate buffer was used to separate bound and free prostaglandins. The supernatant was decanted into 10 ml of scintillation fluid and counted in a scintillation counter for 2 min. Results are expressed in nanograms per milliliter of plasma. The recovery from the extraction and chromatographic separation with ³H-labeled PGE₁ was about 61%.

Materials:

Angiotensin II was obtained from Protein Research Foundation. Prostaglandin E₁, F_{2α}, and anti PGE₁ antibody were gifts from Ono Pharmaceutical Co. ³H-

Fig. 1. Radioimmunoassay of PGE. Folch; the mixture CHCl₃: CH₃OH=2:1. LP, UP; LP means lower phase that formed by the mixture CHCl₃: CH₃OH: HCl(pH 2.0)=200: 100: 75, and UP means upper phase. Developing solvent system; CHCl₃: EtOAc: EtOH: AcOH=200: 200: 40: 10.

labeled PGE_1 was obtained from New England Nuclear.

All samples were assayed in duplicate. Results were expressed as mean $\pm$ standard error. The Student *t* test was used for statistical analysis. A probability (*p*) value of less than 0.05 was taken as a significant difference.

RESULTS

The effects of indomethacin on the pulmonary arterial pressure:

Small but non significant transient increase in pulmonary arterial and aortic pressures was observed after the injection of 5 mg/Kg of indomethacin in 13 dogs. However, the increase in pressure returned toward control value 15 min after the injection (Fig. 2). The modulation by indomethacin of the effect of angiotensin II, $\text{PGF}_{2\alpha}$, and PGE_1 on pulmonary circulation was evaluated in 48 dogs. The increase in pulmonary arterial pressure by the infusion of these substances in the right ventricle was compared with and without administration of indomethacin. With indomethacin, the rise in pulmonary arterial systolic pressure by angiotensin II was augmented significantly at 10 and 15 min after the beginning of the infusion ($p < 0.02$, $p < 0.005$), whereas that by $\text{PGF}_{2\alpha}$ was not augmented significantly. The decrease in pulmonary arterial pressure by PGE_1 did not change with pretreatment of indomethacin (Fig. 3). The rise in total pulmonary resistance (ΔTPR) and the decrease in cardiac output (ΔCO) by angiotensin II changed significantly at 10 min with pretreatment of indomethacin ($p < 0.05$, $p < 0.05$). However, these changes returned to control value at 20 min after the infusion (Fig. 4).

Fig. 2. Effect of indomethacin on pulmonary and systemic blood pressures. Indomethacin 5 mg/Kg was injected into the right ventricle. Ao: Aorta, PA: Pulmonary artery.

Fig. 3. The modulation by indomethacin of the effects of angiotensin II, PGF₂ α and PGE₁ on pulmonary arterial systolic pressure. Indomethacin (5 mg/Kg) was injected into the right ventricle 20 min earlier. Student t test was used for statistical analysis. Δ PA: Difference of pulmonary arterial blood pressure.

Tracings from the experiment were illustrated in Fig. 5. As shown in Fig. 5, the rise in pulmonary arterial pressure by angiotensin II was augmented significantly with pretreatment of indomethacin, while the aortic and right ventricular pressures and max dp/dt of RV by these substances did not change significantly with pretreatment of indomethacin (Table I).

The modulation by indomethacin of the effect of angiotensin II on the pulmonary arterial plasma PGE levels:

Fig. 4. Effects of angiotensin II (0.5 µg/Kg/min, 10 min) on total pulmonary resistance and cardiac output the with and without administration of indomethacin. Student t test was used for statistical analysis. TPR: Total pulmonary resistance, CO: Cardiac output.

Table I. Effects of Angiotensin II, PGF_{2α}, and PGE₁ on Pulmonary Arterial Pulmonary Resistance, and Cardiac Output With

		Angiotensin II (0.5 µg/Kg/min, 10 min)		
		control (n=7)	Indomethacin (n=7)	p
Pa pressure(s)	(ΔmmHg)	4.1 ± 0.6	8.6 ± 1.4	<0.02
Pa pressure(d)	(ΔmmHg)	2.4 ± 1.3	4.6 ± 1.7	n.s.
RV pressure(s)	(ΔmmHg)	0.3 ± 0.6	3.1 ± 1.7	n.s.
Ao pressure(s)	(ΔmmHg)	35.6 ± 5.3	42.3 ± 5.0	n.s.
Ao pressure(d)	(ΔmmHg)	42.1 ± 7.1	39.0 ± 4.5	n.s.
maxRVdp/dt	(%)	110.6 ± 9.9	106.6 ± 7.4	n.s.
TPR	(Δdyne/sec/cm ⁻⁵)	138 ± 36	287 ± 55	<0.05
CO	(ΔL/min)	-0.28 ± 0.05	-0.49 ± 0.07	<0.05

All values were expressed as mean ± SE. Pa=Pulmonary artery, RV=Right ventricle, Ao=Aorta, s=systolic, d=diastolic, TPR=Total pulmonary resistance, CO=Cardiac output, n=

Pulmonary arterial plasma PGE levels rose significantly from 0.52 ± 0.10 (SE) ng/ml to 0.85 ± 0.07 by the infusion of angiotensin II. This increase was not observed when 5 mg/Kg of indomethacin was given 20 min before the infusion of angiotensin II (Table II, Fig. 6). There was an augmentation

Fig. 5. Effects of angiotensin II on pulmonary arterial pressure with and without the administration of indomethacin. Indomethacin (5 mg/Kg) was injected into the right ventricle 20 min earlier. PA: Pulmonary artery.

Pressure, Right Ventricular Pressure, Aortic Pressure, MaxRVdp/dt, Total and Without Administration of Indomethacin

PGF _{2α} (1.0 µg/Kg/min, 10 min)			PGE ₁ (1.0 µg/Kg/min, 10 min)		
control (n=10)	Indomethacin (n=7)	P	control (n=10)	Indomethacin (n=7)	P
7.2±1.6	10.6±2.2	n.s.	-3.1±0.7	-5.7± 2.7	n.s.
5.0±1.0	6.4±2.0	n.s.	-1.6±0.7	-3.4± 2.2	n.s.
1.8±2.0	2.8±1.5	n.s.	-2.0±2.2	1.2± 2.4	n.s.
2.0±4.3	5.0±4.3	n.s.	-26.1±3.9	-16.0± 5.2	n.s.
0.6±3.3	9.7±3.4	n.s.	-22.3±4.4	-15.3± 4.3	n.s.
115.1±6.4	107.4±3.9	n.s.	95.1±7.7	113.5±14.3	n.s.

number of dogs. Statistical significance was determined by a Student t test.

of the pulmonary vasoconstrictor action of angiotensin II as indomethacin blocked an increase of pulmonary arterial PGE level.

Fig. 6. Effects of angiotensin II (0.5 µg/Kg/min, 10 min) on pulmonary arterial plasma PGE levels with and without administration of indomethacin. Indomethacin (5 mg/Kg) was injected into right ventricle 20 min earlier. Student t test was used for statistical analysis.

Table II. Effects of Angiotensin II and Indomethacin on Pulmonary Arterial Plasma Prostaglandin E Levels (as PGE₁)

	before	Angiotensin II (0.5 µg/Kg/min)		before	Indomethacin (5 mg/Kg)	Angiotensin II (0.5 µg/Kg/min)
No. 1	0.37	0.50	No. 7	0.52	0.24	0.37
No. 2	0.75	0.90	No. 8	0.47	0.80	0.44
No. 3	0.52	1.00	No. 9	0.50	0.62	0.23
No. 4	0.29	0.82	No. 10	0.26	0.33	0.21
No. 5	0.34	0.94	No. 11	0.47	0.44	0.24
No. 6	0.86	0.96	No. 12	0.55	0.47	0.50
Mean	0.52	0.85	Mean	0.46	0.48	0.33
±SE	0.10	0.07	±SE	0.04	0.08	0.05
p	<0.025		p	n.s.		n.s.

All values were expressed in ng/ml. Statistical significance was determined by a Student t test.

DISCUSSION

It has been reported that lungs have marked biosynthetic capacity and release prostaglandins in response to a number of physiologic and pathophysiologic stimuli including hyperinflation, anaphylaxis, hypoxia, and pulmonary embolization.^{8),13)} In addition to synthesis and release, the lungs are an important organ for metabolism, and approximately 90% of E and F-series of

prostaglandins are metabolized during a single injection or continuous infusion, whereas A-series prostaglandins are somewhat more resistant.^{14),15)}

Prostaglandins have been shown to have the ability to modulate the actions of the adrenergic nervous system and of pressor hormones in a number of peripheral vascular bed.^{9),16),17)} Prostaglandins are released by sympathetic nerve stimulation from a variety of organs. Prostaglandins E-series, which represent the major type released by nerve stimulation, usually inhibit adrenergic transmission by depressing effects on the adrenergic transmitter at a postjunctional level, and sometimes specifically by inhibiting release of transmitter from nerve terminals. Opposite effects are usually seen with prostaglandins F-series, which facilitate responses produced by sympathetic nerve stimulation and occasionally increase the responsiveness of effectors to norepinephrine.¹⁷⁾ Pressor hormone angiotensin II has been shown to stimulate the release of PGE like material into the venous effluent of the isolated, perfused rabbit kidney⁵⁾ and into the renal venous blood of the intact canine kidney.⁶⁾ Gimbrone and Alexander demonstrated a dose dependent increase in the production of immunoreactive PGE by endothelial cells of cultured umbilical vein when angiotensin II was added to the medium.⁷⁾ In addition, indomethacin has been shown to enhance pressor responses to norepinephrine and angiotensin in the canine renal vascular bed,⁶⁾ and in the perfused rabbit mesenteric vascular beds.⁹⁾

In the pulmonary vascular bed, Kadowitz et al reported that indomethacin enhanced the constrictor response to angiotensin II and $\text{PGF}_{2\alpha}$, and the dilator response to PGE_1 in dogs using a catheterization technique designed for pump perfusion of a lobe, which was hemodynamically separate from the main pulmonary artery, at a constant rate of flow.⁸⁾ There is no report illustrating the effects of angiotensin II and indomethacin on the pulmonary arterial blood levels of prostaglandins.

Our findings indicated that the concentration of pulmonary arterial plasma PGE rose by the infusion of angiotensin II. Furthermore, our results showed that there was an augmentation of the pulmonary vasoconstrictor actions by angiotensin II at the same time when indomethacin blocked the increase of pulmonary arterial PGE levels. On the other hand, the increase in pulmonary arterial pressure by intra-right ventricular infusion of $\text{PGF}_{2\alpha}$ was not augmented significantly and the decrease in pulmonary arterial pressure by PGE_1 did not change with pretreatment of indomethacin. The explanation for differences in pulmonary vascular responses to $\text{PGF}_{2\alpha}$ and PGE_1 with administration of indomethacin is not clear. Differences may be due to experimental methods, because they used a catheterization technique designed for pump perfusion of a lobe at constant rate of flow, and we used intact dogs.

The responses of the pulmonary vessels of intact dogs to prostaglandins and angiotensin II are usually affected by the cardiac and peripheral effects of these agents. It has been known that angiotensin II exerts a positive inotropic action.¹⁹⁾ On the other hand, prostaglandins have been reported to exert both positive^{20),21)} and negative¹⁶⁾ inotropic actions. The rise in pulmonary arterial pressure and total pulmonary resistance in response to angiotensin II was augmented significantly with the pretreatment of indomethacin, while the aortic and right ventricular pressures and max dp/dt of RV did not change significantly. Furthermore the decrease in cardiac output by angiotensin II was augmented significantly with the pretreatment of indomethacin. From these findings, it was suggested that the rise in pulmonary arterial pressure by angiotensin II with the pretreatment of indomethacin seemed to indicate the vasoconstriction of the pulmonary vessels.

Plasma PGE levels of pulmonary artery did not change at 20 min after the administration of 5 mg/Kg of indomethacin. Speckart reported that a single dose of indomethacin did not change basal unstimulated immunoreactive (IR) PGA levels, but chronic administration lowered IR-PGA levels in man.¹⁰⁾ Therefore chronic administration may lower basal unstimulated pulmonary arterial plasma PGE levels.

The productive site of vasodilatory prostaglandin is speculative, but the contracting vascular wall (endothelium) and kidney appear to be most likely organs.^{6),7),9)} Endogenous prostaglandins are generally thought to act locally in or near the tissue in which they are formed.^{7),16)} Therefore pulmonary arterial prostaglandins seem to be produced mainly by the pulmonary vascular wall.

Prostaglandins (especially PGE) may play an important physiologic role in homeostasis of pulmonary arterial pressure antagonizing the vasoconstriction of endogenous angiotensin II. The production of PGE may increase in response to increasing endogenous levels of angiotensin II and act as antagonist to vasoconstrictor activity of the renin-angiotensin system. Thus, our findings suggest that endogenous prostaglandins have an important role in regulation of the pulmonary circulation.

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