Influence of Renal Prostaglandins and Dietary Linoleate on Hypertension in Dahl S Rats

Louis Tobian, M.D., Mukul Ganguli, Ph.D., Mary Ann Johnson, and Junichi Iwai, M.D.

SUMMARY Kidney prostaglandins appear to have powerful influences on NaCl-induced hypertension. In quick-frozen kidneys, the concentration of prostaglandin E₂ (PGE₂) in the renal papilla is 60% lower in Dahl S rats than in Dahl R rats (17 ng/100 mg vs 42 ng/100 mg, p < 0.01) when both S and R rats are fed a 0.3% low NaCl diet. When S and R rats eat a 4% high NaCl diet for 4 weeks or 11 weeks, the PGE₂ concentration doubles in both strains (p < 0.05) but the papillary PGE₂ concentration in the S rats is always about half that in the R rat (p < 0.01). Through effects on sodium (Na) excretion and papillary plasma flow, the low PGE₂ in S papillae may account in part for the large rises in the blood pressure (BP) of S rats after eating a high NaCl diet. This proposition was explored by utilizing high fat diets with either normal or high linoleic acid content. Arachidonic acid is the precursor of PGE₂ and linoleic acid is the precursor of arachidonic acid. It turned out that the low PGE₂ level in S papillae could be tripled by a diet high in linoleic acid. Sixteen S rats on a 16-week diet of 5% NaCl and 1.5% linoleic acid had a mean papillary PGE₂ level of 30 compared to a level of 89 in 15 other S rats on a diet of 5% NaCl and 16% linoleic acid. The 16% high linoleic diet tripled the PGE₂ concentration in S papillae (p < 0.005). It also increased the PGE₂ concentration in R papillae 2 1/2 times, 137 vs 53 (p < 0.02). In rats on either high or normal linoleic diets, the PGE₂ in S papillae was always at least 35% less than that in R papillae. However, the 16% high linoleic diet did raise the papillary PGE₂ level in S rats up to that found in normal rats on regular rat chow of equivalent NaCl content. Moreover, this change in PGE₂ level was associated with greatly reduced BP rises in S rats. The BP of S rats on a 5% NaCl-1.5% linoleic diet began to rise after 5 weeks on the diet and reached 183 mm Hg after 16 weeks. The BP of S rats on a 5% NaCl-16% linoleic diet did not begin to rise until 12 weeks on the diet and reached only 166 after 16 weeks. The high linoleic diet greatly delayed the onset of the rise in BP and significantly reduced the ultimately attained level (p < 0.001). In fact, on a low 0.3% NaCl diet, S rats of comparable age reached approximately the same mildly hypertensive level of 166. Thus, in S rats, the high linoleic diet brings papillary PGE₂ up to normal and also prevents the large rises in BP usually related to a high NaCl Intake. These two changes may well be causally related. (Hypertension 4 (suppl II): II-149-II-153, 1982)

KEY WORDS • sodium excretion • papillary plasma flow • prostaglandin E₂ • linoleic acid

HUMAN essential hypertension clearly has a sodium aspect to it. Life-long low-salt diets can prevent hypertension even in susceptible individuals. Diets very low in sodium can reduce the blood pressure (BP) in established hypertension. Drugs such as thiazides, which slightly reduce body Na content, can do the same. On the other hand, very high intakes of NaCl can raise BP in humans. The way in which Na affects the BP in humans is still not completely understood. To approach this question, we have made some observations in the two strains of Dahl rats. The Dahl S strain weighing below 350 g has a BP within the normal range on a low salt diet but becomes quite hypertensive on a high intake of NaCl. The Dahl R strain remains normotensive on either the high or low NaCl diets. There is evidence that the Dahl S rat has a reduced capacity for brisk natriuresis and possesses circulating antinatriuretic humoral agents. The S rat also has a greatly reduced plasma flow to the renal papillae, which may also tend to retard natriuresis. Since flow to the renal papillae and Na transport in the distal nephron are influenced by prostaglandin E₂ in the renal medulla, we carried out renal prostaglandin studies in the Dahl rats. There is also evidence for the participation of the nervous system in the hypertension of S rats. Cutting peripheral sympathetic nerves can abolish half of the increased vascular resistance in the hindquarters of

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hypertensive S rats on a high NaCl diet. Moreover, the normotensive S rat has pressor responses two to three times greater than those of R rats when angiotensin II or hypertonic saline is introduced into the lateral brain ventricle. The S rat also has a significantly higher norepinephrine concentration in the hypothalamus.

**Methods**

**Experiment A**

Dahl S and R rats were weaned at 4 weeks of age and studied at 15 weeks of age after 11 weeks on a special diet. One group of S and R rats ate Purina chow containing 0.3% NaCl for 11 weeks, which might be considered a normal dietary Na level. Another group of S and R rats were fed for 11 weeks a similar diet containing 4% NaCl; this is a moderately high NaCl intake. A third group of S and R rats ate the 0.3% NaCl diet for 7 weeks and were then switched to the 4% NaCl diet for the final 4 weeks.

At the end of the feeding periods, the rats were anesthetized with pentobarbital on a round-robin schedule, and the left kidney was quickly excised and frozen in liquid nitrogen. The kidney was slowly warmed to —5°C. No new prostaglandins are biosynthesized at this temperature. The papilla was dissected out at —5°C and homogenized in a cold chloroform-methanol solvent. The acidic lipid fraction was separated from the chloroform-methanol extract and put on a silicic acid minicolumn to separate the E prostaglandins, which were then assayed by radioimmunoassay. Tritiated prostaglandin E$_3$ (PGE$_3$) was used to correct for losses during the procedure. We sought to measure the PGE$_3$ concentration in quick-frozen papillae since this is the concentration that is exerting a physiological effect on the tissues. This is very likely a valid assumption since PGE$_3$ is considered not to be stored in depots in the various tissues of the body.

**Experiment B**

We sought to determine the effects of feeding high levels of linoleic acid on the PGE$_3$ concentration in renal papilla. PGE$_3$ is synthesized from arachidonic acid, which in turn is synthesized from linoleic acid, which is a common ingredient of various foodstuffs. In this study, both S and R rats weighing about 375 g were first fed diets containing 20% fat and 0.24% NaCl, a low NaCl diet. Half the S rats and half the R rats were on a diet containing 20% coconut oil. The other half of the S and R rats were on a similar diet with 20% safflower oil. The other 80% of both diets consisted of regular low-salt Purina rat chow. The coconut oil diet contained 1.5% linoleic acid, enough to prevent an essential fatty acid deficiency. The safflower oil diet contained 16% linoleic acid, thereby providing a very generous amount of this essential fatty acid. Body weights were the same on the two contrasting diets over 18 weeks of feeding. After 2 weeks on these 0.24% NaCl diets, both S and R rats were then switched to their same 1.5% or 16% linoleic diet containing this time 5.0% NaCl instead of 0.24% NaCl. These 5% high NaCl diets were maintained for 16 weeks. The BP was measured without anesthesia in all S and R rats every 2 weeks, using the microphonic method. At the end of the 18 weeks of feeding, PGE$_3$ concentration was measured in quick-frozen renal papillae by the method outlined in Experiment A above.

**Results**

**Experiment A**

Table 1 gives the concentration of PGE$_3$ per 100 mg of papilla solids. Two principal findings were evident. First, the S rat, which is genetically susceptible to salt hypertension, had much lower PGE$_3$ levels than the R rat, which is resistant to salt hypertension. When both strains were normotensive on a low salt diet, the papillary PGE$_3$ in the S rat was 60% lower than that in the R rat. After either 4 or 11 weeks of a high salt intake, with S rats hypertensive and R rats normotensive, the PGE$_3$ in the S papillae was still half that in R papillae. All these differences were statistically significant.

The second main finding (table 1) indicated that rats on the 4% high salt diet for either 4 or 11 weeks had about twice the PGE$_3$ concentration as did control rats on the low salt diet. This happened in both S and R rats, and the differences were significant. However, when the S rats more than doubled their papillary PGE$_3$ while on a high salt diet, their PGE$_3$ concentration still rose only to the level found in the R rats on a low salt diet. Thus, on any of the diets, the S rat always showed a PGE$_3$ level only half as high as that of the R rat.

**Experiment B**

As seen in table 2, the 16 S rats on the 1.5% linoleic diet had an average papillary PGE$_3$ concentration of 30 ng/100 mg of solids, whereas the 15 S rats eating the 16% linoleic diet had a PGE$_3$ concentration of 89 ng/100 mg of solids. Thus, the high linoleic diet tripled the concentration of PGE$_3$ in the papillae, and brought it up to slightly above the level seen in R rats eating regular Purina chow with the same NaCl content. The same pattern also occurred in R rats, only at a much higher level. The 15 R rats on the 1.5% linoleic diet had 53 ng of PGE$_3$/100 mg of solids, while the 18 R rats on the 16% linoleic diet had 137 ng of PGE$_3$. Thus, in R rats the 16% linoleic diet increased the PGE$_3$ concentration by 2½ times. On either diet, however, the PGE$_3$ in S rats was at least 35% lower than that in comparable R rats.

Since the 16% linoleic diet produced such a rise of PGE$_3$ in S papillae, we were interested in its effects on NaCl-induced hypertension. As seen in the top line of
TABLE 1. Concentrations of Prostaglandin E\(_2\) in the Renal Papilla of Dahl S and R Rats on High (4%) and Low (0.3%) NaCl Diets

<table>
<thead>
<tr>
<th>Rat group</th>
<th>0.3% NaCl diet for 11 wks</th>
<th>0.3% NaCl diet for 7 wks</th>
<th>4% NaCl diet for 11 wks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dahl R rats, NaCl resistant</td>
<td>42 ± 8 (n = 18; BP = 124)</td>
<td>80 ± 17 (+90% ( p &lt; 0.005)* (n = 17; BP = 127)</td>
<td>78 ± 12 (+89% ( p &lt; 0.025)) (n = 19; BP = 126)</td>
</tr>
<tr>
<td>Dahl S rats, NaCl sensitive</td>
<td>17 ± 3 (n = 15; BP = 140)</td>
<td>41 ± 9 (+141% ( p &lt; 0.05)) (n = 20; BP = 159)</td>
<td>39 ± 8 (+129% ( p &lt; 0.05)) (n = 22; BP = 183)</td>
</tr>
<tr>
<td>Percent reduction</td>
<td>-60%</td>
<td>-49%</td>
<td>-50%</td>
</tr>
<tr>
<td>( p )</td>
<td>&lt; 0.01</td>
<td>&lt; 0.05</td>
<td>&lt; 0.01</td>
</tr>
</tbody>
</table>

Values are means ± SEM. BP is the mean systolic blood pressure in mm Hg at the end of study.

TABLE 2. Concentrations of Prostaglandin E\(_2\) in the Renal Papilla of Dahl S and R Rats Fed 5% NaCl Diets Containing Either 1.5% or 16% Linoleic Acid

<table>
<thead>
<tr>
<th>Rat group</th>
<th>1.5% linoleic-5% NaCl</th>
<th>16% linoleic-5% NaCl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dahl R rats, NaCl resistant</td>
<td>53 ± 11 (n = 15)</td>
<td>137 ± 30 (+156% ( p &lt; 0.02)* (n = 18)</td>
</tr>
<tr>
<td>Dahl S rats, NaCl sensitive</td>
<td>30 ± 7 (n = 16)</td>
<td>89 ± 21 (+197% ( p &lt; 0.005)) (n = 15)</td>
</tr>
</tbody>
</table>

Values are means ± SEM.

*Indicates % increase compared to animals fed 1.5% NaCl diet.

FIGURE 1. Blood pressures of Dahl S and R rats as influenced by 5% NaCl diets containing coconut oil (1.5% linoleic acid diet) or safflower oil (16% linoleic acid diet).

Discussion

Experiment A

It has been shown that S rats have a reduced capacity for natriuresis for given levels of renal arte-
mrial inflow pressure;\textsuperscript{18} for several reasons, one can consider that the low papillary PGE\textsubscript{2} in S rats contributes to their intrinsic inability to excrete Na rapidly. When either strain has a high salt intake, the papillary PGE\textsubscript{2} doubles in concentration. This would appear to be one of many physiological responses that permit the rat to meet the high Na challenge. However, the S rat is unable to bring about the full rise in PGE\textsubscript{2} level. Lacking this, the capacity for rapid Na excretion may be compromised. For instance, Stokes et al.\textsuperscript{60, 61} have shown that, in both the ascending limb of the loop of Henle and in the collecting tubule, PGE\textsubscript{2} inhibits the transport of sodium through the tubular wall. Wilson et al.\textsuperscript{62} also found this same inhibition in the medullary collecting duct. According to this, a reduced concentration of prostaglandin E\textsubscript{2} would be expected to enhance transport through these epithelia.\textsuperscript{21} Hence, if the Dahl S rats have a reduced concentration of PGE\textsubscript{2} in the papilla, this would tend to encourage sodium retention and would somewhat retard rapid sodium excretion. This could be a partial explanation of the inability of the kidneys of S rats to excrete sodium rapidly. And any change that retards natriuresis would also facilitate the development of NaCl-induced hypertension. Along this same line, Ferris (personal communication) has produced a low PGE\textsubscript{2} state in the rat kidney by reducing essential fatty acids in the diet. These rats have a diminished capacity to excrete a Na load and develop hypertension when fed a high salt diet. The low papillary PGE\textsubscript{2} level in S rats might enhance NaCl hypertension in still another way. When normal rats are fed a high NaCl diet for 5 to 7 days, the plasma flow to the renal papilla is increased by 32\% to 45\%.\textsuperscript{19} This increased plasma flow is one of the many physiological responses that help the rat respond appropriately to a high NaCl challenge. Moreover, during the Na retention phase in caval dogs or salt-depleted dogs, a reduced plasma flow to the renal papilla was the only significantly abnormal renal hemodynamic or GFR alteration.\textsuperscript{44} R. Osgood et al.\textsuperscript{48} have also produced evidence that increasing papillary plasma flow during volume expansion favors natriuresis by reducing Na reabsorption in the ascending limb of deep nephrons. Thus, high papillary plasma flows are associated with enhanced natriuresis and reduced papillary plasma flows with reduced natriuresis. Moreover, Dahl S rats always have greatly reduced papillary plasma flows compared to Dahl R rats, regardless of whether the diet contains low or high amounts of NaCl.\textsuperscript{14} Hence, the S rat is not able to bring about the full increase in papillary plasma flow during high NaCl diets, a handicap that would tend to produce a diminished excretion of Na. We have recently found strong evidence that prostaglandins act as vasodilator substances upon the vessels supplying the rat renal papilla.\textsuperscript{77} The low level of PGE\textsubscript{2} in the papillae of S rats would therefore encourage relative vasoconstriction and reduced papillary flow; as mentioned above, this would increase the tendency to Na retention which, in turn, would increase susceptibility to NaCl hypertension.

**Experiment B**

The precursor for prostaglandin E\textsubscript{2} biosynthesis is arachidonic acid. Moreover, the body makes arachidonic acid mainly from the linoleic acid in the diet. It is possible that S rats might need a very high level of dietary linoleic acid in order to synthesize a normal amount of papillary prostaglandin E\textsubscript{2}. It does appear that a diet rich in linoleic acid will raise PGE\textsubscript{2} levels in both S and R papillae. Such a rise of PGE\textsubscript{2} in S papillae should enhance the capacity for brisk natriuresis and reduce the susceptibility of S rats to NaCl hypertension. The BP course of S rats fed a high NaCl-high linoleic diet seems to bear this out. The high linoleic diet prevents about half of the hypertension. Moreover, S rats of this age develop a mild hypertension of between 165 and 170 mm Hg even when they have eaten a life-long 0.3\% low NaCl diet. The S rats on the high linoleic-high NaCl regimen have about this same BP. Thus, the high linoleic diet appears to overcome the hypertensive effects of high NaCl feeding but cannot overcome the intrinsic tendency to hypertension that a middle-aged S rat gets even when eating a low NaCl diet. Seemingly, reduced PGE\textsubscript{2} in the renal papilla appears to contribute to NaCl hypertension in S rats, whereas raising PGE\textsubscript{2} levels with linoleic appears to ameliorate it. Furthermore, the interstitial cells of the renal papilla have a high arachidonic acid content. The high linoleic feeding may enhance the antihypertensive actions of these cells.

**References**

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