Prostanoids and Aldosterone-Induced Mild Experimental Hypertension in Rats

Rutai Hui, John H. Grose, Marcel Lebel, and Pierre Falardeau

The goal of this study was to determine the role of prostanoids in a new model of mineralocorticoid-dependent hypertension induced by the subcutaneous infusion of aldosterone (1 μg/hr) to normal male Sprague-Dawley rats. This regimen caused a mild and gradual increase in systolic pressure over a period of 4 weeks (113±1 vs. 137±3 mm Hg) and was associated with an increase in the in vivo formation of prostaglandins I₂ and E₂ and of thromboxane A₂ in the kidney. High sodium intake induced a fall in the urinary levels of prostaglandin E₂ and a rise in the arterial pressure of control rats (126±1 vs. 113±1 mm Hg) but did not influence aldosterone-induced hypertension. Indomethacin (3.0 mg/kg/day) caused a profound inhibition of the in vivo synthesis of prostaglandin I₂ and thromboxane A₂ without modifying the renal production of prostaglandin E₂. Although indomethacin exerted no effect on aldosterone-induced hypertension in rats fed a normal diet, it caused a further rise in systolic pressure in aldosterone-treated rats fed a high sodium diet (157±6 vs. 140±4 mm Hg).

The results of this study in a model of aldosterone-induced mild hypertension in the rat indicate that 1) aldosterone exerts a stimulatory effect on the renal synthesis of prostanoids, particularly prostaglandin E₂; 2) thromboxane A₂ and prostaglandin I₂ do not seem to play a role in aldosterone-induced hypertension under conditions of normal dietary salt intake, whereas the role of prostaglandin E₂ is unclear; 3) there is enough sodium in a normal diet to allow for the maximal expression of the hypertensive effect of aldosterone; 4) prostaglandin I₂ seems to play a significant role in modulating the cardiovascular impact of a high sodium diet in aldosterone-treated rats; and 5) the renal biosynthesis of prostaglandin E₂ is particularly resistant to the inhibitory effect of indomethacin in vivo. (Hypertension 1990;15:198-203)

Although mineralocorticoids have been reported to stimulate the renal synthesis of prostaglandin (PG) E₂ and thromboxane (TX) A₂ in vivo,¹-³ the role of prostanoids in mineralocorticoid-induced hypertension is unclear. In two studies, in which the in vivo biosynthesis of prostanoids was not monitored, the administration of cyclooxygenase inhibitors aggravated the hypertension in humans or in rats treated with mineralocorticoids.⁴,⁵ Recently, Roman et al⁶ reported that the acute administration of a thromboxane receptor antagonist (but not that of a thromboxane synthesis inhibitor) reduced mineralocorticoid-induced hypertension in rats and that the acute addition of meclofenamate to such a regimen induced a further fall in blood pressure, suggesting that the concomitant formation of a pressor prostanoid could contribute to mineralocorticoid-induced severe hypertension. However, these models, which combine mineralocorticoid administration with uninephrectomy or the addition of sodium chloride to drinking water at an isosmolar or hyperosmolar concentration, are situations rarely encountered in clinical medicine.

The objective of the present study was to investigate the role of prostanoids in a model of mineralocorticoid-induced hypertension that resembles more closely human primary aldosteronism. The results include the influence of aldosterone, dietary salt, and indomethacin on the systolic arterial pressure and the in vivo biosynthesis of PG I₂ and PG E₂ and of TXA₂, in a model of hypertension induced by the chronic subcutaneous administration of aldosterone in normal Sprague-Dawley rats.

Methods

Male Sprague-Dawley rats (130–150 g) were purchased from Taconic Farms Inc. (Germantown, New York) and were allowed a 1-week equilibration period before initiation of the experiments.
d-Aldosterone (Sigma Chemical Co., St. Louis, Missouri), dissolved in polyethylene glycol, was administered subcutaneously at a rate of 1 μg/hr via osmotic Alzet pumps (model 2002, ALZA Corp., Palo Alto, California) according to Garwitz and Jones. The pumps were implanted subcutaneously under light ether anesthesia and were changed after 2 weeks. Indomethacin (Sigma Chemical Co.), dissolved in a solution of 0.9% sodium chloride plus 70 mg% Na₂CO₃, was administered intraperitoneally at a dosage of 3.0 mg/kg/day in two divided doses (at 8:00 AM and 5:30 PM).

The diets were made according to our specifications by Ralston Purina Co. (Richmond, Indiana). The normal diet contained 1.0% sodium chloride and 2.0% potassium chloride by weight (0.4% sodium; 1.0% potassium) and the high salt diet contained 5.9% sodium chloride and 2.0% potassium chloride by weight (2.3% sodium). The rats were allowed to eat and to drink tap water ad libitum for the entire period of the study including the days of urine collection.

Systolic arterial pressure was measured in unanesthetized rats by the tail-cuff method. Twenty-four hour urinary collections were carried out in individual metabolic cages as already described.

Plasma and urinary sodium and potassium were measured by flame photometry. Plasma aldosterone was measured by a direct radioimmunoassay without prior extraction. Urinary TXB₂ was analyzed by radioimmunoassay after extraction and purification of the samples by column chromatography. Urinary 2,3-dinor-6-oxo-PGF₁α, 6-oxo-PGF₁α, and PGE₂ were analyzed by capillary gas chromatography-negative ion chemical ionization-mass spectrometry (CGC-NCI-MS) according to a procedure based on a stable isotope dilution method described elsewhere.

The urinary excretion of 2,3-dinor-6-oxo-PGF₁α was used as an index of the systemic production of PGI₃ whereas the urinary excretion of PGE₂, 6-oxo-PGF₁α, and TXB₂ was used as an index of the respective formation of PGE₂ and PGI₃ and of TXA₂ in the kidney. Although there is some controversy about the origin of 6-oxo-PGF₁α in urine (renal ± systemic), it appears that in the rat it originates predominantly from the kidney.

Protocols

Study 1. A pilot study was conducted to determine the pattern of rise in blood pressure in a model that had never been studied previously. Alzet pumps were implanted subcutaneously on the back of 20 rats; half the rats received a pump containing vehicle only and the other half a pump containing aldosterone. On the day after the implantation of the pumps, all the rats were put on a high salt diet. Systolic arterial pressure was monitored before the implantation of the pumps and after 1, 2, 3, and 4 weeks.

Study 2. After an equilibration period of 1 week with a normal diet, 60 rats were equally distributed in six groups. Rats from groups 1 and 2 received a normal diet plus aldosterone and either indomethacin (group 2) or vehicle (group 1) intraperitoneally. Rats from groups 3 and 4 were put on a high salt diet plus aldosterone and either indomethacin (group 4) or vehicle (group 3) intraperitoneally. Rats from groups 5 and 6 were used as control animals and received a high salt diet (group 5) or a normal diet (group 6); they were also injected with vehicle intraperitoneally. However, no pumps were implanted in rats from groups 5 and 6; previous studies have shown that the presence of a pump with vehicle did not influence the blood pressure or the urinary levels of prostanoids in rats (P. Falardeau, unpublished observation).

Two rats from group 1 were removed from the study because their blood pressure did not change (perhaps because of Alzet pump failure); two rats from group 3 and from group 4 were also removed because they died before completion of the study.

Special diets and indomethacin administration were initiated at the beginning of the experimental period (the day after the implantation of the pumps), which lasted 28 days. Systolic arterial pressure was measured at days 18 and 25, whereas 24-hour urinary collections were obtained at day 26. The rats were killed by decapitation at days 27 and 28, and the blood was collected in heparinized tubes and immediately centrifuged. Plasma was kept frozen at −80°C until further processed.

Statistics

Group comparisons were done by two-way analyses of variance according to the method of Bonferroni.

Results

Study 1

The chronic subcutaneous infusion of aldosterone to normal rats on a high sodium diet induced a gradual rise in systolic arterial pressure over a period of 4 weeks (Figure 1). At the end of the experimental period, the systolic arterial pressure of the rats submitted to the aldosterone–high sodium regimen averaged 144±12 mm Hg (mean±SD) compared
with 121 ± 7 mm Hg in control rats on a high sodium diet and vehicle only (p = 0.0001).

Study 2

Systolic arterial pressure (Table 1). The administration of aldosterone was associated with a rise in systolic arterial pressure even in the presence of a normal diet (137 ± 3 vs. 113 ± 1 mm Hg, mean ± SEM, p = 0.0003). The superimposition of a high sodium diet did not modify the urinary excretion of sodium and potassium monitored after 4 weeks of treatment.

Plasma sodium levels were within the normal range in aldosterone-treated rats but were slightly above normal in rats receiving aldosterone plus indomethacin. Plasma potassium levels were slightly above normal values, probably the result of a mild degree of hemolysis during sampling or processing of the blood. The plasma samples of rats of groups 5 and 6 were unavailable for analysis. There were no statistically significant differences between the plasma electrolytes in the various groups.

Urinary prostaglandins. The urinary levels of 2,3-dinor-6-oxo-PGF1α were not affected by the diet or by aldosterone treatment (Figure 3). On the other hand, the chronic administration of aldosterone was

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**TABLE 1. Biological Data in Control and Aldosterone-Treated Rats on a Normal or a High Salt Diet After Four Weeks**

<table>
<thead>
<tr>
<th>Group</th>
<th>Systolic pressure (mm Hg)</th>
<th>Body weight (g)</th>
<th>Volume (ml/24 hr)</th>
<th>Sodium (meq/24 hr)</th>
<th>Potassium (meq/24 hr)</th>
<th>Aldosterone (ng/dl)</th>
<th>Sodium (meq/l)</th>
<th>Potassium (meq/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>137±3</td>
<td>316±7</td>
<td>30±2</td>
<td>2.4±0.1</td>
<td>6.4±0.4</td>
<td>176±11</td>
<td>145±3</td>
<td>5.4±0.2</td>
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<td></td>
<td>(8)</td>
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<td>(6)</td>
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</tr>
<tr>
<td>2</td>
<td>139±4</td>
<td>312±8</td>
<td>39±2</td>
<td>2.3±0.1</td>
<td>5.4±0.4</td>
<td>215±13</td>
<td>148±1</td>
<td>5.5±0.2</td>
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</tr>
<tr>
<td>3</td>
<td>140±4</td>
<td>293±8</td>
<td>112±6</td>
<td>17.3±0.7</td>
<td>4.2±0.2</td>
<td>199±13</td>
<td>144±4</td>
<td>4.8±0.4</td>
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<tr>
<td>4</td>
<td>157±6</td>
<td>288±5</td>
<td>115±10</td>
<td>16.3±1.7</td>
<td>4.1±0.3</td>
<td>215±25</td>
<td>149±1</td>
<td>4.9±0.2</td>
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<tr>
<td>5</td>
<td>126±1</td>
<td>296±9</td>
<td>53±3</td>
<td>16.0±0.7</td>
<td>4.1±0.2</td>
<td>70±15</td>
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</tr>
<tr>
<td>6</td>
<td>113±1</td>
<td>328±5</td>
<td>11±2</td>
<td>2.0±0.2</td>
<td>4.4±0.4</td>
<td>77±10</td>
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Values are mean ± SEM. Number of animals or samples in parentheses. Systolic pressure: groups 1, 2, 3: NS; 4 vs. 1, 2, or 3 and 5 vs. 6: p<0.05. Body weight: groups 1, 2, or 6: NS; 3, 4, or 5 vs. 1, 2, or 6: p<0.05. Urine volume: groups 1 vs. 2 or 3 vs. 4: NS; 1 or 2 vs. 3, 4, or 5: p=0.0001. Plasma aldosterone: groups 1 vs. 2, 3, or 4 and 5 vs. 6: NS; 5 or 6 vs. 1, 2, 3, or 4: p<0.05. Urine sodium: groups 1 vs. 2 or 6 and 3 vs. 4 or 5: NS; 1, 2, or 6 vs. 3, 4, or 5: p=0.0001. Urine potassium: groups 2 vs. 6 and 6 vs. 3, 4, or 5: NS; 1 vs. 2, 3, 4, 5, or 6 and 2 vs. 3, 4, or 5: p<0.05. Plasma aldosterone: groups 1 vs. 2, 3, or 4 and 5 vs. 6: NS; 5 or 6 vs. 1, 2, 3, or 4: p=0.0001. Plasma sodium or potassium: no significant differences between groups.

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**FIGURE 2. Bar graph showing influence of indomethacin on the systolic arterial pressure of aldosterone-treated rats given high sodium diet. *p<0.05 when compared with control group. †p<0.05 when compared with high salt+aldosterone group.**
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HORHAL DIET   HI6H SALT
DffiT ALDOSTERONE AND NORMAL DIET ALDOSTERONt AND  

FIGURE 3. Bar graph showing urinary excretion of 2,3-dinor-6-oxo-prostaglandin F12 (PGI-M), 6-oxo-prostaglandin F12 (6-oxo-PGF1a), and thromboxane B2 (TXB2) in control and in aldosterone-treated rats given normal or high sodium diet. \( *p<0.05; \) \( tp=0.06 \) when compared with corresponding control group.

associated with a moderate rise in the urinary excretion of 6-oxo-PGF1a and TXB2 and a large increase in the urinary levels of PGE2 (Figures 3 and 4). Although the high sodium diet had no statistically significant influence on the urinary excretion of both 6-oxo-PGF1a and TXB2, it exerted a marked inhibitory effect on the urinary levels of PGE2 in both control and aldosterone-treated rats (Figure 4) (103±12 vs. 36±4 ng/24 hr in untreated rats, \( p=0.0006; \) 442±31 vs. 154±10 ng/24 hr in aldosterone-treated rats, \( p=0.0055 \)).

At a dosage of 3.0 μg/kg/day, indomethacin exerted a marked inhibitory effect on the urinary excretion of 2,3-dinor-6-oxo-PGF1a, 6-oxo-PGF1a, and TXB2, but did not lower the urinary levels of PGE2 (Figures 5 and 6).

Discussion

We have described what we believe to be a new model of mineralocorticoid-induced mild experimen-
tal hypertension that simulates human primary aldosteronism more closely than the usual animal models resorting to uninephrectomy and substitution of salt solutions as drinking water. In this model, the chronic subcutaneous infusion of aldosterone to adult male Sprague-Dawley rats maintained on a normal or a high sodium diet was shown to cause a mild and gradual increase in systolic arterial pressure over a period of 4 weeks.

The chronic administration of aldosterone was also associated with a rise in the urinary levels of PGE2, 6-oxo-PGF1a, and TXB2, an observation that suggests aldosterone stimulates the biosynthesis of PGE2 and PGI2 and of TXA2 in the kidney. These results are in agreement with the conclusions of most reports on

FIGURE 4. Bar graph showing urinary excretion of prostaglandin E2 (PGE2) in control and in aldosterone-treated rats given normal or high sodium diet. \( *p<0.05 \) when compared with the corresponding control group. \( tp<0.05 \) when compared with the corresponding group on normal diet.

FIGURE 5. Bar graph showing influence of indomethacin on urinary excretion of 2,3-dinor-6-oxo-PGF1a (PGI-M), 6-oxo-prostaglandin F12 (6-oxo-PGF1a), and thromboxane B2 (TXB2) in control and in aldosterone-treated rats given normal or high sodium diet. \( *p=0.00025; \) \( tp=0.008 \); and \( sp=0.03 \) when compared with the corresponding control group.

FIGURE 6. Bar graph showing lack of influence of indomethacin on the urinary excretion of prostaglandin E2 (PGE2) in control and in aldosterone-treated rats on normal or high sodium diet.
the biosynthesis of prostanoids in various models of mineralocorticoid-induced hypertension.\(^1\) On the other hand, aldosterone did not seem to exert any significant effects on the systemic production of PG\(_{1\alpha}\) as judged by its lack of influence on the urinary excretion of 2,3-dinor-6-oxo-PGF\(_{1\alpha}\). The mechanism by which aldosterone stimulates the biosynthesis of prostanoids in the kidney in vivo is unclear but may be related to its known effects on the activity of phospholipase A\(_2\) and the deacylation/reacylation of phospholipids in vitro.\(^20\),\(^21\)

The rather high plasma levels of aldosterone registered in this study are undoubtedly the consequence of measuring aldosterone directly in unextracted plasma samples, a method that yields high background values.\(^9\) Even if this approach lacked the power to discriminate between actual normal and low plasma levels of aldosterone, it confirmed that the various groups of rats receiving an infusion of aldosterone had increased and comparable plasma levels of aldosterone.

Although the high sodium diet caused a significant increase in the systolic arterial pressure of normal rats, it did not aggravate aldosterone-induced hypertension. This observation implies that the normal diet used in our study already contained sufficient sodium (0.4 g/100 g diet) to allow for the maximal expression of the hypertensive effects of aldosterone in this model. Sodium-deficient diets (0.07 and 0.24 g/100 g diet) have been reported to prevent mineralocorticoid-induced hypertension in rats.\(^1\),\(^22\) The administration of a high sodium diet exerted no significant influence on the biosynthesis of PG\(_{1\alpha}\) and TXA\(_2\) but induced a large reduction in the urinary levels of PGE\(_2\), both in control and in aldosterone-treated rats. Although unequivocal, this latter observation is difficult to explain teleologically if we assume that the urinary level of PGE\(_2\), a natriuretic substance, is an index of its renal synthesis in vivo.\(^15\) It remains possible that dietary sodium could influence, in some way, the proportion of renal PGE\(_2\) escaping the kidney via the blood or the urine.\(^23\),\(^24\) Another possible explanation for this phenomenon may be related to the effect of high dietary sodium on intrarenal PGE\(_2\)-9-ketoreductase activity, which favors the conversion of PGE\(_2\) to PGF\(_{2\alpha}\).\(^25\) This latter possibility cannot be verified as urinary PGF\(_{2\alpha}\) was not measured in this study. Whether the slight but significant increase in systolic arterial pressure observed in normal rats fed a high sodium diet was the consequence of the concomitant reduction in the renal synthesis of PGE\(_2\) is not known.

The observed influence of a high intake of sodium on the urinary levels of prostanoids is also difficult to put in perspective in view of the conflicting literature dealing with this topic: a number of contradictory reports on the positive, the negative, and the lack of influence of either salt intake or urinary volume on the urinary excretion of PGE\(_2\) in both humans and rats have been published over the years.\(^26\)–\(^30\) These discrepancies, which remain difficult to explain, might reflect specie or strain differences, methodological inaccuracies in measuring prostanoids, and great disparities in the models studied in terms of duration of experiments, modes of administration of sodium chloride and, perhaps more importantly in terms of the initial "extracellular volume status" of the subjects. Uehara et al\(^31\) recently isolated from the plasma of uninephrectomized rats submitted to a high sodium intake a factor that exerts an inhibitory effect on the biosynthesis of PGE\(_2\) and TXA\(_2\) in vitro.

Indomethacin, at a dosage of 3.0 mg/kg/day exerted a profound inhibitory effect on the formation of PG\(_{1\alpha}\) and TXA\(_2\) without modifying the renal synthesis of PGE\(_2\) in vivo. A similar observation has recently been described in aspirin-treated rats.\(^32\) Further experiments in our laboratory have shown that doses of indomethacin equal to or greater than 5 mg/kg/day are required to obtain a significant reduction in the urinary levels of PGE\(_2\) in the rat.\(^33\) However, the administration of such high doses of indomethacin to rats for more than a few days proved to be lethal to the animals (R. Hui, P. Falardeau, unpublished observations).

Although inducing a significant reduction in the urinary levels of 2,3-dinor-6-oxo-PGF\(_{1\alpha}\), 6-oxo-PGF\(_{1\alpha}\) and TXB\(_2\), the administration of indomethacin did not modify the hypertensive effect of aldosterone in rats fed a normal diet. This observation seems to preclude a role for PG\(_{1\alpha}\) or TXA\(_2\) in this model of aldosterone-induced mild hypertension under conditions of normal dietary salt intake. On the other hand, the role of PGE\(_2\) in this model of experimental hypertension remains unclarified as our pharmacological intervention failed to reduce its formation in aldosterone-treated rats.

In contrast to its lack of influence on aldosterone-induced hypertension in rats fed a normal diet, indomethacin caused a further increase in arterial pressure in treated rats fed a high sodium diet. Because indomethacin curtailed the synthesis of both PG\(_{1\alpha}\) and TXA\(_2\) but not the renal production of PGE\(_2\), we conclude that the deleterious influence of indomethacin on the arterial pressure of aldosterone-treated rats on a high sodium diet can be ascribed to its inhibitory effect on the formation of vasodilator PG\(_{1\alpha}\) (rather than to the inhibition of the synthesis of vasoconstrictor TXA\(_2\)). These observations imply that PG\(_{1\alpha}\) plays a significant role in modulating the cardiovascular effects of a high sodium intake in aldosterone-treated rats.

The results of this study in a model of aldosterone-induced mild hypertension in the rat indicate that 1) aldosterone exerts a stimulatory effect on the renal synthesis of prostanoids, particularly PGE\(_2\); 2) TXA\(_2\) and PG\(_{1\alpha}\) do not seem to play a role in aldosterone-induced hypertension under conditions of normal dietary salt intake, whereas the role of PGE\(_2\) is unclear; 3) there is a sufficient amount of sodium in a normal diet to allow for the maximal expression of the hypertensive effect of aldosterone; a further increase in dietary sodium intake does not aggravate aldoste-
ron-induced hypertension; 4) PG\textsubscript{12} (from renal or systemic origin) seems to play a significant role in modulating the cardiovascular impact of a high sodium diet in aldosterone-treated rats; and 5) the renal biosynthesis of PG\textsubscript{2} is particularly resistant to the inhibitory effect of indomethacin in vivo.

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KEY WORDS • prostaglandins • mineralocorticoid hypertension \* aldosterone \* salt \* indomethacin

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