SUMMARY The effect of high salt intake on vascular and renomedullary prostaglandin (PG) synthesis was compared in Sprague-Dawley and salt-sensitive (S) and -resistant (R) Dahl rats. Animals were given a diet containing either 0.6% or 8% NaCl starting at 5 weeks of age, and were sacrificed 6 weeks later. Systolic blood pressure of S rats increased to 220 ± 7 mm Hg but was unaffected in R and Sprague-Dawley rats. Prostaglandin synthesis was studied in aortic rings and renomedullary microsomes using [14C]-arachidonate as substrate. [3H]PGE$_2$ degradation was measured in the renocortical cytosol.

In Sprague-Dawley and R rats, aortic PGI$_2$ synthesis was not affected by high salt intake, while a significant increase compared to animals on 0.6% NaCl (from 608 ± 84 to 992 ± 108 pmoles/60 min, $p < 0.05$) was noted in S rats. Enhancement of PGI$_2$ synthesis in S rats may be secondary to the hypertension.

Salt-loading consistently stimulated renomedullary PGE$_2$ synthesis in all three animal groups. S rats, however, had the lowest PG synthesis in renal medulas compared to Sprague-Dawley and R rats when placed on either diet. Thus, even after 6 weeks on high salt, S rats did not reach the levels of PGE$_2$ synthesis seen in R or Sprague-Dawley rats on regular diet.

The activity of cortical 15-hydroxyprostaglandin dehydrogenase was increased by salt-loading in S and Sprague-Dawley, but not in R rats. R rats had lower dehydrogenase activity than the other two groups when placed on either diet.

The observed differences in PG synthesis and catabolism will tend to maintain the net output of renal PGs highest in R and lowest in S rats. These differences correlate with the reported differences in renal papillary flow between these two rat strains and may be relevant to their susceptibility or resistance to hypertension in response to salt. (Hypertension 3: 219-224, 1981)

KEY WORDS • salt-loading • prostaglandin synthesis • Sprague-Dawley rat • Dahl rat • renal medulla • hydroxyprostaglandin dehydrogenase • aortic rings

EXTENSIVE epidemiologic studies in humans have shown a positive correlation between habitual salt intake and the prevalence of hypertension. The mechanisms by which increased dietary sodium initiates and maintains hypertension are not known, however. Studies on experimental animals have convincingly demonstrated that genetic factors are involved, and it is likely that such factors also operate in human essential hypertension. The physiological and biochemical pathways through which genetic factors exert their influence on blood pressure (BP) are unclear. Because of the pivotal role of the kidney in regulating BP by maintaining sodium and water balance, attempts have been made to demonstrate genetically determined differences in renal function between animals prone to develop hypertension (salt-sensitive) and those that maintain normal BP (salt-resistant) when fed high salt diets. It is also possible that BP elevation in response to salt is the result of vascular changes not necessarily related to the expansion of extracellular fluid volume.

Prostaglandins are involved in the regulation of both sodium balance and vascular smooth muscle tone. It is possible that differences in the pattern of renal and vascular prostaglandin metabolism mediate, in part, the variable response of BP to salt. The study of the role of prostaglandins in salt-induced hypertension has been facilitated by the development of two strains of rats, one sensitive (S) and one resistant (R) to the effects of salt. Induction of hypertension in S rats by a high salt diet is associated with decreased renal papillary flow, decreased natriuretic capacity, and increased systemic vascular resistance compared to R rats. The present study explores the possibility that these differences between the two rat strains reflect disparate responses of the prostaglandin system to salt-loading.
Materials and Methods

Experiments were carried out on male Sprague-Dawley rats (BioLab Company) and Dahl salt-sensitive and -resistant rats (from the colony maintained at Brookhaven National Laboratories, Upton, New York). Systolic BP was measured under light methoxyflurane anesthesia with a tail-cuff sphygmomanometric technique between 8:30 and 9:30 A.M. at weekly or biweekly intervals. At age 5 weeks, the animals were randomized into the high (8% NaCI) or normal (0.6% NaCl) salt-diet group (10 to 14 animals in each of the six experimental groups). Their diets were otherwise identical and were obtained from Purina Company. The animals were allowed free access to food and water. The amounts of water and food consumed were measured daily, and the body weight weekly. The animals were sacrificed at 11 weeks of age.

For measurement of prostaglandin synthesis by aortic rings, segments of the thoracic aorta (40 mm long from the origin of the left subclavian artery) were cleaned meticulously of periadventitial tissue and sliced to 1 mm thickness with a McIlwain tissue slicer; the tissues were prepared in the cold (0°-4°C) and were kept wet in cold Krebs solution. The aortic segments (two aortas per tube) were placed in 1.5 ml Krebs, then incubated at 37°C under 95% O₂ + 5% CO₂ for 15 minutes, and then transferred to tubes containing 0.2 μCi [14C]-arachidonate (Amersham/Searle, Arlington Heights, Illinois, sp. activity 55 mCi/mmol) in 1.5 ml Krebs solution. Incubation was carried out for 1 hour with shaking at 37°C under 95% O₂ + 5% CO₂. The reaction was stopped by acidifying to pH 3 with 0.5 M citric acid. Then 1 ml of the reaction medium was extracted twice with 2.5 ml ethyl acetate, and the combined extracts were dried under nitrogen.

The extracts were then taken up in 100 μl of benzene-ethanol (4:1 v/v) for thin layer chromatography on silica gel plates. Standard solutions of arachidonate, prostaglandins, and their metabolites were spotted together with the specimens. The upper phase of ethyl acetate: isooctane:acetic acid:water (110:50:20:100, by vol) was used as solvent, and the plates were developed to 15 cm from the origin. After drying, the standards were visualized with iodine vapors, and the corresponding radioactive spots were visualized by autoradiography and scraped off for radioactivity measurement. Conversion of [14C]-arachidonate to 6-keto-PGF₁α, the stable metabolite of PG₁α, was used as an index of aortic PG₁α synthesis. For renomedullary prostaglandin synthesis, microsomes were prepared as previously described. Briefly, renal medullae were homogenized in 4 vol of 50 mM tris-HCl (pH 8.0) and spun at 700 g for 10 minutes. The supernatant was centrifuged at 8500 g for 15 minutes and the resultant supernatant at 104,000 g for 60 minutes. The microsomal pellet was resuspended in tris-HCl buffer (50 mM, pH 8.0). Prostaglandin synthesis was assayed in a medium containing 50 mM tris-HCl (pH 8.0), 1 mM L-epinephrine, 0.5 mM reduced glutathione, 0.3 mg microsomal protein, and 0.1 μCi [14C]-arachidonate in a total volume of 0.5 ml. Incubation was carried out at 37°C for 15 minutes, and the reaction was stopped by acidification to pH 3. Extraction and chromatography of prostaglandins were carried out as described above. Enzymatic activity was calculated from the percentage of [14C]PGF₁α converted to 15-keto-PGF₂α, 13,15 diketo PGE₂ was less than 5% to 10% of 15-keto-PGE₂.

Results

The effect of salt on the BP of Dahl rats is shown in figure 1. After 3 weeks of excess salt intake the systolic BP in salt-sensitive rats increased progressively and reached 220 ± 7 mm Hg at 6 weeks. The S rats on regular (0.6% NaCl) diet had an average of 15 mm Hg higher BP than R rats but this difference did not increase with time. High salt diet had no effect on the BP of R rats. Sprague-Dawley rats had a systolic BP of 126 ± 3 mm Hg and this was not affected by salt-loading. Food intake was similar in the S and R rats on either diet. The amount of water consumed by the
animals on high salt diet was 2.7 times that consumed by animals on regular diet, and there were no differences between S and R rats. At 5 weeks of age, the body weight was 127 ± 5 g in R and 127 ± 7 g in S rats, and at 11 weeks it was 340 ± 7 g and 336 ± 8 g respectively. Eleven-week-old R rats that had been on 8% NaCl for 6 weeks weighed 339 ± 8 g, and the similarly treated S rats, 334 ± 6 g. The dry weights of the thoracic aortae (40 mm length) for 11-week-old animals fed 8% salt, the dry aortic weights were: Sprague-Dawley, 11.6 ± 0.3 mg; Dahl R, 13.6 ± 0.5 mg; and Dahl S, 15.1 ± 0.5 mg. A significant increase (appr. 13%) of aortic weight was noted only in R rats, and this was probably related to the development of hypertension.

In all experimental groups, the aortic rings synthesized predominantly PGI2. The time course of prostaglandin synthesis by aortic rings of normal animals is shown in figure 2. The effect of excess salt intake on aortic prostaglandin synthesis is shown in table 1. In S rats, PG synthesis increased significantly when excess salt was added to the diet, while this diet had no effect on the aortas of R and Sprague-Dawley rats.

The effect of salt-loading on prostaglandin synthesis by renomedullary microsomes is shown in table 2. Among animals on regular diet, S rats had significantly lower PGE2 synthesis. After 6 weeks on salt, renomedullary PG synthesis increased in all three experimental groups, but S rats remained well below the levels of either R or Sprague-Dawley rats. High salt diet enhanced the synthesis of the major prostaglandin classes (E2, F2α, and D) both in S and R Dahl rats without significantly altering their ratios. Medullae from Sprague-Dawley rats showed a great variability in their capacity to synthesize PGF2α, as well as in the responses of this prostaglandin to salt-loading. This may reflect greater genetic heterogeneity among commercially available Sprague-Dawley rats than is present among S and R rats selectively bred in a closed colony.

The time course of PG synthesis by aortic rings of 11-week-old Sprague-Dawley rats on 0.6% NaCl diet. Two thoracic aortic segments were incubated in Krebs solution (pH 7.4) with 0.2 μCi 14C-arachidonate at 37°C, as described in the text.
TABLE 3. Effect of High Salt Diet on Renocortical NAD<sup>+</sup>-Dependent 15-Hydroxy-Prostaglandin Dehydrogenase

<table>
<thead>
<tr>
<th>Diet</th>
<th>NAD&lt;sup&gt;+&lt;/sup&gt;-dependent 15-hydroxy-prostaglandin dehydrogenase (pmol 15-keto-PGE&lt;sub&gt;2&lt;/sub&gt;/mg prot/15 min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.6% NaCl</td>
<td>Dahl Sensitive: 266 ± 16, Dahl Resistant: 154 ± 24, Sprague-Dawley: 340 ± 46</td>
</tr>
<tr>
<td>8.0% NaCl</td>
<td>Dahl Sensitive: 628 ± 43*, Dahl Resistant: 183 ± 20†, Sprague-Dawley: 646 ± 85*</td>
</tr>
</tbody>
</table>

Mean ± SE for five paired experiments.
*<i>p<0.01</i>
†No significant difference, compared to controls (0.6% NaCl).

Figure 3. Time course of PGE<sub>2</sub> oxidation by NAD<sup>+</sup>-dependent 15-hydroxy-prostaglandin dehydrogenase from renal cortex of Sprague-Dawley rats on 0.6% (○) or 8.0% NaCl (●) for 6 weeks.

Discussion

The results of the present study demonstrate disparate effects of salt-loading on aortic and renomedulary prostaglandin synthesis. Only salt-sensitive Dahl rats responded to excess salt intake with an increase in aortic PG<sub>I</sub><sub>2</sub> synthesis. Since this experimental group was also the only one to develop hypertension after salt feeding, the enhancement of vascular prostaglandin synthesis can be viewed as a secondary response to the hypertensive process rather than a direct effect of salt. Increased PG synthesis by the aortas of these hypertensive animals could not be accounted for solely on the basis of increased cell mass; the per cent difference (approximately 63%) of PG<sub>I</sub><sub>2</sub> synthesis between the two groups of S rats far exceeded the difference in aortic dry weights (approximately 13%).

A similar enhancement has been shown in other models of induced or spontaneous hypertension<sup>11, 12, 13</sup> and may represent a compensatory mechanism by which the hypertensive process is contained to some extent. Enhancement of vascular prostaglandin synthesis could attenuate the hypertensive process as is implied by the aggravation of hypertension when prostaglandin synthesis is inhibited.<sup>14, 15</sup> It should be noted that even on regular salt diet, S rats had higher BP (15 mm Hg difference) and lower aortic PG synthesis than R rats. In view of the fact that increased intraluminal pressure may stimulate PG synthesis, it is possible that the differences between S and R rats were blunted to some extent.

Although high salt intake stimulated renomedulary prostaglandin synthesis in all three experimental groups, important quantitative differences were noted. The sensitive Dahl rat had the lowest prostaglandin synthetic capacity while on a regular diet and, despite the significant increase induced by excess salt, did not reach that seen in normotensive animals.

The activity of NAD<sup>+</sup>-dependent dehydrogenase responded to salt-loading in a pattern different from that of endoperoxide synthetase. Of the three experimental groups, Dahl R rats had the lowest activity and failed to respond to the high salt diet. Both Dahl S and Sprague-Dawley rats showed a marked increase in the activity of this prostaglandin-degrading enzyme when given excess salt. The contrast between the response of endoperoxide synthetase and dehydrogenase in Dahl rats suggests that salt affects these two enzymes independently. The combination of the observed effects of excess salt on prostaglandin synthesis and catabolism would result in the highest increment of prostaglandin output in the R rat and lowest in the S Dahl rat.

The effect of salt-loading on prostaglandins has been the subject of previous investigations with variable results.<sup>16-23</sup> It should be noted that most of these studies were short-term (less than 48 hours), and the effects of salt were gauged by measuring changes in the plasma, renal venous effluent, or urinary prostaglandin concentrations. Usually, the changes reported were concordant in all major prostaglandin classes with the exception of Weber et al.<sup>24</sup> who found increased urinary PGF<sub>2</sub><sub>α</sub> and decreased PGE<sub>2</sub> output in rabbits in response to salt. Such differential effect has not been confirmed by subsequent studies.<sup>25</sup> The discrepancies in the literature may be partly due to differences in the experimental design, animal species, and methodology for assessing prostaglandin synthesis. The present study shows unequivocal changes in renal prostaglandin metabolism after long-term salt-loading. If these in vitro findings are representative of the in vivo situation, the pattern of response of renal prostaglandin metabolism to chronic salt excess may be related to the susceptibility or resistance to
hypertension. Tan et al.20 have recently reported that sodium-loading of Dahl rats resulted in a transient increase in urinary PGE\textsubscript{2} excretion which was higher in S rats and had returned to control values by the 5th week. Differences in methodology may account for the discrepancy between their findings and other studies in both humans23 and the Dahl sensitive rats.24

The interest in the influence of salt on renal prostaglandins is based on the effects of prostaglandins on sodium-handling by the kidney. Prostaglandins may modify renal hemodynamics and tubular sodium reabsorption. Although the role of prostaglandins in the regulation of renal blood flow is probably minor under physiological conditions, it may become critical in pathological situations, such as hypertension. These substances affect salt and water balance both directly and indirectly. Indirect effects include those secondary to interference with blood flow distribution.25 ADH action on the distal nephron26 and interaction with angiotensin and kallikrein-kinin systems.27 Direct effects on tubular functions have been demonstrated by Kauker28 who observed inhibition of sodium reabsorption after injection of PGE\textsubscript{2} within the lumen of rat kidney tubules and by Stokes and Kokko29 in isolated perfused collecting ducts.

In view of the natriuretic properties of prostaglandins, their increased synthesis following sodium-loading could be viewed as a compensatory mechanism designed to maintain sodium homeostasis. Failure of this compensation would facilitate the inductive and/or maintenance of hypertension. From this point of view, our findings in the Dahl rats are of particular interest since these animals have been inbred for their susceptibility or resistance to the prohypertensive effect of salt. Ganguli et al.30 showed low renal papillary plasma flows in Dahl S rats prior to the initiation of a high salt diet. R rats responded to the excess salt by increasing renal papillary flow while S rats failed to increase flow to the same level. In view of the role of prostaglandins in regulating the medullary blood flow, these hemodynamic results are strikingly similar to the directional changes in renal prostaglandin metabolism reported in this study. Thus, deviations in prostaglandin metabolism may be implicated in the inability of S rats to increase their natriuresis in response to excess salt intake. Hypertension may thus ensue to achieve homeostasis by "pressure natriuresis." It is interesting to note that the low renomedullary PGE\textsubscript{2} synthesis in S rats on 0.6% NaCl is associated with a small rise in BP compared to R rats (fig. 1). When S rats are placed on 8% NaCl, sodium chloride toxicity in the albino rat. II Occurrence of hypertension and of a syndrome of edema and renal failure. J Exp Med 115: 1173, 1962


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