Papillary Collecting Tubule Synthesis of Prostaglandin E₂ in Dahl Rats

GERALD M. REID, RICHARD G. APPEL, AND MICHAEL J. DUNN

SUMMARY Isolated kidneys of Dahl salt-sensitive rats (DS) excrete sodium less readily than those of Dahl salt-resistant rats (DR). The collecting tubule is an important source of papillary prostaglandin E₂ and is a site of significant sodium reabsorption. We cultured renal papillary collecting tubule cells from 5-week-old, prehypertensive DS and DR on a low salt diet and also after 14 weeks of high salt feeding, and we measured prostaglandin E₂ synthetic capacity. Unstimulated renal papillary collecting tubule cells from 5-week-old DS produced 62 ± 5% less prostaglandin E₂ than did comparable cells from DR (p<0.001). The cells from DS also synthesized less prostaglandin E₂ after stimulation with the calcium ionophore A23187 (67 ± 6% of control; p<0.001) or the addition of exogenous arachidonate (74 ± 7% of control; p<0.01). Urinary prostaglandin E₂ excretion was also diminished in the 5-week-old DS compared with their salt-resistant counterparts (18.1 ± 1.3 vs 23.9 ± 1.7 ng/24 hr; p<0.025). After high salt feeding, the DS became hypertensive but the DR remained normotensive. Renal papillary collecting tubule cells cultured from these DS continued to produce less prostaglandin E₂ than those from control rats, both in the basal state (60 ± 12% of control; p<0.09) and after stimulation with ionophore (62 ± 2% of control; p<0.002). In these older animals, the DS continued to underexcrete prostaglandin E₂ compared with the DR (29.7 ± 3.2 vs 42.2 ± 6.1 ng/24 hr; p<0.08). The underproduction of prostaglandin E₂ in the papillary collecting tubule of DS may play a role in their inadequate renal natriuretic capacity and contribute to the onset and maintenance of salt-induced hypertension in this strain. (Hypertension 11: 179-184, 1988)

KEY WORDS  • Dahl rats • prostaglandin E₂ • collecting tubule • cell culture

DAHL salt-sensitive rats (DS) become hypertensive when fed a diet rich in NaCl, whereas Dahl salt-resistant rats (DR) remain normotensive on a high salt diet. The kidney is of major importance in determining this response. Renal cross-transplantation¹,² experiments between DS and DR have shown that salt-sensitivity transfers with the genotype of the donor. Using isolated perfused kidneys, Tobian et al.³ and Maude and Ko⁴ found that, at similar perfusion pressures, DR kidneys display a greater natriuresis than DS kidneys and that, to quantitatively equalize natriuresis, DS kidneys need a higher perfusion pressure. This defect in natriuresis probably leads to hypertension through a more complex mechanism than simple volume expansion.⁵ Natriuretic substances may be released that have diffuse effects on ion transport, leading to increased vascular reactivity.

A number of observations suggest that the synthetic capacity for renal prostaglandin E₂ (PGE₂) may be different between the DS and DR strains, especially in the medulla. Urinary PGE₂ excretion, which is predominantly medullary in origin,⁶ was decreased in the DS as compared with the DR, at prehypertensive and hypertensive phases.⁷ The PGE₂ content of snap-frozen renal papillae was also less in the DS than in the DR, both before and after high salt diets.⁸ Renal medullary microsomes from DS also underproduced PGE₂ as compared with microsomes from DR.⁹ Reduction of medullary PGE₂ may be important in the defective natriuresis observed in the DS strain. Prostaglandins have a role in promoting natriuresis in the inner medullary collecting duct in volume-expanded rats,¹⁰ a situation somewhat analogous to high salt feeding. Indeed, the inner medullary collecting duct may reabsorb up to 6% of the filtered sodium load¹¹ in volume expansion. This segment is a rich source of medullary PGE₂,¹²,¹³ Microdissected rabbit nephron segments from the medullary collecting tubule synthesized greater amounts of PGE₂ than any other segment of the tubule.¹⁴
As cells from the renal papillary collecting tubule (RPCT) can be cultured and produce notable amounts of PGE$_2$, we hypothesized that RPCT cells from DS might express a genetic defect in PGE$_2$ synthesis as compared with RPCT cells from DR. We tested this hypothesis using cultured RPCT cells from prehypertensive and hypertensive DS and age-matched DR controls. We found significantly lower PGE$_2$ synthesis by RPCT cells from DS, whether prehypertensive or hypertensive.

Materials and Methods

Animals
Weanling 3½-week-old male DS and DR were purchased from Brookhaven National Laboratories (Upton, NY, USA). They were maintained on 0.3% NaCl rat chow until 5 weeks of age. Initial urine collections and blood pressure measurements were performed, and the animals were either killed for cell culture experiments or begun on 4% NaCl rat chow for a total of 14 weeks. Color-coded 0.3% NaCl and 4% NaCl rat chow were obtained from ICN Biochemicals (Cleveland, OH, USA).

Blood Pressures
Blood pressures for the 5-week-old rats were obtained directly through an intra-aortic catheter with the rats under pentobarbital anesthesia (40 mg/kg i.p.). Indirect tail-cuff measurements obtained with the rats under light ether anesthesia were used after 9 and 14 weeks of high salt feeding. Each culture was obtained with the rats under pentobarbital anesthesia (40 mg/kg i.p.), and kidneys were rapidly excised and placed in ice-cold Hanks balanced salt solution. Medullary slices were prepared from DS and DR animals were killed, and their kidneys were placed in ice-cold phosphate-buffered saline. Papillae were carefully dissected and minced. After a 2-hour collagenase (CLS II Lot 4176, Worthington, Freehold, NJ, USA) digestion at 37°C, the incubates were exposed to a brief hypotonic shock followed by a 10% albumin density centrifugation. Cells were initially seeded in a Hams F-12–Dulbecco’s minimal essential medium containing 10% fetal bovine serum to facilitate attachment. After 24 hours this medium was changed to a freshly prepared, defined, K-1 epithelial growth medium.

After 72 hours of culture, the cells were nearly confluent and were used for biochemical study. The rate of cell growth was similar for the two strains. Culture wells were washed twice and then incubated for 30 minutes in a 5% CO$_2$, 95% O$_2$ atmosphere at 37°C in either minimal essential medium alone (basal) or in medium containing a final concentration of 2 μM Ca$^{2+}$ ionophore A23187 (Calbiochem-Behring, San Diego, CA, USA) or arachidonic acid (Sigma Chemical, St. Louis, MO, USA), 5 μg/ml. At the end of the incubation, the supernates were aspirated and quickly frozen. The cell cultures were digested in 1 N NaOH and then assayed by the technique of Lowry et al. for total cell protein per well. PGE$_2$ was measured on unextracted samples by radioimmunoassay.

Renal Papillary Collecting Tubule Cultures
RPCT cultures were done on 5-week-old rats or after 14 weeks of high salt feeding. Each culture was obtained from the pooled kidneys of two or three rats from the same strain. The cultures were carefully paired prospectively such that kidney isolations and preparation were done concurrently from groups of DR and DS, all isolation vehicles and media were identical for each parallel culture, and each culture dish contained wells from both DR and DS cultures.

Cell cultures were perfused as previously described. Animals were killed, and their kidneys were placed in ice-cold phosphate-buffered saline. Papillae were carefully dissected and minced. After a 2-hour collagenase (CLS II Lot 4176, Worthington, Freehold, NJ, USA) digestion at 37°C, the incubates were exposed to a brief hypotonic shock followed by a 10% albumin density centrifugation. Cells were initially seeded in a Hams F-12–Dulbecco’s minimal essential medium containing 10% fetal bovine serum to facilitate attachment. After 24 hours this medium was changed to a freshly prepared, defined, K-1 epithelial growth medium.

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Urine Studies
Twenty-four-hour urine samples were collected in metabolic cages at 5 weeks of age and again after 14 weeks of high salt feeding. Sodium azide, 25 mg, and meclofenamate, 250 mg, were added to each urine container, and 2.5% dextrose was added to the drinking water of the young animals to ensure adequate urine volume. Aliquots (1–2 ml) from the urine samples were acidified to pH 3.0 to 3.5 with citric acid, and [H]$^3$POGE$_2$ (2000 cpm, specific activity, 160 Ci/mmol, Amersham Arlington Heights, IL, USA) was added to each sample to determine recovery. The samples were then extracted twice with ethyl acetate, dried, and dissolved in toluene/ethyl acetate/methanol, 60:40:2. Silicic acid (Mallinckrodt, McGaw Park, IL, USA) columns were prepared with toluene/ethyl acetate/methanol, 60:40:20. After an elution with toluene/ethyl acetate/methanol, 60:40:20, the eluates were adjusted to pH 3.0 to 3.5 with citric acid, and PGE$_2$ was eluted with the 60:40:20 solvent. Recoveries were 50 to 75%. PGE$_2$ was measured by radioimmunoassay using PGE$_2$ antisera from Institut Pasteur (Paris, France).

Results
At 5 weeks of age, while fed only the 0.3% NaCl diet, both DS and DR groups were normotensive. Intra-aortic mean pressure was higher in the DS than in

Statistical Analyses
All results were analyzed using independent t tests except those for Tables 2 and 3, where statistical calculations were based on randomized block analysis of variance on log-transformed data.
the DR (97.4 ± 2.9 vs 85.3 ± 1.0 mm Hg; p < 0.001). Blood pressures rose in both groups after high salt feeding, more dramatically in the DS strain. After 14 weeks of a 4% NaCl diet, the DS became frankly hypertensive compared with age-matched DR (160 ± 5.8 mm Hg vs 109.8 ± 1.6 mm Hg; p < 0.001).

In the young, prehypertensive animals, the DS excreted less urinary PGE<sub>2</sub> than the DR (Table 1), although total urinary volumes were similar between the groups. After high salt feeding, PGE<sub>2</sub> excretion increased in both 19-week-old DS and DR. However, DS continued to excrete less PGE<sub>2</sub> than DR, again with no difference between the strains in urinary volume (see Table 1).

RPCT PGE<sub>2</sub> production was assessed in the basal condition (i.e., unstimulated RPCT cells) and after stimulation with the Ca<sup>2+</sup> ionophore A23187 (2 μM) or arachidonic acid (5 μg/ml). The means of replicate wells for each experiment are shown in Table 2. In the basal, as well as in each of the stimulated conditions, the RPCT cells from DS rats synthesized less PGE<sub>2</sub> than the cells from DR (expressed as pg/μg cell protein). The cell protein content was similar between each of the paired, cultured strains.

### Table 1. 24-Hour Urinary Collections in 5- and 19-Week-Old DS and DR

<table>
<thead>
<tr>
<th>Strain</th>
<th>PGE&lt;sub&gt;2&lt;/sub&gt; (ng/24 hr)</th>
<th>Urine volume (ml/24 hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-week-old</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DR (n = 22)</td>
<td>23.9 ± 1.7</td>
<td>44.5 ± 2.0</td>
</tr>
<tr>
<td>DS (n = 22)</td>
<td>18.8 ± 1.3&lt;sup&gt;*&lt;/sup&gt;</td>
<td>43.6 ± 2.4</td>
</tr>
<tr>
<td>19-week-old</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DR (n = 18)</td>
<td>42.2 ± 6.1†</td>
<td>20.1 ± 2.0</td>
</tr>
<tr>
<td>DS (n = 17)</td>
<td>29.7 ± 3.2‡</td>
<td>22.7 ± 2.2</td>
</tr>
</tbody>
</table>

*All values are means ± SEM.
†p < 0.025, compared with values for 5-week-old DR; †p < 0.005, compared with values for same strain at 5 weeks of age; ‡p < 0.08, compared with values for 19-week-old DR.

In an effort to negate the week-to-week variability in cultured-cell PGE<sub>2</sub> synthesis and stimulability, we decided to assess more carefully each weekly experiment to isolate the effect of strain origin alone. For each culture pair, in each of the three assay conditions, the mean production in the DS wells can be expressed as a percentage of the concurrent DR well production. These data are graphically displayed in Figures 1, 2, and 3. When looked at in this way, the DS cells again produced less PGE<sub>2</sub> than their DR counterparts in basal and in both stimulated states.

RPCT cultures were obtained from three additional sets of DS and DR animals after 14 weeks of high salt feeding. PGE<sub>2</sub> synthetic capacity was tested under both basal and A23187-stimulated conditions for each culture. The means for the six replicate wells in each experiment are shown in Table 3. DS cultures produced less PGE<sub>2</sub> than DR in all cultures under both conditions. This observation was statistically significant when the cells were stimulated, but it failed to reach significance in the basal state because of the small number of studies performed. When the data are expressed as a percentage of PGE<sub>2</sub> production for each culture, the DS cells synthesized 60 ± 12% of DR production in the basal state (p < 0.09) and 62 ± 2% of DR production after stimulation (p < 0.002). Finally, medullary slices were stimulated with 30 μM arachidonic acid from five 19-week-old rats of each strain, after the high salt diet. The DS produced 2551 ± 624 pg PGE<sub>2</sub>/min/mg dry weight, while the DR produced 4389 ± 578 pg PGE<sub>2</sub>/min/mg dry weight (p < 0.06).

### Table 2. Renal Papillary Collecting Tubule PGE<sub>2</sub> Production in 5-Week-Old Rats on Low Salt Intake

<table>
<thead>
<tr>
<th>Experiment no.</th>
<th>PGE&lt;sub&gt;2&lt;/sub&gt; production (pg/μg cell protein/30 min)</th>
<th>A23187 (2 μM)</th>
<th>Arachidonate (5 μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DS</td>
<td>DR</td>
<td>DS</td>
</tr>
<tr>
<td>1</td>
<td>2.4</td>
<td>4.8</td>
<td>27.8</td>
</tr>
<tr>
<td>2</td>
<td>0.9</td>
<td>1.7</td>
<td>11.6</td>
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<tr>
<td>3</td>
<td>4.0</td>
<td>4.5</td>
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<td>1.6</td>
<td>2.7</td>
<td>10.4</td>
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<td>7</td>
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<td>2.0</td>
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<tr>
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<td>1.7</td>
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</tr>
<tr>
<td>9</td>
<td>1.5</td>
<td>2.4</td>
<td>10.9</td>
</tr>
</tbody>
</table>

Mean ± SEM: 2.0 ± 0.3* 3.2 ± 0.4 14.8 ± 2.8† 22.2 ± 3.9 51.1 ± 14.8‡ 70.8 ± 22.5

*F = 39.16, p < 0.001; †F = 22.52, p < 0.002; ‡F = 15.37, p < 0.01; compared with respective values for DR.

### Discussion

The kidneys of Dahl rats are important determinants of the blood pressure response to a high salt diet. The DS have defective sodium chloride excretion as compared with the DR strain. Kidneys from DS also have decreased medullary PGE<sub>2</sub> content. PGE<sub>2</sub>, an

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**Note:** The above text is a representative sample of the content from the provided page. For a comprehensive understanding, it is recommended to review the original document.
abundant medullary autacoid, has been shown to inhibit sodium chloride transport in the thick ascending limb of Henle of the mouse \(^{23}\) and the cortical collecting tubule of the rabbit. \(^{24}\) There is also indirect evidence, in vivo, of prostaglandin-mediated inhibition of sodium reabsorption in the medullary collecting tubule of the rat. \(^{10}\)

To investigate the onset phase of hypertension in Dahl rats, we conducted our initial studies in young, prehypertensive animals. Urinary PGE\(_2\) excretion, which is predominantly of renal inner medullary origin, \(^{6}\) was clearly decreased in the DS compared with the DR, confirming the work of others. \(^{7}\) We next looked at RPCT cells, cultured from the inner medullary collecting tubules of DS and DR, and showed that RPCT cells derived from DS synthesized less PGE\(_2\) than those from DR. The reduced PGE\(_2\) synthesis (26–38% decrement) was detectable in both basal and stimulated (calcium ionophore or arachidonic acid) conditions. We believe that this finding may represent a genetically inherited difference between the strains and may play a role in the defective natriuresis of the DS kidneys. The inner medullary collecting tubule is the final site for salt transport in the nephron and can play a critical role in determining the final electrolyte content of the urine. In the studies of Stein et al., \(^{11}\) up to 6% of the filtered sodium load was reabsorbed in this segment in young, volume-expanded rats.

We also felt that a defect in PGE\(_2\) production may persist in older animals and thereby be important in the maintenance phase of salt-induced hypertension in the DS. After 14 weeks of high salt feeding, the DS were hypertensive whereas the DR remained normotensive. At this time, urinary PGE\(_2\) excretion was decreased in

![Figure 1](image1.png)  
**Figure 1.** Unstimulated PGE\(_2\) synthesis in concurrent paired DS and DR renal papillary collecting tubule (RPCT) cultures. In each pair, the DR culture synthesis was set at 100% and DS synthesis expressed as a percentage of DR synthesis. The data are shown for nine separate cultures. The actual PGE\(_2\) production is also given. PGE\(_2\) synthesis was significantly different between groups (p<0.001, DS vs 100% for DR values, by independent t test).

![Figure 2](image2.png)  
**Figure 2.** PGE\(_2\) synthesis in concurrent paired DS and DR renal papillary collecting tubule (RPCT) cultures stimulated with Ca\(^{2+}\) ionophore A23187 (2 \(\mu\)M). The data are shown for nine separate cultures. Actual PGE\(_2\) production is also given. PGE\(_2\) synthesis was significantly different between groups (p<0.001, DS vs 100% for DR values, by independent t test).

![Figure 3](image3.png)  
**Figure 3.** PGE\(_2\) synthesis in concurrent paired DS and DR renal papillary collecting tubule (RPCT) cultures stimulated with arachidonic acid (5 \(\mu\)g/ml). The data are shown for eight separate cultures. Actual PGE\(_2\) production is also given. PGE\(_2\) synthesis was significantly different between groups (p<0.01, DS vs 100% for DR values, by independent t test).

![Table 3](image4.png)  
**Table 3.** Renal Papillary Collecting Tubule PGE\(_2\) Production in 19-Week-Old Rats After High Salt Feeding

<table>
<thead>
<tr>
<th>Experiment no.</th>
<th>Basal</th>
<th>A23187 (2 (\mu)M)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DS</td>
<td>DR</td>
</tr>
<tr>
<td>1</td>
<td>1.8</td>
<td>4.0</td>
</tr>
<tr>
<td>2</td>
<td>1.6</td>
<td>1.9</td>
</tr>
<tr>
<td>3</td>
<td>0.9</td>
<td>1.8</td>
</tr>
</tbody>
</table>

Mean ± SEM: 1.4 ± 0.3 \(*\) 2.6 ± 0.7 \(*\) 15.8 ± 1.3 \(*\) 25.5 ± 1.6

*F = 8.13, p<0.11; \(t\) = 3.71, \(p<0.002\) vs respective values for DR.
the DS compared with the DR. RPCT cultures from the DS synthesized 40% less PGE₂ than cultures from the DR in the basal and 38% less in Ca⁺⁺ ionophore-stimulated experiments. In addition, medullary slices from 19-week-old DS released less PGE₂ after the addition of exogenous arachidonic acid than slices from DR, further supporting the cell culture findings.

Work from the laboratory of Tobian et al. underscores the possible importance of the PGE₂ deficit in the DS strain. When these rats were placed on a high linoleic acid diet, renal papillary PGE₂ content rose and the salt-induced rise in blood pressure was significantly blunted. Linoleic acid is chain-elongated and desaturated to the prostaglandin precursor arachidonic acid. Augmented PGE₂ synthesis in peripheral vasculature as well as the kidney may be important in the decreased salt sensitivity in these studies. It should be noted that both linoleic acid, which increased renal prostaglandins, and linolenic acid, which decreased renal prostaglandins, decreased blood pressure in rats with renovascular hypertension.

Although patients with essential hypertension are a heterogeneous group, renal prostaglandin synthesis may be important in some instances. Urinary PGE₂ excretion studies in these patients have yielded conflicting results. However, there appears to be a group of essential hypertensive patients with markedly subnormal urinary PGE₂ excretion, and these patients may also belong to the subgroup that is highly salt-sensitive. Indeed, in one well-studied case, urinary PGE₂ was extremely low and hypertension was salt-sensitive. Furthermore, linoleic acid diets have lowered blood pressure in essential hypertensive humans, although the mechanism of blood pressure reduction is unknown.

Our results, demonstrating decreased PGE₂ synthesis by the RPCT cells from DS in culture, do not unambiguously indicate which enzymatic step in the prostaglandin biosynthetic sequence may be involved. Critical enzymatic sites that regulate the eventual production of PGE₂ include the acylation and deacylation of arachidonic acid in membrane phospholipids (acyltransferase, phospholipase), the cyclooxygenation of arachidonic acid to endoperoxides (cyclooxygenase), and the conversion of endoperoxides to PGE₂ (PGE₂ isomerase). The reduced PGE₂ synthesis in the DS strain in the basal state is consistent with an alteration at any of these sites. The reduced stimulability of PGE₂ by the Ca⁺⁺ ionophore A23187 is consistent with defective phospholipase activation or deficient availability of arachidonate but is also consistent with reduced activity of the enzymes beyond the phospholipase step. Abnormalities of these more distal enzymes are further suggested by reduced PGE₂ synthesis in response to arachidonic acid in the DS-derived RPCT cells, especially from the younger, prehypertensive animals. Evaluation of the PGE₂ isomerase would only be possible from the younger, prehypertensive animals. Evaluation of the PGE₂ isomerase would otherwise be possible with the addition of PGH₂ to the cells in culture. Hence, we must conclude that the defect of PGE₂ synthesis may be at multiple enzymatic steps or could be solely attributable to defective cyclooxygenase or PGE₂ isomerase.

In summary, we postulate that defective medullary PGE₂ synthesis in DS may be one factor leading to a decreased capacity to excrete salt, especially in response to an increased dietary salt load. The decreased salt excretion leads to higher blood pressure, probably through a series of complex interactions. The higher perfusion pressure brings the DS back into salt balance, at the expense of increased systemic pressure. This mechanism may be operative in both the genesis and maintenance phases of salt-induced hypertension in this model.

Acknowledgments

The authors thank Cheryl Subjeck and Michael Simonson for technical assistance and Linda Goldberg and Norma Minear for preparation of the manuscript.

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G M Reid, R G Appel and M J Dunn

_Hypertension_. 1988;11:179-184
doi: 10.1161/01.HYP.11.2.179

_Hypertension_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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