Low Renal Papillary Plasma Flow in Both Dahl and Kyoto Rats with Spontaneous Hypertension

MUKUL GANGULI, M.V.SC, PH.D., LOUIS TOBIAN, M.D., AND LEWIS DAHL, M.D.

SUMMARY Abnormally low plasma flow to renal papilla characterizes Dahl hypertension. When eating a normal Na diet (0.3% NaCl) both hypertension-sensitive (S) rats and hypertension-resistant (R) rats, 16 weeks old, have fairly normal blood pressure (BP), averaging 144 and 129 mm Hg, respectively. However, even in this barely hypertensive state, 18 S rats had a 31% lower papillary plasma flow (Lilienfeld method) than 22 R rats, 19.2 ml/100 g of papilla per min compared to 25.6 (P < 0.001). When a high (8%) NaCl diet was fed for 7 days, R rats increased papillary plasma flow from 25.6 on 0.3% NaCl to 33.2 on 8% NaCl, a 32% rise (P < 0.001). S rats increased papillary flow from 20.4 to 24.8, a 22% rise (P < 0.05). When a high (8%) NaCl diet was fed for 4 weeks, R rats increased papillary plasma flow from 25.7 ml/100 g per min on 0.3% NaCl to 29.5 ml/100 g per min on 8% NaCl, a 15% rise (P < 0.025).

THE RENAL MEDULLA has several possibly important connections to hypertension. For instance, the renal interstitial cells are all located in the inner medulla.1 These cells appear to be secretory in nature and are known to secrete prostaglandin E₃.² Moreover, when implants of these cells are placed subcutaneously in a hypertensive rat or rabbit, some humoral agent issuing from them lowers the level of arterial pressure.³ ⁴ These interstitial cells also contain a distinctly reduced number of cytoplasmic lipid granules in five separate forms of experimental rat hypertension.⁵ ⁶ ⁷ These cells not only secrete prostaglandins but also appear to elaborate Muirhead's antihypertensive neutral lipid.² ⁸ ⁹ Moreover, in three forms of experimental hypertension in rats the sodium concentration in the papilla was shown to be significantly lower than that in normotensive controls.¹ ¹ ¹ ¹ • These interstitial cells are all located in the inner medulla.¹ ¹ ² ¹ ³ ¹ ⁴ Moreover, the reduction in sodium concentration in the papilla paralleled the reduction in the number of interstitial cell granules, with a fairly strong correlation.¹ ¹ ² ¹ ³ ¹ ⁴ Moreover, prostaglandins are very powerful vasoactive agents; in the kidney, they are synthesized (90%) mainly in the renal medulla,¹ five largely by the interstitial cells and the collecting duct cells.¹ ¹ ⁶ The renal medulla also contains the collecting ducts and they are powerful regulators of ultimate sodium excretion.¹ ¹ ⁷ ¹ ⁸ Since hypertension is profoundly affected by the sodium balance in the body, the collecting ducts of the medulla conceivably could have an important connection to hypertension.

With this background, we became interested in measuring plasma flow to this important region in hypertensive rats. It has been reported by several investigators that although renal cortical blood flow is well autoregulated, renal medullary blood flow is not well autoregulated. Kramer et al.¹⁴ and Miyamoto and Gordon¹⁵ found that during a sudden rise in the perfusion pressure, the blood flow through the renal medulla increased, while the cortical and total renal blood flow remained constant. If this were indeed true under all circumstances, then in hypertensive rats papillary plasma flows could well be very large. Ganguli and Tobian¹⁶ noted in rats with “post-salt” hypertension that the renal papilla does indeed chronically “autoregulate” its flow, because a reduced renal papillary plasma flow was found in rats with post-salt hypertension. Since the post-salt hypertensive kidneys showed a considerable amount of nephrosclerosis which led to the atrophy of some nephron units, we further endeavored to test this hypothesis in Kyoto spontaneously hypertensive rats (SHR) and Dahl salt-sensitive (S) rats. Both of these strains have almost no nephrosclerosis at 17 weeks of age if fed a normal salt intake. Furthermore, the SHR and S rats are recognized as models resembling human essential hypertension.¹⁷ ¹⁸

Methods

The renal papillary plasma flow was measured in rats anesthetized with ethyl(-methylpropyl)malonothiourea (Inactin) (100 mg/kg) by a method essentially similar to that of Lilienfeld et al.¹⁹ but incorporating slight modifications by Ganguli and Tobian¹⁶ and subsequently by Solez et al.²⁰ to make it applicable to the rat. In brief,¹²¹ I-labeled albumin at the concentration of 4 µCi/ml in normal saline (0.9%) is steadily infused into the right atrium. Then for about 60 seconds in the dog and for about 36 seconds in the rat, the radioactive counts in the papilla increase approximately linearly with time after the start of the infusion. Because of the small amount of mixing and slow flow...
and long length of the vessels in the papilla, this represents the time that elapses before plasma entering the papilla actually leaves it.

During the precise time interval in which radioactive albumin was entering the kidney, blood in the carotid artery was slowly but continuously sampled for radioactivity at approximately the same rate of withdrawal as fluid containing radioactive albumin entered the right atrium. After a time interval of 24 seconds, the kidney pedicle was instantaneously ligated and the collection of blood from the carotid artery was terminated. Since the time interval for linear accumulation of radioactive albumin in the papilla lasts 36 seconds, an accumulation time of 24 seconds is well within the linear period and should provide an accurate index of plasma flow to the papilla.

At the end of the prescribed time interval, we quickly dissected out the papilla of the kidney and placed it in an air-tight preweighed weighing bottle to obtain the wet weight of the papilla. Then the radioactivity of the papilla was ascertained in a gamma scintillation counter. The samples of blood from the carotid artery were similarly weighed and counted. Tissue radioactivity is expressed as counts per minute per gram of tissue. Blood radioactivity is expressed as counts per minute per milliliter of plasma after making corrections for the hematocrit. When the radioactive counts per gram of papilla are divided by counts per milliliter of plasma, one has an estimate of the volume of plasma accumulating in a gram of papilla during 24 seconds of elapsed time. This should represent papillary plasma flow.

Just prior to infusion of radioactive albumin, we measured mean blood pressure directly from the carotid artery with a mercury manometer. For statistical analyses of the data we used Student's t-test.

**EXPERIMENT 1**

We used the two strains of rats developed by Dahl. One group, the S strain, invariably becomes hypertensive when fed a high NaCl intake (8% NaCl in dry chow). The other group, the R strain, never becomes hypertensive when fed an 8% high salt intake. The S strain is so named because it is sensitive to salt hypertension. The R strain is so named because it is resistant to salt hypertension.

A total of 77 female rats of S and R strains, about 16 weeks of age and weighing 235–285 g, were used. They were divided into eight groups, according to duration of feeding period, amount of NaCl fed, and type of rat (Table 1).

The two levels of dietary NaCl, 0.3% and 8.0%, were considered as either "normal" or "high salt," respectively. All rats had received 0.3% NaCl chow since weaning. After that, high salt feeding was carried out in both S and R rats for 1- and 4-week periods. Concomitant feeding of a diet with 0.3% NaCl was carried out in other S and R rats. The papillary plasma flow then was measured as described above. This was done in a "round-robin" fashion among groups I through IV, and among groups V through VIII.

**EXPERIMENT 2**

Twenty-six male Kyoto spontaneously hypertensive (SHR) rats with blood pressure (BP) = 170–200 mm Hg, and 24 Kyoto normotensive controls (BP = 100–130), weighing between 280 and 360 g and all about 17 weeks of age, were used. The mean arterial pressure for the Kyoto SHR was 182 mm Hg; the mean pressure for the Kyoto normotensives was 118 mm Hg. All rats had received 0.3% NaCl chow since weaning. The plasma flow to the renal papilla was measured by the method described above. The measurements on Kyoto hypertensive and Kyoto normotensive rats were alternated.

**Results**

**EXPERIMENT 1 (TABLE 1)**

When eating a normal Na diet with 0.3% NaCl, 18 hypertension-sensitive (S) rats and 22 hypertension-resistant (R) rats (all 20 weeks old) had blood pressures close to the normal range, the two groups averaging 144 and 129 mm Hg, respectively. The S rats could be considered to have borderline hypertension because their average mean blood pressure was about 15 mm Hg above that of the R rats. After both groups had been on the normal (0.3%) NaCl intake for several weeks, the borderline hypertensive S rats (groups I and V) had a mean papillary plasma flow of 19.2 ml/100 g of papilla per min, which was 25% lower than the 25.6 ml/100 g per min plasma flow of the normotensive R rats (groups III and VII). The difference in flow here was highly significant, with a P value much less than 0.001.

**Table 1:** Effects of NaCl Intake on Blood Pressure and Papillary Plasma Flow

<table>
<thead>
<tr>
<th>Group</th>
<th>Type of rat</th>
<th>No. of rats</th>
<th>% NaCl in diet</th>
<th>Blood pressure (mm Hg)</th>
<th>Papillary plasma flow (ml/100 g per min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-wk diet</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>S</td>
<td>10</td>
<td>0.3</td>
<td>145 ± 4.2</td>
<td>20.4 ± 1.30</td>
</tr>
<tr>
<td>II</td>
<td>S</td>
<td>9</td>
<td>8.0</td>
<td>152 ± 3.1</td>
<td>24.8 ± 1.56</td>
</tr>
<tr>
<td>III</td>
<td>R</td>
<td>13</td>
<td>0.3</td>
<td>130 ± 2.4</td>
<td>25.6 ± 0.81</td>
</tr>
<tr>
<td>IV</td>
<td>R</td>
<td>12</td>
<td>8.0</td>
<td>128 ± 1.8</td>
<td>33.8 ± 1.84</td>
</tr>
<tr>
<td>4-week diet</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V</td>
<td>S</td>
<td>8</td>
<td>0.3</td>
<td>142 ± 3.3</td>
<td>17.7 ± 1.13</td>
</tr>
<tr>
<td>VI</td>
<td>S</td>
<td>7</td>
<td>8.0</td>
<td>162 ± 3.3</td>
<td>20.0 ± 1.74</td>
</tr>
<tr>
<td>VII</td>
<td>R</td>
<td>9</td>
<td>0.3</td>
<td>127 ± 2.9</td>
<td>25.7 ± 0.72</td>
</tr>
<tr>
<td>VII</td>
<td>R</td>
<td>9</td>
<td>8.0</td>
<td>127 ± 2.5</td>
<td>29.5 ± 1.41</td>
</tr>
</tbody>
</table>

S = hypertension-sensitive rats; R = hypertension resistant rats.

Results are expressed as mean ±SEM. Bracketed P values for bracketed pairs of means indicate a significant difference between those two means. Where no P values are listed, the difference between means was not statistically significant.
When the R rats were challenged with a high (8%) NaCl diet for 7 days, the papillary plasma flow rose to 33.8 ml/100 g per min, whereas it was only 25.6 ml/100 g per min in comparable R rats that remained on the normal (0.3%) NaCl intake. Thus, exposure to the high salt intake led to a 32% increase in papillary flow in the R rats. The P value of the difference was less than 0.001. The same 8% high salt feeding was carried out for 1 week in S rats. After the 1-week challenge with a high salt intake, the S rats had an average papillary flow of 24.8 ml/100 g per min compared to a flow of 20.4 ml/100 g per min in S rats on a normal salt intake. The high salt intake increased papillary flow by 22% in these S rats, with a P value less than 0.05.

We next studied the effect of a 4-week challenge with the high salt feeding. In R rats on the high salt intake, the flow was 29.5 ml/100 g per min, compared to 25.7 ml/100 g per min in R rats on a normal salt intake. Thus, even after 4 weeks of high salt feeding, there was an increase of 15% in papillary flow above control, with a P value of 0.025. This is a considerably smaller rise than the 32% increase seen after 1 week on the high salt intake. Evidently, some degree of adaptation to the high salt intake had taken place.

When the 4-week salt challenge was tested on S rats, we found a small rise in flow compared to the control group. The difference was not statistically significant. Thus the S rats appear to demonstrate little increase of papillary flow after 4 weeks of high salt feeding, a much smaller response than was present after 1 week of high salt feeding.

A contrast between change in blood pressure and papillary flow was found in the two strains of rats after 1 week of high salt feeding. The R rats showed no increase in blood pressure. It was still 128 mm Hg. In the S rats BP increased to 152 mm Hg after only 1 week of salt feeding. The R rats had a papillary flow of 33.8 ml/100 g per min, whereas the S rats had a flow of only 24.8 ml/100 g per min. This is a 27% difference in papillary flow, with a P value less than 0.005. The same comparison was made after 4 weeks of high salt feeding. The blood pressure of the R rats still remained low at 127 mm Hg. The blood pressure of the S rats had risen to 162 mm Hg. The papillary flow of the R rats was 29.5 ml/100 g per min, whereas that of the S rats was only 20.0 ml/100 g per min. This is quite a large difference, with the S rats having a flow 32% lower than that of the R rats. The P value of this difference was less than 0.001.

**EXPERIMENT 2**

On a normal salt intake, the papillary flow of 26 Kyoto spontaneously hypertensive rats (age = 17 weeks, BP = 182 mm Hg) was compared with that of 24 Kyoto normotensive rats (age = 17 weeks, BP = 118 mm Hg). The Kyoto hypertensives had an average papillary flow of 29.5 ± 0.7 (SEM) ml/100 g per min, while the Kyoto normotensives averaged 33.2 ± 0.7 ml/100 g per min. Thus the Kyoto hypertensives had a papillary flow 11% lower than the Kyoto normotensives (P ≤ 0.001).

**Discussion**

In previous studies of S and R rats on either 0.3% or 8.0% NaCl by Ben-Ishay et al., the PAH clearance was virtually the same in either strain on either high or normal salt diets. Thus the total renal blood flow in the S rat is not reduced, compared to the R rat. The elevation of total renal vascular resistance just matches the rise in arterial pressure, so that the total renal blood flow in the S rat remains normal. This is in striking contrast to our finding of a markedly lower papillary plasma flow in the S rat. The vascular resistance of vessels supplying the papilla in the S rat must be disproportionately much greater than the vascular resistance in the S kidney as a whole.

This disproportionate vasoconstriction in vessels supplying the S papilla when the S rat has only borderline hypertension well may be related to this rat’s susceptibility to NaCl hypertension. Our findings indicate that papillary plasma flow significantly increases in normotensive R rats and in ordinary Holtzman and Sprague-Dawley rats when they are challenged with a high salt intake. This increase in papillary flow may be a physiological aid in the expeditious excretion of the large NaCl load. It is well demonstrated by Stein et al., Sonnenberg, and Diezi et al. that the collecting duct participates very importantly in the excretion of a large Na load. The increase in papillary plasma flow during a high sodium intake may be related to this collecting duct action. The S rat has an abnormally low papillary flow while on a normal sodium intake. However, as long as the sodium intake is fairly low, the reduced papillary flow of the S rat may be of little importance in sodium homeostasis, and the rat’s blood pressure does not rise above the borderline hypertensive range. However, when the S rat begins to eat the high salt diet, there is only a limited increase in papillary flow, which remains a striking 32% below the papillary flow of the R rat after 4 weeks on the same high salt intake. One might theorize that the limited papillary flow in the S rat somehow brings about a limited capacity of the kidney to excrete sodium rapidly. If this were indeed the case, other, alternative natriuretic forces might then be needed to expedite a brisk Na excretion. It is well established that an increase in renal arterial pressure will bring about a natriuresis in an isolated kidney. Thus one can further theorize that the low papillary flow of the S rat limits the usual rapid excretion of Na, and thereby necessitates an increase in arterial pressure to bring about Na excretion through the mechanism of a "pressure natriuresis." We hasten to add that this formulation is at present only a working hypothesis, and further information on several key points is needed before it can be accepted or rejected with any confidence.

If indeed low papillary flow retards the rate of sodium excretion and higher papillary flow facilitates it after a sodium load, this could very well be related to the development of hypertension. In a previous study ordinary rats were fed a diet containing 8% NaCl, and 40% of them became hypertensive. Another group of similar rats were fed the same high salt diet and at the same time were given a thiazide diuretic in their drinking water. None of the rats in this group became hypertensive. Both of these groups came into sodium balance on the high salt intake but one group had a high prevalence of hypertension and the other group had no hypertension. The group on the high salt diet developed an increase of extracellular fluid (ECF) volume 5% above control after 1 week on the diet. In the group on
the high salt diet plus thiazide the ECF volume was very close to control levels. Even though rats in both groups excreted the ingested NaCl, the thiazide facilitated this excretion in one group, thereby achieving Na balance with a normal ECF volume. In the other group without thiazide, Na balance was achieved in association with an expanded ECF volume. Hypertension was frequently present in the group with the expanded ECF volume but was absent in the group with the normal ECF volume. When the ECF volume is expanded for a considerable period of time, as in mineralocorticoid or renal parenchymal hypertension, hypertension often supervenes. It is conceivable that the marked difference in papillary plasma flows in the S and R rats may have had a differential effect on sodium excretion just as the presence or absence of thiazide did.

The low plasma flow to the renal papilla in S rats and Kyoto hypertensive rats may have another implication. The low flow indicates an unusual degree of increase in vascular resistance, more than is found in other vascular beds in hypertensive. The hypertensive state, in general, is associated with narrow vascular lumina and increased vascular resistance. It could be that the vessels supplying the papilla are especially sensitive to this "hypertensive" effect, more sensitive than any other set of vessels in the body. They could be so sensitive that they produced a 25% reduction of papillary plasma flow even in S rats with borderline hypertension eating a normal salt diet. The vessels supplying the papilla in these rat could be narrowed quite a bit while other vessels are scarcely narrowed at all. The supersensitivity of papillary vessels in S rats also could affect and retard sodium excretion during a high sodium load, which in turn would have the effect of increasing the severity of the hypertension, which in turn could further narrow papillary vessels. Thus, during a large sodium load the key elements might exist for a mildly vicious cycle.

If the vessels to the papilla are especially narrowed in S rats, one might suspect an excess or deficit of vasoactive humoral agents which are elaborated in close proximity to these vessels. Substances secreted by the interstitial cells could very well be causing the exaggerated narrowing of vessels supplying the papilla of the S rat. These would include prostaglandins as well as Muirhead's "neutral lipid" as well as other unrecognized secretory materials elaborated by the interstitial cells. Moreover, the collecting ducts also synthesize prostaglandins which could be acting on these blood vessels.

In regard to the increased papillary plasma flow after a high salt intake, one is reminded that volume expansion causes the elaboration in the rat of a humoral agent of nonrenal, nonadrenal origin which dilutes renal blood vessels and heightens natriuresis. It would be of interest to know whether this factor is stimulated by a high NaCl diet and whether it affects the caliber of blood vessels supplying the renal papilla.

In the Kyoto spontaneously hypertensive rats papillary flow was lower by 11% than in the Kyoto normotensive controls. This is of particular interest in relation to the finding by Nishiyama et al. that the blood flow to the Kyoto hypertensive kidney as a whole is the same as that of the Kyoto normotensive kidney as a whole. Thus, here again the resistance in vessels supplying the papilla is disproportionately higher than the vascular resistance to the kidney as a whole. The vessels supplying the papilla appear to be especially sensitive to the narrowing associated with the hypertensive process. The flow to the papilla in Kyoto hypertensives is only 11% below Kyoto controls. This is a smaller difference than was seen between Dahl S and R rats and is consistent with the observation that the Kyoto hypertensive strain shows a much more modest rise in blood pressure in response to a high salt intake than that of the Dahl S rat.

In a previous study it was noted that the papillary flow in rats with "post-salt" hypertension is 15% lower than that of normotensive controls. Thus we have studied three distinct types of experimental rat hypertension, all of which have significantly reduced papillary plasma flow. It is possible that this low papillary flow is a genuine repeating hallmark for all types of experimental rat hypertension. More studies will definitely be needed to get a firm answer to this question.

References

The Effect of the Pattern of Cardiac Sympathetic Activity on Myocardial Contractile Force and Norepinephrine Overflow in the Dog Heart

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With the technical assistance of Herrick Finkelstein

SUMMARY

The left or right cardiac sympathetic nerves in open-chest, anesthetized dogs were stimulated at mean frequencies of 2 or 4 Hz. The stimuli were applied intermittently, in patterns with repetition rates of either 60/min or 15/min, to simulate the spontaneous patterns of sympathetic neural activity that occur synchronously with the cardiac or respiratory cycles, respectively. With either repetition rate, intermittent stimulation of the left sympathetic nerves was about 10–20% less effective in enhancing myocardial contractile force (CF) and about 10% less effective in increasing coronary sinus blood flow than was steady stimulation at the same mean frequency. With right-sided stimulation, there was no appreciable difference between steady and intermittent stimulation patterns with respect to the effect on heart rate. With either left- or right-sided stimulation, the rate of norepinephrine (NE) overflow into the coronary sinus blood was 20–40% less with intermittent than with steady stimulation. Cocaine administration did not materially affect this difference in NE overflow. It was concluded that the higher instantaneous frequencies that prevail during intermittent stimulation result in a reduction in the rate of NE release at the sympathetic postganglionic nerve endings in the heart.

The Pattern of efferent activity in sympathetic nerve fibers varies considerably. In some fibers and under certain conditions, the activity appears to be random. Frequently, however, there are distinct groupings of activity, synchronous with either the heart beat or the respiration. Aside from introducing some rhythmical variations in heart rate, myocardial contractility, and peripheral resistance, the influence of the grouping of efferent impulses in sympathetic neurons has not been established. It is not known, for example, whether a given number of sympathetic neural impulses will produce different responses depending upon whether the activity is steady or intermittent. The present study was designed to answer this question with respect to certain cardiac responses. The changes in ventricular contractile force (CF) and heart rate were measured with different patterns of sympathetic stimulation, and these changes were correlated with the rates of overflow of norepinephrine (NE) into the coronary sinus blood. Two repetition rates were arbitrarily selected for the stimulation patterns delivered to the cardiac sympathetic nerves in order to simulate the frequency of the cardiac and respiratory groupings of impulses in the resting, anesthetized dog.

Methods

All experiments were conducted on mongrel dogs with an average weight of 22.4 ± 2.8 (SD) kg. The mean heart weight was 157 ± 24 (SD) g. The dogs were anesthetized with sodium pentobarbital (30 mg/kg, iv) and the chest was opened through an incision in the 4th intercostal space. Heparin (500 U/kg, iv) was injected to prevent blood coagulation, and a modified Morawitz cannula was introduced into the coronary sinus via theazygos vein. The tip of the cannula was fixed in position by means of a suture placed in the posterior wall of the right atrium and around the coronary sinus, within 1 cm of the ostium of the coronary sinus. The coronary venous blood was conducted from this cannula through the extracorporeal probe of an electromagnetic flowmeter (Biotronix) and was returned to the venous system by way of the right external jugular vein.

Both stellate ganglia were decentralized and bipolar platinum electrodes were placed about the two limbs of either the left or the right ansa subclavia. In most experi-
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Circ Res. 1976;39:337-341
doi: 10.1161/01.RES.39.3.337

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