SUMMARY Development of hypertension in Dahl salt-sensitive rats (DS) is accompanied by reduced renomedullary prostaglandin synthesis, which may be responsible for their lower natriuretic capacity. To examine the changes in renomedullary prostaglandin E_2 synthesis, the effects of high (8.0%) and normal (0.6%) NaCl diets were examined in DS and in Dahl salt-resistant rats (DR). In response to an 8.0% NaCl diet, the number of prostaglandin E_2 receptors in the renal outer medulla of DR increased (2.97 ± 0.2 vs 2.18 ± 0.2 pmol/mg on 0.6% NaCl diet) while no change was noted in their affinities (K_a, 9.5 ± 0.2 vs 9.4 ± 0.3 nM). Receptor number and affinity in the renal cortex, inner medulla, and liver of DR were not affected. In contrast, renomedullary receptors of DS had a lower affinity than those of age-matched DR (K_a, 13.9 ± 0.2 nM on 0.6% NaCl diet and 14.0 ± 0.3 nM on 8.0% NaCl diet) and did not increase in number after a high salt diet. This apparent inability of DS to modulate prostaglandin receptors may contribute to their susceptibility to salt-induced hypertension. (Hypertension 8: 566-571, 1986)

KEY WORDS • prostaglandins • receptors • natriuresis • adenylate cyclase • Dahl rats • hypertension

A n important epidemiological feature of the prevalence of human essential hypertension is its dependence on the level of dietary salt intake. Clues about the biochemical and physiological basis for this dependency have come from studies of Dahl salt-sensitive rats (DS), which become hypertensive in response to high dietary salt. In this strain, the kidney's decreased ability to excrete a sodium load is closely linked to the pathogenesis of hypertension. We, as well as others, have demonstrated that the capacity of renomedullary membranes from DS to synthesize prostaglandins is significantly lower than that of Dahl salt-resistant rats (DR) on either regular (0.6%) or high (8.0%) NaCl diets. In view of the important role of prostaglandins in modulating natriuresis and renal blood flow, these between-strain differences in renal prostaglandin synthesis may explain the disparate susceptibilities of the two strains to the effects of salt.

Prostaglandins exert their physiological effects on responsive tissues by interacting with specific membrane-bound receptors. The numbers and properties of these receptors can be altered by physiological conditions or pathological processes and either amplify or restrict the cellular effects of ambient prostaglandins. Therefore, the physiological importance of altered renal prostaglandin synthesis in DS should be interpreted within the context of concomitant changes in renal prostaglandin receptors. In the present study, we examined the response of prostaglandin E_2 (PGE_2) receptors to salt in DS and DR in an effort to explain the propensity of DS to become hypertensive.

Materials and Methods

Age-matched male DS and DR (Brookhaven National Laboratories, Upton, NY, USA) and Sprague-Dawley rats (Bio-Lab, St Paul, MN, USA) were kept in groups of two or three in filter-protected boxes in an air-conditioned room under constant temperature (75°F) and controlled light time (11-hour light, 13-hour dark cycle). Systolic blood pressure was measured between 0800 and 1000 using a tail-cuff sphygmomanometer with the rats under light methoxyflu-
per group), which were fed 0.6% or 8.0% NaCl diets for 5 to 6 weeks.

Immediately after death, the kidneys of each rat were collected in ice-cold buffer containing 0.01 M tris (hydroxymethyl)aminomethane (Tris) HCl (pH 7.6), 0.15 M NaCl, and 30 μM indomethacin. The inner and outer medulla and cortex from each pair of kidneys were then dissected and homogenized separately in the buffer. In some experiments, portions of the liver were also processed similarly. The homogenate then was centrifuged at 700 g for 15 minutes, the supernatant was collected, the pellet was rehomogenized, and centrifugation was repeated. The combined supernatants were centrifuged at 40,000 g for 30 minutes, and the resultant pellet was resuspended in homogenization buffer to which MgCl₂ (0.002 M) had been added. The protein content was determined by the method of Lowry et al., and the concentration was adjusted to 1 mg/ml.

Chemicals were obtained from Sigma Chemical Company (St. Louis, MO, USA), [³²P]PGE₁ (160 Ci/mmol) from Amersham/Searle (Arlington Heights, IL, USA), and [α-³²P] adenosine 5' -triphosphate (ATP; 33 Ci/mmol) from New England Nuclear Company (Boston, MA, USA).

For the PGE₁ binding assay, the medium contained 0.2 ml of the sample (0.15–0.25 mg protein), 0.1 to 0.2 μCi [³²P]PGE₁, and unlabeled PGE₁ (total PGE₁ concentration, 7–14 nM) in a total volume of 0.21 ml. The reaction mixture was incubated at 37°C for 20 minutes, and the assay was terminated by immersing the tubes in an ice-cold bath and adding 2.0 ml of cold 0.05 M Tris HCl (pH 7.5). The mixture was passed through Whatman GF/C filters (Clifton, NJ, USA), which were then washed twice and dried, and retained radioactivity was counted. Non specific binding, determined in the presence of excess (1.3 μM) unlabeled PGE₁, averaged 5% of total. Maximal number of receptors and affinities were calculated from Scatchard plots of the binding data.

For adenylate cyclase assays, the medium contained 25 mM Tris acetate (pH 7.6), 5 mM Mg²⁺ acetate, 1 mM dithiothreitol, 0.01 mM guanosine 5'-triphosphate, 0.5 mM [³²P]ATP, 5 mM creatine phosphate, 250 U/ml creatine phosphokinase, 0.05 mM cyclic adenosine 3',5'-monophosphate (cAMP), 0.5 mg/ml bovine serum albumin, and 20 to 30 μg of membrane protein in a total volume of 0.05 ml. When added, PGE₁ was present at 10⁻⁸ to 10⁻³ M and NaF at 6 mM final concentrations. The mixture was incubated at 37°C for 15 minutes, and the reaction was stopped by adding 2% sodium dodecyl sulfate, 45 mM ATP, and 1.3 mM cAMP followed by [³²P]cAMP (8000–10,000 cpm; New England Nuclear). Recovery of generated [α-³²P]cAMP was performed according to the method of Salomon et al.

Results

Table 1 provides the final body weight and systolic blood pressure measurements in the DS and DR killed 2 weeks after randomization to either normal or high salt diets. The DS on a high salt intake showed a significant rise in blood pressure and gained less weight than did DS on regular salt diet. In contrast, neither body weight nor blood pressure was affected by the high salt intake in DR. The histology of the renal medulla was unchanged in both strains. Vascular changes were observed as previously described.

As shown in Table 2, the high salt diet significantly increased PGE₁ binding in the outer medulla of DR and Sprague-Dawley rats but not in DS. Scatchard plots of the binding data revealed that the high salt diet increased the number of PGE₁ receptors in the renal outer medulla of DR without affecting the affinity, as indicated by the unchanged Kᵣ (Figure 1). In contrast, variations in dietary salt intake did not influence either the number or the affinity of the PGE₁ receptors in the DS rat (Figure 2). The data for all experimental groups are summarized in Table 3. The affinity of outer medullary receptors from DS on either diet was considerably lower than that of age-matched and diet-matched DR or Sprague-Dawley rats. Salt loading did not affect the affinity of the outer medullary receptors in any of the groups, and this indicates that the higher PGE₁ binding in DR and Sprague-Dawley rats on the high salt diet was due to an increase in total receptor numbers. Although a high salt diet was ineffective in increasing PGE₁ binding in DS as a group, a significant difference in PGE₁ binding was noted between DS that responded to the high salt diet with an increase of systolic blood pressure to or above 160 mm Hg (n = 8; 844 ± 16 fmol/mg) and those that had a smaller hypertensive response (n = 10; 1218 ± 52 fmol/mg; p < 0.01). There was a statistically significant negative correlation (r = -0.68, p < 0.01) between PGE₁ binding by outer medullary membranes and the blood pressure at death in DS fed an 8.0% NaCl diet (Figure 3).

The mechanism of the effect of a high salt diet on renal prostaglandin receptors was further investigated. There appeared to be a regional specificity within the kidney because variations of NaCl intake did not change PGE₁ binding activity in the cortex (DS: 74.4 ± 3 on 8.0% NaCl vs 76.1 ± 3 fmol/mg on 0.6% NaCl; DR: 79.7 ± 5 on 8.0% NaCl vs 80.2 ± 5 fmol/mg on 0.6% NaCl) or inner medulla (DS: 102 ± 45 on 8.0% NaCl vs 108 ± 30 fmol/mg on 0.6% NaCl; DR: 110 ± 50 on 8.0% NaCl vs 118 ± 40 fmol/mg on 0.6% NaCl). The organ specificity of the effect of salt was demonstrated by the finding that the

### Table 1. Body Weights and Blood Pressure of Dahl Rats 24 to 48 Hours Before Death

<table>
<thead>
<tr>
<th>Group</th>
<th>Body weight (g)</th>
<th>Blood pressure (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.6% NaCl (n = 17)</td>
<td>246 ± 3</td>
<td>113 ± 3</td>
</tr>
<tr>
<td>8.0% NaCl (n = 17)</td>
<td>243 ± 3</td>
<td>118 ± 3</td>
</tr>
<tr>
<td>DS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.6% NaCl (n = 17)</td>
<td>233 ± 7</td>
<td>134 ± 5</td>
</tr>
<tr>
<td>8.0% NaCl (n = 18)</td>
<td>215 ± 5</td>
<td>162 ± 6</td>
</tr>
</tbody>
</table>

Values are means ± SE.
**TABLE 2. Prostaglandin E₂ Binding in Outer Renal Medulla of Dahl and Sprague-Dawley Rats**

<table>
<thead>
<tr>
<th>NaCl in diet (%)</th>
<th>PGE₂ binding (fmol/mg protein)</th>
<th>Sprague-Dawley</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DR</td>
<td>DS</td>
</tr>
<tr>
<td>0.6</td>
<td>946 ± 25 (n = 17)</td>
<td>956 ± 41 (n = 16)</td>
</tr>
<tr>
<td>8.0</td>
<td>1250 ± 41 (n = 17)*</td>
<td>1052 ± 54 (n = 18)†</td>
</tr>
</tbody>
</table>

Results are means ± SE. PGE₂ = prostaglandin E₂.

*p < 0.001, †p > 0.1, ‡p < 0.05, compared with the group on 0.6% diet.

**TABLE 3. Prostaglandin E₂ Receptor Densities and Binding Constants in Dahl and Sprague-Dawley Rats**

<table>
<thead>
<tr>
<th>NaCl in diet (%)</th>
<th>Bₘₐₓ * (pmol PGE₂ per mg protein)</th>
<th>Kₐ (nM)</th>
<th>Bₘₐₓ * (pmol PGE₂ per mg protein)</th>
<th>Kₐ (nM)</th>
<th>Bₘₐₓ * (pmol PGE₂ per mg protein)</th>
<th>Kₐ (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n = 6)</td>
<td>2.18 ± 0.2</td>
<td>9.4 ± 0.3</td>
<td>2.75 ± 0.2</td>
<td>13.9 ± 0.2</td>
<td>2.37 ± 0.2</td>
<td>8.6 ± 0.3</td>
</tr>
<tr>
<td>8.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n = 6)</td>
<td>2.97 ± 0.2*</td>
<td>9.5 ± 0.2</td>
<td>2.97 ± 0.2*</td>
<td>14.0 ± 0.3</td>
<td>2.91 ± 0.3*</td>
<td>8.4 ± 0.2</td>
</tr>
</tbody>
</table>

Results are means ± SE for six experiments in each group. Maximal binding activity (Bₘₐₓ) expressed as pmol PGE₂/mg protein. PGE₂ = prostaglandin E₂.

*p < 0.05, compared with the group on 0.6% NaCl diet.

Various salt intakes had no effect on maximal PGE₂ binding by liver membranes in either DS or DR (500 and 550 fmol/mg, respectively, on either diet; Kₐ = 53 nM for groups on either salt diet; data not shown).

The possibility of a direct effect of NaCl on outer medullary PGE₂ receptors was then considered. To demonstrate the in vitro effect of NaCl, tissues were collected and homogenized in buffer containing sucrose instead of NaCl. The resuspension medium contained various concentrations of sucrose and NaCl so that the osmolality was kept at 350 mosm. Compared with NaCl-free medium, medium containing 0.15 M NaCl resulted in a 30% increase in the number of prostaglandin receptors but had no effect on receptor affinity. The extent of prostaglandin binding stimulation by in vitro addition of NaCl was similar in DS and DR (Table 4). However, membranes from DR and DS prepared in the presence of 0.33 M sucrose instead of 0.15 M NaCl in the homogenization buffer still showed between-strain differences in the response of renal prostaglandin receptors to in vivo salt administration. Therefore, factors other than a direct influence of NaCl on the binding process appear to account for the disparate responses of the two strains to a high dietary salt intake. The increased PGE₂ binding in the outer renal medulla of DR was sustained after 5 to 6 weeks of...
TABLE 4. In Vitro Effect of 0.15 M NaCl on Prostaglandin E₂ Binding by Outer Medullary Membranes in Dahl Rats

<table>
<thead>
<tr>
<th>NaCl in diet (%)</th>
<th>DR 0.33 M Sucrose</th>
<th>0.15 M NaCl</th>
<th>DS 0.33 M Sucrose</th>
<th>0.15 M NaCl</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.6 (n = 9)*</td>
<td>626 ± 46</td>
<td>850 ± 40</td>
<td>608 ± 61</td>
<td>820 ± 50</td>
</tr>
<tr>
<td>8.0 (n = 9)*</td>
<td>812 ± 45†</td>
<td>1156 ± 42†</td>
<td>655 ± 52†</td>
<td>909 ± 55†</td>
</tr>
</tbody>
</table>

Values are means ± SE. *Number of individual animals and comparisons for each experimental group. †p < 0.05, ‡p > 0.1, compared with group on 0.6% diet.

Discussion

The first step in the interaction of prostaglandins with responsive tissues is their binding to specific sites on the cell membrane. We have recently demonstrated the presence of prostaglandin receptors in the rat kidney and have described their properties. Prostaglandin receptors are believed to play a crucial role in the regulation of blood pressure and cardiovascular function. In this study, we have focused on the correlation between systolic blood pressure and prostaglandin E₂ (PGE₂) binding to renal outer medullary membranes in Dahl rats fed different sodium chloride (NaCl) diets.

High salt intake (1340 ± 91 on 8.0% NaCl diet vs 946 ± 81 fmol/mg on 0.6% NaCl diet; p < 0.01). In contrast, the increase in PGE₂ binding observed in DS after long-term salt loading did not reach statistical significance (1120 ± 75 on 8.0% NaCl diet vs 940 ± 63 fmol/mg on 0.6% NaCl diet). All DS maintained for 5 to 6 weeks on 8% NaCl had systolic blood pressure over 160 mm Hg; the average was 182 mm Hg.

Adenylate cyclase activities in membranes from outer medullae of DS and DR on both diets were compared. As shown in Table 5, there were no significant between-group differences in basal, PGE₁-stimulated, or NaF-stimulated activities on either salt diet. The concentration dependence of the PGE₁-induced stimulation of adenylate cyclase activity was comparable in all groups (Figure 4).

Figure 3. Correlation between systolic blood pressure and prostaglandin E₂ (PGE₂) binding to renal outer medullary membranes in DS on an 8.0% NaCl diet.

Figure 4. Prostaglandin E₁ (PGE₁) dependence of adenylate cyclase activity in the renal medullae of DR and DS. Results (mean ± SE) for six experiments in each group are expressed as percent of control (activity in the absence of PGE₁).
Prostaglandin receptors are associated with membranes and show a nonuniform distribution along the nephron; the highest density of binding sites is found in the outer medulla. If these receptors have physiological significance, they should be subject to homologous and heterologous regulation. Indeed, we have previously shown that in vivo administration of the slowly metabolized analogue 16,16'-dimethyl-PGE, leads to a rapid and concentration-dependent decline in prostaglandin receptors, which is consistent with a process of down-regulation. Down-regulation has also been described for liver PGE receptors after in vivo dimethyl-PGE administration and contrasts with the up-regulation seen in response to prostaglandin synthesis inhibitors.

The process of homologous desensitization is a well-studied mechanism through which the magnitude of agonist-mediated effects is limited and is particularly relevant when cells are presented with agonist concentrations above the physiological range. This type of regulation is in striking contrast to the enhanced prostaglandin binding that occurred following a high salt diet in this study. Since, as we have demonstrated previously, this dietary manipulation results in enhanced renomedullary prostaglandin synthesis, which would expose the receptor to increased agonist concentrations, the opposite result might have been anticipated. In view of an unchanged $K_c$, and the expected higher endogenous prostaglandin levels, the increase in binding sites suggests that NaCl also potentiates the effect of prostaglandins in vivo by increasing their binding to effector cells. From the teleological point of view, this pattern of response to NaCl is precisely the one needed to support or amplify the physiological consequences of enhanced prostaglandin synthesis, since the number of the receptors is expected to be rate-limiting. The Sprague-Dawley rats had the greatest interindividual variability, which could be explained by a wider spectrum of genetic characteristics in these animals compared with the more homogeneous Dahl strains. This observation emphasizes the difficulty in demonstrating biological responses to environmental factors when genetically heterogeneous subjects are studied. For example, the wide spectrum of genetic background has been a major problem in human studies on the effect of dietary salt.

The results shown in Tables 2 and 3 are better understood in light of previous observations on renomedullary prostaglandin synthesis in DR and DS. The capacity for prostaglandin synthesis in the renal medulla of DS is markedly reduced even when they are maintained on 0.6% NaCl, and it remains well below that of DR despite a stimulation induced by 8.0% NaCl. Therefore, if prostaglandin binding was simply reflecting the endogenous prostaglandin levels it should be higher in DS on either diet. The present results indicate that DS fed 0.6% NaCl diets have a greater PGE receptor density (maximal binding activity, 2.75 pmol/mg protein), which is compatible with their lower level of prostaglandin synthesis, and that DS fed 8.0% NaCl diets have a blunted receptor response compared with that in DR. Because this depressed receptor affinity may also compromise the effector cell response to the low levels of endogenous prostaglandin present in this strain, the in vitro binding data shown in Table 1 for the in vivo conditions should be interpreted with caution. The in vitro conditions provide optimal binding under fixed ligand concentrations, while in vivo conditions may be quite dissimilar in the two strains.

Of greater importance are the directional changes noted following dietary manipulation, which clearly indicate a restricted response in the DS. Furthermore, the DS that had a sharper rise in blood pressure also had a more sluggish response of prostaglandin binding to dietary sodium load. A longitudinal study might be helpful in establishing whether the rate of blood pressure elevation is related to the magnitude of receptor modulation.

The mechanism of enhanced prostaglandin binding in salt-loaded animals currently is conjectural; however, a high salt diet has been reported to stimulate other receptors, including $\beta$-adrenergic and angiotensin receptors. Recent observations in our laboratory suggest that prostaglandin receptors have a short half-life and that maintenance of their numbers depends on continued synthesis. It is tempting, therefore, to speculate that the effect of dietary salt is mediated through an enhanced rate of prostaglandin receptor synthesis. Whatever the mechanism involved, the prostaglandin receptor changes are concordant with the lower renomedullary prostaglandin synthesis in DS and limit the functional effectiveness of the renal prostaglandin system. This limitation, in turn, places severe restrictions on the kidney's ability to handle an increased sodium load and may partly account for the susceptibility of DS to salt-induced hypertension. This concept is supported by the demonstration that dietary enhancement of renal prostaglandin synthesis in DS attenuates the course of blood pressure rise in response to a high salt diet. In the present study it was also noted that, among DS on a high salt diet, those with the highest increments in prostaglandin receptors had a lesser degree of hypertension.

The enhancement of prostaglandin binding to outer medullary membranes of salt-loaded DR was not accompanied by statistically significant differences in adenylyl cyclase activities. There are two possible explanations for this observation. First, prostaglandin-receptor interactions may have physiological effects that do not involve adenylyl cyclase stimulation. The precedent for this suggestion is provided by the report that PGE, affects Mg$^{2+}$ transport in cultured cells independently of adenylyl cyclase stimulation. Second, it is possible that appearance of increased prostaglandins receptors on the cell membrane precedes their coupling to the adenylyl cyclase and that enhanced PGE-stimulated activity may indeed be present later in the evolution of the hypertensive process. This possibility is currently under investigation in our laboratory.
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C Limas and C J Limas

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