In Vivo Production of Prostaglandin I$_2$ in Dahl Salt-Sensitive and Salt-Resistant Rats

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SUMMARY Prostaglandin I$_2$ (PGI$_2$, prostacyclin), a potent vasodilator synthesized by the blood vessels, has been postulated to play a role in hypertension. The purpose of our study was to test this hypothesis by monitoring the in vivo production of PGI$_2$ in Dahl salt-sensitive (S) and salt-resistant (R) rats under normal and high sodium intake. The 24-hour urinary excretion of two endogenous metabolites of PGI$_2$, 2,3-dinor-6-oxo-PGF$_{1\alpha}$ and 2,3-dinor-13,14-dihydro-6,15-dioxo-PGF$_{1\alpha}$, was measured by combined gas chromatography-mass spectrometry (GC-MS) and used as an index of the total production of PGI$_2$ by the animals. The pattern of urinary excretion of these two metabolites in the R and the S rats during the control period indicated that, under normal conditions, early in life the basal production of PGI$_2$ was the same in both groups of rats. Following the chronic administration of a high sodium diet (8.1% sodium chloride, starting at 36 days of age), a significant and sustained increase in the urinary excretion of 2,3-dinor-6-oxo-PGF$_{1\alpha}$ was documented in the R rats (from 37 ± 7 ng/24 hrs at age 35 days to 63 ± 7, 52 ± 4, and 56 ± 10 ng/24 hrs at 50, 60, and 80 days, respectively), whereas the urinary levels of this metabolite decreased slightly in the S rats (from 41 ± 7 ng/24 hrs at age 35 days to 25 ± 5, 30 ± 6, and 28 ± 9 ng/24 hrs at 50, 60, and 80 days, respectively). During the same period, the R rats remained normotensive (103 ± 5 mm Hg, systolic pressure) while the arterial pressure of the S rats increased gradually (to 142 ± 8 and 180 ± 19 mm Hg at ages 60 and 80 days, respectively). After the age of 35 days, the urinary levels of 2,3-dinor-13,14-dihydro-6,15-dioxo-PGF$_{1\alpha}$ decreased sharply and independently of the diet in all groups, suggesting further transformation of this metabolite by ω-oxidation, a well-known age-dependent phenomenon.

Results of this study indicate that a high sodium intake is associated with a rise in the overall production of PGI$_2$ in the salt-resistant but not the salt-sensitive rats. This observation points to the existence of a defect in the production of PGI$_2$ in the salt-sensitive animals, a defect that is uncovered by high sodium intake and that may be causally related to the development of hypertension.

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KEY WORDS • sodium chloride • genetic hypertension • mass spectrometry • metabolite • prostaglandin

PROSTAGLANDIN I$_2$, the major prostaglandin synthesized by the blood vessels and the renal cortex, has potent vasodilator and natriuretic properties, among others. Evidence so far indicates that it is the most likely prostaglandin involved in the modulation of the vascular tone in vivo and, indirectly, the arterial pressure. Consequently, it has been proposed that PGI$_2$ might play a role in hypertension, either as a cause or as an effect. One hypothesis is that a basic defect in the production of PGI$_2$ may contribute to the development and/or maintenance of essential hypertension. Another opposite hypothesis proposes that, in hypertensive individuals, the synthesis of PGI$_2$ may increase secondarily to the rise in arterial pressure, as a protective mechanism for reducing the severity of the hypertension.

The purpose of the present study was to test these hypotheses by monitoring the in vivo production of PGI$_2$ in Dahl salt-sensitive and salt-resistant rats under normal or high dietary sodium intake. This model of experimental hypertension originally developed by Dahl et al. best illustrates the importance of the interrelations between genetic and environmental factors in determining the level of arterial pressure. Indeed, this model shares similarities with human essential hypertension where both the genetic background and environmental factors such as dietary sodium interact strongly to determine the expression of the syndrome.
Materials and Methods

Dahl salt-sensitive and salt-resistant male rats were purchased from the Brookhaven National Laboratory (Upton, New York). The 40 animals included in the study were born during the same week and arrived at our unit a few days after weaning. After a period of equilibration of 10 days under a normal diet (control period), the rats, then aged 36 days, were randomly and evenly distributed into four groups (experimental period): R and S rats, with a normal or a high sodium diet.

The normal diet consisted of standard rat Purina chow containing 0.36% sodium and 1.08% potassium by weight. The high sodium diet was prepared by Ralston Purina Company (Richmond, Indiana), by adding 8 g of sodium chloride per 100 g of normal diet. Food and water were provided ad libitum during the entire period of the study, including the days of urine collections.

The systolic arterial pressure was measured at ages 60 and 80 days in unanesthetized animals by the tail-cuff method. Urinary creatinine was measured by a standard colorimetric method.

To evaluate the in vivo production of PGI₂ by the animals, we monitored the 24-hour urinary excretion of two major endogenous metabolites of PGI₂ in the rat, 2,3-dinor-6-oxo-PGF₁α and 2,3-dinor-13,14-dihydro-6,15-dioxo-PGF₁α. It has been recently demonstrated that the urinary levels of these two metabolites can be used as an index of the total production of PGI₂ in the organism.

Twenty-four-hour urinary collections free of fecal contamination were obtained at ages 35, 50, 60, and 80 days by housing the rats in individual metabolic cages (Nalgene 650-0100). After completion of the collections, the volumes of urine were measured and aliquots were “spiked” with 100 ng each of the following deuterated internal standards: [8,10,10-²H₃]-2,3-dinor-6-oxo-PGF₁α and [8,10,10-²H₃]-2,3-dinor-13,14-dihydro-6,15-dioxo-PGF₁α. Samples were kept frozen at -80°C until analyzed.

The quantitative determination of these two dinor metabolites of PGI₁ was carried out by combined gas chromatography-mass spectrometry according to a procedure based on a stable isotope dilution method fully described elsewhere. Briefly, a urine sample containing the deuterated analogs was acidified to pH 3 and eluted through a “Clin Elut” column (Analytichem) with a solvent mixture of dichloromethane-ethyl acetate (8:2, vol/vol). The eluate was washed with 0.05 M sodium borate buffer (pH 8). The aqueous phase was discarded and the organic solvent evaporated under a stream of nitrogen. The residue was then dissolved in two to three drops of pyridine, and 1 ml of 0.05 M Tris buffer (pH 8) was added. After standing at room temperature for 15 minutes, the mixture was extracted twice with ethyl acetate and the organic phases discarded. The aqueous phase was again acidified to pH 3 and the metabolites finally extracted into dichloromethane. After evaporation of the organic solvent, the metabolites were converted into O-methylxime-trimethylsilyl ether-methyl ester derivatives and analyzed on a Hewlett Packard 5985 GC-MS, by selectively monitoring the M-31 fragments. A column (180 cm x 2 mm) packed with 3% SP-2250 on Supelcoport (100-120 mesh) was used and flushed with helium at a flow rate of 30 ml/min. The oven temperature was maintained at 235°C, the ion source temperature at 200°C, and the electron energy at 70 eV.

Statistics were conducted according to a factorial design, taking into account the repeated nature of the observations. The factors considered were the groups, the times of the observations, and the individuals within each group. The global effect of each factor was assessed by the F statistic. The differences between specific groups were analyzed according to the method of Newman-Keuls whereas the test of Dunnett was used when comparing one group to each of the others.

Results

Urinary Levels of 2,3-Dinor-6-Oxo-PGF₁α and of 2,3-Dinor-13,14-Dihydro-6,15-Dioxo-PGF₁α

During the control period, the patterns of urinary excretion of the two dinor metabolites of PGI₂ were similar in the R and the S rats (tables 1 and 2). Following chronic administration of a high sodium diet, a significant and sustained increase in the urinary excretion of 2,3-dinor-6-oxo-PGF₁α, in the order of 40% to 70% was observed in the R rats, whereas the urinary levels of this metabolite decreased slightly in the S rats (fig. 1). Statistical analysis of these data indicated a highly significant difference in the urinary excretion of 2,3-dinor-6-oxo-PGF₁α between the two groups during high sodium intake (p < 0.01). Normalization of the 24-hour urinary excretion of 2,3-dinor-6-oxo-PGF₁α per milligram of creatinine did not affect the degree of significance of this difference (3.8 ± 0.5, 2.6 ± 0.7, and 2.5 ± 0.3 ng/mg creatinine at 50, 60, and 80 days respectively in the R rats and 1.2 ± 0.3, 1.8 ± 0.3, and 1.6 ± 0.7 ng/mg creatinine at 50, 60, and 80 days respectively in the S rats p < 0.01).

Urinary excretion of 2,3-dinor-6-oxo-PGF₁α remained unchanged throughout the entire study in the R rats fed a normal diet, whereas it decreased slightly in the S rats (table 1). There was no statistically significant difference in the pattern of urinary excretion of this metabolite with time between the S rats fed a normal or a high sodium diet. However, a highly significant difference was observed between the R rats fed a normal diet or a high sodium diet (p < 0.01).

After the control period, the urinary levels of 2,3-dinor-13,14-dihydro-6,15-dioxo-PGF₁α fell sharply in the four groups of rats and became difficult to determine at 60 days (table 2). However, a statistically significant difference in the urinary excretion of this metabolite during the experimental period could still be demonstrated between the R and the S rats fed a high sodium diet (p < 0.01), and between the R rats fed a normal or a high sodium diet (p < 0.05).
PROSTAGLANDIN I₂ AND HYPERTENSION/Falardeau and Martineau

### Table 1. Urinary Excretion of 2,3-Dinor-6-Oxo-PGF₁α (ng/24 hrs) in Dahl Salt-Resistant and Salt-Sensitive Rats Under Normal and High Sodium Diet

<table>
<thead>
<tr>
<th></th>
<th>Control period</th>
<th>Experimental period</th>
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<tr>
<td></td>
<td>35 days old</td>
<td>Experimental group</td>
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<tr>
<td></td>
<td>(t₁)</td>
<td>Group 1: high sodium diet</td>
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<tr>
<td>Salt-resistant</td>
<td>37 ± 7* (n = 20)</td>
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<td>Group 2: normal diet</td>
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<td>(n = 10)</td>
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<tr>
<td>Salt-sensitive</td>
<td>41 ± 7* (n = 10)</td>
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<td>Group 3: high sodium diet</td>
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<td>(n = 7)</td>
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<td></td>
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<td>Group 4: normal diet</td>
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<td>(n = 10)</td>
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</table>

Values are expressed as means ± SD.

*t₁ = R vs S (p > 0.05).

**t₂ + t₃ + t₄ = Group 1 vs 2 (p < 0.01), 1 vs 3 (p < 0.01), 2 vs 4 (p < 0.01), 3 vs 4 (p > 0.05).

### Arterial Pressure

During the entire period of the study, the R rats fed either a normal or a high sodium diet remained normotensive (table 3). Following administration of a high sodium diet, the systolic arterial pressure of the S rats increased gradually (142 ± 8 and 180 ± 19 mm Hg at 60 and 80 days respectively). The S rats fed a normal diet throughout the study did not become hypertensive but their systolic pressure was consistently higher than the systolic pressure of their R counterparts (131 ± 6 and 138 ± 10 vs 110 ± 12 and 109 ± 11 mm Hg at 60 and 80 days; p < 0.001).

![Figure 1. Urinary excretion of 2,3-dinor-6-oxo-PGF₁α in Dahl salt-resistant (R) and salt-sensitive (S) rats under high sodium diet. The values are expressed in ng/24 hrs (means ± sd). Open circles = salt-resistant rats. Black circles = salt-sensitive rats.](http://hyper.ahajournals.org/)

![Figure 2. Systolic blood pressure in Dahl salt-resistant and salt-sensitive rats under normal and high sodium diet. The values are expressed as means ± SD. *t₃ + t₄ = Group 1 vs 3 (p < 0.001), 2 vs 4 (p < 0.001), 1 vs 2 (p > 0.05), 3 vs 4 (p > 0.05).](http://hyper.ahajournals.org/)

### Table 2. Urinary Excretion of 2,3-Dinor-13,14-Dihydro-6,15-Dioxo-PGF₁α (ng/24 hrs) in Dahl Salt-Resistant and Salt-Sensitive Rats Under Normal and High Sodium Diet

<table>
<thead>
<tr>
<th></th>
<th>Control period</th>
<th>Experimental period</th>
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<tr>
<td></td>
<td>35 days old</td>
<td>Experimental group</td>
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<tr>
<td></td>
<td>(t₁)</td>
<td>Group 1: high sodium diet</td>
</tr>
<tr>
<td>Salt-resistant</td>
<td>36 ± 11* (n = 20)</td>
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<tr>
<td></td>
<td></td>
<td>Group 2: normal sodium diet</td>
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<td></td>
<td>(n = 10)</td>
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<tr>
<td>Salt-sensitive</td>
<td>31 ± 7* (n = 10)</td>
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<td></td>
<td></td>
<td>Group 3: high sodium diet</td>
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<td>(n = 10)</td>
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Values are expressed as means ± SD.

*t₁ = R vs S (p > 0.05).

**t₂ + t₃ = Group 1 vs 2 (p < 0.01), 1 vs 3 (p < 0.05), 2 vs 4 (p > 0.05).

### Table 3. Systolic Blood Pressure in Dahl Salt-Resistant and Salt-Sensitive Rats Under Normal and High Sodium Diet

<table>
<thead>
<tr>
<th></th>
<th>Experimental group</th>
<th>60 days</th>
<th>80 days</th>
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<tbody>
<tr>
<td>Salt-resistant</td>
<td>Group 1: high sodium diet</td>
<td>110 ± 8</td>
<td>103 ± 3</td>
</tr>
<tr>
<td></td>
<td>Group 2: normal sodium diet</td>
<td>110 ± 12</td>
<td>109 ± 11</td>
</tr>
<tr>
<td>Salt-sensitive</td>
<td>Group 3: high sodium diet</td>
<td>142 ± 8</td>
<td>180 ± 19</td>
</tr>
<tr>
<td></td>
<td>Group 4: normal sodium diet</td>
<td>131 ± 6</td>
<td>138 ± 10</td>
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</table>

Values are expressed as means ± SD.

*t₃ + t₄ = Group 1 vs 3 (p < 0.001), 2 vs 4 (p < 0.001), 1 vs 2 (p > 0.05), 3 vs 4 (p < 0.001).
Discussion

Levels of the major urinary metabolites of a given prostaglandin have been used by many investigators as an index of the total production of that prostaglandin by the organism. 14-18 The validity of the assumption was unequivocally established several years ago for prostaglandins of the E and F series 19 and, more recently, for prostaglandin I 2.12> 13 In the present study with Dahl salt-sensitive and salt-resistant rats, we used that approach, the only one presently available to indirectly evaluate the overall production of PGI 2 in vivo by the intact animal.

The pattern of urinary excretion of 2,3-dinor-6-oxo-PGF 2a and of 2,3-dinor-13,14-dihydro-6,15-dioxo-PGF 2a in the R and the S rats during the control period indicates that, under normal conditions, early in life the basal production of PGI 2 was the same in both groups of rats (fig. 1). Following the chronic administration of a high sodium diet, the urinary levels of 2,3-dinor-6-oxo-PGF 2a increased markedly and in a sustained fashion in the R rats whereas they decreased slightly in the S rats. The large fall in the urinary levels of 2,3-dinor-13,14-dihydro-6,15-dioxo-PGF 2a observed in the four groups of rats is obviously independent of the diet and is most likely due to an age-dependent activation of the enzymes involved in the ω-oxidation of prostaglandins, with further transformation of this metabolite into 20-hydroxy- and 20-carboxy-derivatives.20 Nevertheless, even though the levels of this metabolite were low, they were still significantly higher in the R compared to the S rats during high sodium intake.

These results indicate that a high sodium intake was associated with a rise in the overall production of PGI 2 in the R but not in the S rats. We think that the lack of increase in the urinary levels of 2,3-dinor-6-oxo-PGF 2a in the S rats reflects a lack of increase in the production of PGI 2 and that it cannot be explained simply in terms of a possible renal failure to excrete the metabolite. Although abnormalities of the renal function of the S rats have been described in vitro, no defect severe enough to explain a 40% to 70% discrepancy in the urinary levels of this metabolite between the R and the S rats has ever been reported, especially at an early phase of high sodium intake. 21-24 The discrepancy already existed at the beginning of the experimental period, at a time when the arterial pressure of the S rats was only slightly elevated and when secondary renal damage should have been mild, at the most.25 Indeed, the clearances of inulin and paraaminohippurate have been reported normal in Dahl salt-sensitive rats after 6 weeks on a high sodium diet, at a time when the blood pressure was moderately elevated.26 These observations argue strongly against the probability that a simple reduction in the renal clearance of this metabolite in the S rats could explain the differences observed.

Taken together, our data point to the existence of a defect in the production of PGI 2 in Dahl salt-sensitive rats. This observation contrasts with the results obtained by other investigators with the Okamoto-Kyoto strain of spontaneously hypertensive rats (SHR), in which increased vascular synthesis of prostaglandins, compared to normal Wistar rats, was observed in vitro.27, 28 This led to the proposal that, in these animals, the increase in PGI 2 formation is secondary to the rise in arterial pressure and contributes to reduce the severity of the hypertension. However, no quantitative determination of the in vivo production of PGI 2 in this strain of genetically hypertensive rats has yet been published to substantiate this hypothesis.

Conclusions

Our results point to the existence of a defect in the production of PGI 2 in Dahl salt-sensitive rats, a defect that is uncovered by high sodium intake and that may be causally related to the development of hypertension. We propose that, following a prohypertensive stimulus like high sodium intake, the salt-resistant rat remains normotensive because of its capacity to increase its vascular synthesis of PGI 2, which, by virtue mainly of its vasodilator properties, can prevent a rise in the arterial pressure (fig. 2). The salt-sensitive rat, lacking this potential to achieve a compensatory increase in PGI 2 synthesis, gradually becomes hypertensive under these circumstances. The nature of the defect remains speculative. However, recent observations of a deficient renal production of PGE 2 in the salt-sensitive rat 29, 30 suggest that a more generalized abnormality in prostaglandin biosynthesis may well be present in these animals. Since prostaglandins are known to act as modulators of the action of several vasoactive hormones,1, 3, 4, 32 a defect in the vascular synthesis of prostaglandins could perhaps explain the inappropriately high peripheral and renal vascular resistance observed in the salt-sensitive compared to the salt-resistant rat after high sodium intake, with a consequent rise in the arterial pressure. 33, 34
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References


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