Role of prostacyclin in blood pressure regulation and aldosterone production in conscious rabbits

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Summary

1. The effects of subdepressor infusion of prostacyclin (PGI2, 5.3 pmol min⁻¹ kg⁻¹) on arterial pressure and aldosterone production induced by angiotensin II (ANG II) were studied in conscious rabbits.

2. Indomethacin pretreatment caused an augmented blood pressure response after ANG II infusion, which returned to near control level after concomitant infusion of a subdepressor dose of PGI2.

3. Aldosterone production after ANG II was significantly attenuated after pretreatment with indomethacin. PGI2 infusion restored this reduced response to near control level.

4. These results may suggest that PGI2 in the circulation could also serve to modulate the pressor and hormonal action(s) of ANG II.

Key words: aldosterone, blood pressure, prostacyclin.

Introduction

In various experimental models, the prostaglandin E series [1, 2] and prostacyclin [3] have been reported to attenuate the vasoconstrictor responses to angiotensin II or noradrenaline. Since indomethacin or like-acting drugs have been shown to augment the pressor responses induced by these vasoactive hormones [4–6], possibly through inhibitory action on cyclo-oxygenase, it has been postulated that the endogenous prostaglandins (PG) may contribute to the modulation of blood pressure interacting with pressor substances at the vessel walls [3]. Recent study in man, however, suggests that the circulating PGI2 is unlikely to have a physiological role in steady states, based on the observation that the plasma levels of 6-oxo-PGF1α, the stable hydrolysis product of PGI2, is in the low range [7], in agreement with the finding that the calculated PGI2 production rate is extremely low [8]. Lack of evidence on plasma levels or production rate of PGI2 in pathophysiological conditions, however, does not preclude a possibility that PGI2 released into the circulation still serves to modulate vascular tone. Steroidogenic potency of PG was originally demonstrated with ox adrenals in vitro [9]. However, the role of PGI2 in steroid production is controversial. We have recently shown that PGI2 may be involved in the aldosterone response after ACTH administration in man [10]. In order to investigate whether the circulating PGI2 might have some role in the regulation of blood pressure, either directly or through its effect on aldosterone synthesis, we studied the effects of subdepressor PGI2 infusion on aldosterone production and arterial pressure in conscious rabbits.

Materials and methods

Male New Zealand white rabbits weighing 2.5–3.5 kg were used. The ear artery was cannulated with a polyethylene tube under local anaesthesia. The tube was connected to a pressure transducer for a continuous recording of arterial pressure. The
rabbits were divided into three groups of seven to nine in random order. In group 1, which acted as control to the other two groups, angiotensin II (ANG II; Hypertensin, Ciba) (19 pmol min\(^{-1}\) kg\(^{-1}\)) dissolved in sodium chloride solution (154 mmol/l) was infused for 30 min. In group 2, indomethacin (8.4 \(\mu\)mol/kg) dissolved in ethanol was given intramuscularly 60 min before the start of ANG II infusion. ANG II infusion was carried out in the same manner as in group 1. In group 3, indomethacin pretreatment and ANG II infusion was done as in group 2, and PGI\(_2\) (Prostacyclin methyl ester, Ono Pharmaceutical, Osaka, Japan) (5.3 pmol min\(^{-1}\) kg\(^{-1}\)) was infused concomitantly with ANG II, through a scalp vein needle in a marginal ear vein. PGI\(_2\) was reconstituted in Tris buffer, pH 8.6, immediately before use. In each experiment, rabbits sat quietly in the resting box for at least 20 min after ear artery cannulation. Blood samples (2 ml) were withdrawn three times from each rabbit from the arterial cannula during the experiment. Plasma renin activity (PRA) and plasma aldosterone were measured by radioimmunoassay as previously described [11]. In a limited number of rabbits, plasma 6-oxo-PGF\(_{1\alpha}\) was determined by radioimmunoassay by the method reported by Salmon [12]. Briefly, 1.0 ml of plasma adjusted to pH 3.0 was extracted twice with 2.5 ml of acetic acid. After thin layer chromatography (Silicagel 60/Kieselguhr F 254, Merck) with the solvent system ethyl acetate/triethylpentane/acetic acid/water (11:5:2:10, by vol.), 6-oxo-PGF\(_{1\alpha}\) was extracted with ethyl acetate and measured by radioimmunoassay using antiserum made available by Seragen (Boston, U.S.A.). Statistical analysis was done by using analysis of covariance for comparison of the data between groups. For the analysis of the results within the same group, Student's t-test was used. Results are expressed as means \(\pm\) SEM.

Results

Effects of infusing incremental doses of PGI\(_2\) on blood pressure and plasma immunoreactive 6-oxo-PGF\(_{1\alpha}\) levels

Results of intravenous infusion of PGI\(_2\) at a dose range of 1.3–21.4 pmol min\(^{-1}\) kg\(^{-1}\) are shown in Fig. 1. Baseline mean arterial pressure (MBP) in seven rabbits was 74.7 \(\pm\) 3.5 mmHg. MBP was not significantly changed up to a dose of 10.7 pmol min\(^{-1}\) kg\(^{-1}\). At the highest dose of 21.4 pmol min\(^{-1}\) kg\(^{-1}\), however, MBP decreased to 68.2 \(\pm\) 3.1 mmHg (P < 0.05 vs baseline). In subsequent experiments a fixed infusion of 5.3 pmol min\(^{-1}\) kg\(^{-1}\) was used as a subdepressor dose. Baseline level of plasma immunoreactive 6-oxo-PGF\(_{1\alpha}\) was 1.3 \(\pm\) 0.1 pmol/ml, which increased to 1.4 \(\pm\) 0.2, 1.6 \(\pm\) 0.2, 1.6 \(\pm\) 0.3 and 1.7 \(\pm\) 0.2 pmol/ml after the end of each incremental dose of PGI\(_2\) infusion (P < 0.05 vs baseline). Although the value at the highest dose showed approximately 30% increase in 6-oxo-PGF\(_{1\alpha}\) from the baseline, dose responsiveness was not clearly demonstrated owing to inter-individual variation.

Effects of indomethacin administration on mean blood pressure and plasma 6-oxo-PGF\(_{1\alpha}\)

Indomethacin (8.4 \(\mu\)mol/kg intramuscularly) did not produce significant changes in MBP in conscious rabbits during the 90 min observation period (Table 1). At 60 min after indomethacin treatment, plasma 6-oxo-PGF\(_{1\alpha}\) decreased to 1.0 \(\pm\) 0.1 pmol/ml, an approximately 16% decrease from baseline (P < 0.05). After PGI\(_2\) infusion at 5.3 pmol min\(^{-1}\) kg\(^{-1}\) for 30 min, 6-oxo-PGF\(_{1\alpha}\) increased to 1.2 \(\pm\) 0.1 pmol/ml, near pretreatment level.

Effects of indomethacin and/or PGI\(_2\) on blood pressure response induced by ANG II

Baseline MBP in group 1 rabbits was 74.9 \(\pm\) 4.6 mmHg, which increased to 81.8 \(\pm\) 3.5 and 83.5 \(\pm\) 3.4 mmHg at 10 and 30 min respectively, after ANG II infusion at 19 pmol min\(^{-1}\) kg\(^{-1}\). In group 2, baseline MBP was 74.2 \(\pm\) 4.9 mmHg; after 10 and 30 min of ANG II infusion, MBP increased to
Prostacyclin and blood pressure

TABLE 1. Changes of blood pressure after indomethacin treatment [8.4 μmol/kg (3 mg/kg)] in conscious rabbits

<table>
<thead>
<tr>
<th>Blood pressure (mmHg)</th>
<th>Before</th>
<th>10 min</th>
<th>20 min</th>
<th>30 min</th>
<th>60 min</th>
<th>90 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic</td>
<td>121.0 ± 5.2</td>
<td>122.5 ± 5.5</td>
<td>120.1 ± 4.4</td>
<td>120.0 ± 4.7</td>
<td>124.0 ± 5.9</td>
<td>122.2 ± 4.5</td>
</tr>
<tr>
<td>Diastolic</td>
<td>51.5 ± 3.6</td>
<td>50.8 ± 4.2</td>
<td>52.5 ± 4.9</td>
<td>50.7 ± 3.4</td>
<td>51.6 ± 5.0</td>
<td>50.9 ± 4.2</td>
</tr>
<tr>
<td>Mean</td>
<td>74.4 ± 4.7</td>
<td>74.7 ± 5.6</td>
<td>75.0 ± 5.8</td>
<td>73.0 ± 4.3</td>
<td>75.8 ± 5.5</td>
<td>74.7 ± 4.4</td>
</tr>
</tbody>
</table>

Fig. 2. Changes of mean blood pressure (MBP) after angiotensin II infusion in three groups of conscious rabbits. Indomethacin [8.4 μmol/kg (3 mg/kg)] intramuscularly] significantly augmented MBP increment (●), which returned to near control level after a subdepressor dose of PGI2 (5.3 pmol min⁻¹ kg⁻¹; ▲). Results are shown as means ± SEM of six experiments in each group. ○, Control; ▲, indomethacin and PGI2 together; ●, indomethacin. * P < 0.05 vs control and indomethacin + PGI2. NS, Not significant.

Effects of indomethacin and/or PGI2 on PRA and aldosterone responses induced by ANG II infusion

Basal PRA levels in groups 1, 2 and 3 were 5.5 ± 1.0, 7.7 ± 1.7 and 7.2 ± 1.6 pmol of ANG 1 h⁻¹ ml⁻¹ respectively. After ANG II infusion for 30 min, PRA significantly decreased in all three groups (Table 2). After ANG II infusion, plasma aldosterone increased from the basal concentration of 52.2 ± 7.2 to 73.7 ± 9.1 pmol/dl in group 1, in group 2 from 56.9 ± 3.3 to 61.1 ± 3.3 pmol/dl, and in group 3 from 53.3 ± 2.5 to 66.1 ± 3.1 pmol/dl. Plasma aldosterone response was significantly lower in group 2, showing that 8.4 μmol of indomethacin/kg inhibits the plasma aldosterone stimulatory response induced by ANG II. When the test was repeated while PGI2 was infused continuously (group 3), the reduced response to plasma aldosterone showed a tendency to be restored.

Discussion

Extensive studies have demonstrated the role of endogenous PG in the modulation of vascular tone. The E series of PG is particularly well known for its vasodilatory response in various animal species [1, 2, 13]. Recent evidence indicates that the major PG produced in the vascular wall is prostacyclin [14]. Since PG12 has been shown to be 30-40 times more potent than PGE2 [15] for anti-platelet action and two to eight times for vasodilatation [16], it may be postulated that PG12 is physiologically important for the blood pressure regulation. Furthermore, as the rate of inactivation of PG12 during passage through the lung was shown to be less than 15% [15], this raised the possibility that PG12 might be a circulating hormone [17]. This initial concept is now considered to be unlikely, in view of the finding that the basal plasma levels of 6-oxo-PGF1α measured by gas chromatography has been found to be less than 0.008 pmol/ml in man [7]. The estimated production rate of PG12 in man is also very low, which negates the putative role of PGI in the circulation [8]. In pathophysiological conditions, e.g. in hypoxia or in shock states with vasodilatation,
TABLE 2. Plasma renin activity (PRA) and plasma aldosterone before and after angiotensin II (19 pmol min⁻¹ kg⁻¹) infusion in conscious rabbits

Basal levels of plasma immunoreactive 6-oxo-PGF₁α in four rabbits were also measured. PRA decreased after ANG II in groups 1 and 2. In group 1, basal plasma 6-oxo-PGF₁α and the plasma aldosterone increment were significantly reduced. Means ± SEM are shown: * P < 0.05 vs before ANG II infusion; ** P < 0.05 vs the other two groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>Before</th>
<th>After</th>
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<tbody>
<tr>
<td>1</td>
<td>5.5 ± 1.0</td>
<td>1.0 ± 0.3*</td>
</tr>
<tr>
<td>2</td>
<td>7.7 ± 1.7</td>
<td>4.2 ± 1.6*</td>
</tr>
<tr>
<td>3</td>
<td>7.2 ± 1.6</td>
<td>4.5 ± 1.0*</td>
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<thead>
<tr>
<th>Group</th>
<th>Before</th>
<th>After</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>52.2 ± 7.2</td>
<td>73.3 ± 9.1*</td>
</tr>
<tr>
<td>2</td>
<td>56.9 ± 3.3</td>
<td>61.1 ± 3.3*</td>
</tr>
<tr>
<td>3</td>
<td>53.3 ± 2.5</td>
<td>66.1 ± 3.1*</td>
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<thead>
<tr>
<th>Group</th>
<th>Before</th>
<th>After</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>20.8 ± 8.6</td>
<td>4.4 ± 3.3**</td>
</tr>
<tr>
<td>2</td>
<td>12.5 ± 2.7</td>
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</tr>
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</table>

PGI₂ released into the circulation might still be involved in the development of cardiovascular changes related to these conditions. Our present results suggest that PGI₂ given exogenously into the circulation may modulate the blood pressure response induced by ANG II. The level of 6-oxo-PGF₁α after PGI₂ infusion in group 2 (pretreatment with indomethacin) did not exceed the baseline level, which suggests that this dose is not supraphysiological. The interplay of vascular PG with various vasoconstrictor hormones has been extensively studied, and recently reviewed [18]. Indomethacin and similar drugs have been reported to augment the blood pressure responses induced by ANG II or noradrenaline in man [19]. Attenuation by PG of the vasoconstrictor responses induced by ANG II [20], noradrenaline or sympathetic nerve stimulation has been demonstrated [21,22]. In the anaesthetized dog, ANG II administration alone produced PGI₂-like substances, which were not detected after indomethacin (14 nmol/kg) treatment. Indomethacin administration was accompanied by an attenuated pressor response to ANG II [3]. Perfusion of rabbit mesenteric artery also demonstrates that PGI₂ is released from vascular beds in response to ANG II or noradrenaline [23]. These observations indicate that endogenous PGE₂ and/or PGI₂ is important in the modulation of vascular tone. We tested the role of PGI₂ in the circulation by a continuous subdepressor dose of PGI₂ infused in situations where endogenous PG production was inhibited by pretreatment with indomethacin. The dose of indomethacin we used has been shown to decrease urinary excretion of PGE₂ by 63% in rats [24] and 70% in man [25]. Plasma levels of 6-oxo-PGF₁α in our experiment showed 16% decrease after indomethacin, which did not produce significant changes in blood pressure. Colina-Chouri and colleagues [26] reported that 42 µmol (15 mg) of indomethacin day⁻¹ kg⁻¹ (five times the dose used in the present experiment) raised mean blood pressure in rabbits, suggesting that larger doses of indomethacin than previously considered may be necessary to inhibit endogenous PG. Therefore it could be argued that endogenous PG may not be inhibited completely in our experiment. The blood pressure responsiveness to ANG II, however, was significantly increased and was restored to near control level after PGI₂ infusion. These results suggest that PG produced not only in the vessel wall but also in the circulation may serve in the modulation of blood pressure responses induced by ANG II. Since we have not confirmed whether similar effects will also be observed after other types of cyclo-oxygenase inhibitors, we cannot completely exclude the possibility that PG-unrelated actions of indomethacin might be partly responsible for the present results [27,28]. The mechanism(s) whereby PGI₂ in the circulation may influence the pressor action of ANG II is not apparent from the present study.

PG of the E series have been shown to possess a steroidogenic property in ox adrenal [9]. Indomethacin causes a decrease in basal secretion of mineralocorticoids in man [29], in support of the PG related adrenal steroidogenesis. We have previously reported that aldosterone production induced by ACTH in sodium depleted man is attenuated after indomethacin pretreatment, suggesting a role for endogenous PG, possibly PGI₂ [10]. The role of endogenous PG in aldosterone
production by the adrenal has been demonstrated in rat in vitro [24] and in man in vivo [6], but the group of PG has not been specified. Our results demonstrate that PGI₂ may also be involved in aldosterone production after ANG II stimulation in the rabbit.

Acknowledgments

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References