Renomedullary Deficiency in Partial Nephrectomy-Salt Hypertension

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SUMMARY Partial nephrectomy-salt hypertension (PN-SH) of the rat is associated with Na volume loading. As the hypertensive state evolves, the renomedullary interstitial cells (RIC) of the renal nubbin undergo major changes, decreasing significantly in number while the remaining ones exhibit degenerative changes. The antihypertensive action of the RIC in the renal nubbin, as measured by transplants of fragmented papillae into hypertensive recipients, virtually disappears as the hypertension develops. The changes in the RIC occur whether vascular disease of the kidney is or is not overtly present. It is suggested that deficiency of the antihypertensive action of the RIC allows the prohypertensive effects of Na volume loading to operate without proper control. Thus, the sustained hypertensive state of this model does not appear to be due solely to volume expansion. Rather, it appears due to a combination of the effects of Na and volume and a renomedullary deficiency of hormonal type.

The specific cause(s) of the changes in the RIC was not determined. It seems evident that it is related to the high salt intake since the partial nephrectomy procedure without the added salt load did not alter the appearance of the RIC. (Hypertension 2: 281-290, 1980)

KEY WORDS • renomedullary interstitial cells • antihypertensive action • hormone deficiency • renoprival • transplants • sodium dependency

The hemodynamic characteristics of the hypertensive state have been divided into two general categories, namely, vasoconstriction-dependent and volume-dependent. Vasoconstriction-dependent hypertension is proposed as that type primarily due to the action of vasoconstrictors, predominantly angiotensin, although other vasoconstrictor agents have been considered as possible mediators in some cases. Volume-dependent hypertension is considered to result primarily from the expansion of extracellular fluid volume (ECFV) and is therefore mainly sodium-dependent. The most severe forms of hypertension tend to combine elements of both types.

A major type of sodium volume-dependent hypertension results from bilateral nephrectomy plus volume excess, i.e., renoprival hypertension. This model is useful for short-term experiments but may be extended by chronic dialysis to maintain the experimental animal. A somewhat similar experimental model of sodium volume-dependent hypertension utilizes partial renal ablation along with sodium volume loading. Enough excretory function is preserved so that long-term dialysis is not necessary. This is the Chanutin-Ferris type of hypertension in which one kidney and the poles of the other kidney are removed, resulting in a 66%-75% renal ablation (fig. 1).

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Figure 1. Diagrammatic representation of the manipulations leading to the partial nephrectomy-salt hypertensive model.
Guyton and associates\textsuperscript{10, 11} used the partial nephrectomy-salt model in the dog to demonstrate early transient increase in cardiac output followed by elevation of peripheral vascular resistance and decrease in cardiac output. Ylitalo et al.\textsuperscript{12} supported these findings by further work in the rat.

There are increasing indications that the renovascular interstitial cells (RIC) of the renal papilla exert an endocrine-type of antihypertensive function.\textsuperscript{13-20} This antihypertensive action appears to be especially effective against Na volume-dependent hypertensive states.\textsuperscript{14-18, 21-24} Since partial nephrectomy-salt hypertension (PN-SH) is Na volume-dependent, it appeared worthwhile to investigate the state of the RIC as this hypertensive state evolved.

Materials and Methods

Male Wistar rats, weighing 225-235 g, about 3 months old, and purchased from one supplier (the Pel-Freez Co. of Rodgers, AR) were divided into four groups. In Groups I, II, and III, the systolic arterial pressure was measured by the tail-cuff method using the E and M physiograph apparatus (E and M Co., Houston, TX). The rats were preheated for 10 minutes (Sears Co. heat lamp, Catalog S-15 CR 401 at 25 cm) and were conscious and upright within a plastic cage. The mean aortic pressure of the rats in Group IV was measured by an indwelling aortic catheter, as previously described.\textsuperscript{25} The ECFV was measured by the S\textsuperscript{90} O\textsubscript{2} method of Walker et al.,\textsuperscript{26} as previously described.\textsuperscript{27} Exchangeable sodium (Na\textsubscript{e}) was determined according to Tobian et al.\textsuperscript{28} also as previously described.\textsuperscript{27} The serum Na concentration was determined by flame photometry (Instrumentation Laboratories, Watertown, ME).

Partial Nephrectomy

The partial nephrectomy, entailing removal of the right kidney and the poles of the left kidney (fig. 1), as conducted in this laboratory, has been described elsewhere.\textsuperscript{15} For removal of the poles, a special device was developed that allowed removal of a reasonably defined mass of renal tissue. The procedure, performed under pentobarbital anesthesia (30 ml/kg i.p.), leaves behind a renal nubbin consisting of papilla, outer medulla, and the cortex immediately lateral to these two. Following the partial nephrectomy, the animals were placed on the sodium-free diet of Hartroft and Hartroft,\textsuperscript{29} containing 0.24 mEq/g potassium\textsuperscript{*} (Nutritional Biochemical Corp., Cleveland, OH) and were given distilled water to drink for 5 days. This approach was used to allow rats time for recovery from the operation before being challenged with the salt load (1% NaCl to drink). It is realized that this time interval allowed for some hypertrophy of the renal nubbin so that the remaining renal mass became more than the original estimate. Each day 100 ml of salt solution was given to the salt-receiving animals.

Renal Papillary Transplant (Tr Pap)

We have modified our procedure for transplantation of fragmented renal papilla as follows. The kidney (or renal nubbin) is removed under pentobarbital anesthesia (30 mg/kg i.p.). After delivery, the kidney is sectioned across the convex surface of the cortex slightly to the left of center with a 5.5 cm straight razor blade. This opens the pelvis and exposes the papilla in an intact state. With small curved scissors, the papilla is cut across its base and removed. The papilla(ae) is placed in a Petri dish containing 0.1 ml of Hank’s balanced salt solution. With the same razor blade, the renal tissue is minced for 1½ minutes, using right angle, up-and-down strokes. A curved spatula is used to maintain the minced papilla(ae) in one spot on the Petri dish. After the mincing procedure, the tissue is placed in a 25 ml conical centrifuge tube along with 5 ml of Hank’s solution. The particles are suspended and centrifuged for 1 minute in a table-model centrifuge. The supernatant is decanted and the particles of tissue are taken up in a tuberculin syringe containing 0.2 ml of Hank’s solution plus 500 units of penicillin and 100 \textmu g gentamicin. Operating room type sterile procedures are used throughout. The recipient’s skin is prepared by shaving, scrubbing with PhisoHex, washing with 70% alcohol, and then treating with tincture of iodine for one minute. The iodine is removed with alcohol. With a 21 gauge, 4 cm needle, the particles of papilla are injected. The needle is driven subcutaneously to the hilt and slowly removed as the injection occurs, thereby making a thin long track of tissue. The entire procedure takes 10-15 minutes.

Three points are crucial: 1) the mincing procedure must not be overdone, lest the tissue not take (particles of 1 mm or less are preferable); 2) there must be a track and not a bolus, lest necrosis of much of the transplant occur; and 3) a palpable nodule that appears to expand must develop by the next day or so.

The source kidneys for the Tr Pap were from an outbred Wistar rat line (allogeneic). For this reason, the experiment was conducted for only 3 days. We have previously determined\textsuperscript{30} that allogeneic renomedullary transplants were effective over this time period.

The four groups of animals were studied as follows:

\textbf{Group I (12 rats)}: The rats of this group had an estimated 75% initial partial nephrectomy. After the initial postoperative interval, six animals were switched to a 1% NaCl solution to drink while six paired controls continued to drink distilled water. Following the sodium volume loading, the experimental animals developed hypertension that appeared in two phases, an early phase (120 to 155 mm Hg over 3 days) and a later phase (170-180 mm Hg 20 days later). At 30 to 53 days, ECFV and Na\textsubscript{e} were measured. At the same time, similar measurements were made on the paired controls.

\textsuperscript{*}The average daily intake of K was 2.84 mEq.
Group II (14 rats). The rats of this group also had an estimated 75% initial partial nephrectomy. All rats were placed on 1% NaCl drinking solution. The body weight, oral fluid intake, and arterial pressure were monitored almost daily. Between 28 and 36 days, the animals were killed and a morphometric study was performed on the renal papillae of 10 animals. The results of this study were compared with a similar study of five of the paired controls from Group I that drank distilled water and five normal rats from the same colony.

Group III (11 rats). The original partial nephrectomy of this group was estimated at 66%. The arterial pressure and body weight were monitored almost daily. At 7, 12, and 18 days, three animals of the group were sacrificed and a morphometric study of the renal papilla was performed. The same study was conducted on the two remaining animals at Day 25. Light and electron microscopic studies of the cortex and medulla were done.

Group IV (84 rats). We then wished to determine if the morphologically changed RIC, as demonstrated by the results of Group III, were also altered with respect to their antihypertensive action. A preliminary experiment was done in which normal papillae were transplanted to a one-kidney, one clip Goldblatt hypertensive rat of 3-6 months' duration (produced as previously described\textsuperscript{3}) to determine the least number of normal papillae needed to induce a significantly measurable 3-day antihypertensive effect.

For further experiments, two papillae each from partial nephrectomy-salt hypertensive animals and from normal control animals were transplanted to one-kidney, one clip Goldblatt hypertensive rats (3-6 months' duration) and the pressures of recipient rats were monitored for 3 days. Partial nephrectomy-salt hypertensive rats were used for transplantation purposes at 7, 14, and 25-30 days in order to correspond to varying degrees of RIC damage seen in the previous morphometric study (Group III).

Morphologic and Morphometric Studies

At sacrifice, the renal papilla of either an intact kidney or the remaining renal nubbin was dissected free from the cortex. The cortex and papilla were processed for light microscopy (hematoxylin and eosin stain) in the usual way. The papilla was processed for semi-thin section (0.5 μm) study (toluidine blue stain) and electron microscopy (EM), as previously described.\textsuperscript{80} For the morphometric study, the entire papilla was sectioned. A rectangular area consisting of 10 squares, as indicated by the hatched squares in figure 2, was delineated in the center of the center of the papilla, extending about 0.6 mm from its tip and 0.5 mm from the sides near the tip. A grid containing a square having 100 points, each point representing an intersection of lines partitioning the square, was placed in the microscope eyepiece. The position of the grid was gradually moved up along the area of the papilla shown in figure 2. By this means, 10 squares were covered. The number of RIC touching the points of the square as well as the number of granules in each RIC were counted. By this means, 1000 points per papilla were considered. This approach consisted of a modification of the morphometric study as originally described by Weibel\textsuperscript{11} and used by Bohman and Jensen.\textsuperscript{80} The morphologic appearance of the RIC was also examined by EM.

Statistical analyses were conducted by the paired t test.

Results

Group I

Figure 3 summarizes the results. The test group on salt had an arterial pressure elevation from nearly 115 mm Hg to 160-180 mm Hg. The paired controls drinking Na-free water had no significant change in arterial pressure, from 116 ± 4 at the outset to 122 ± 2 mm Hg at sacrifice. At the time of the volume studies, the test group had an arterial pressure of 172 ± 10 mm Hg.

There was no difference in body weight and serum Na concentration of the two groups. The ECFV and Na\textsubscript{a} of the salt-hypertensive group (47 ± 5% of the body weight and 69 ± 4 mEq/kg respectively) were significantly elevated over that of the control group. The control group had values for these parameters somewhat elevated over those of normal animals of the same colony (for normals, the ECFV was 22.9 ± 1% body weight and Na\textsubscript{a} was 34.8 ± 0.1 mEq/kg).\textsuperscript{19, 80}
FIGURE 3. (Group I). The graph reveals the changes in arterial pressure of two groups of rats: partial nephrectomy-salt (solid line), and partial nephrectomy-no salt (broken line). The "salt" group had an elevation in pressure from nearly 115 to 160 mm Hg or higher. The "no salt" had no change in pressure. Data are mean values ± SEM. The table reveals no change in body weight and serum Na between the two groups but a significant elevation of both extracellular fluid volume and exchangeable sodium (Na,) in the partial nephrectomy-salt hypertension group. The values were derived 30-53 days after the experiments were begun.

PN = partial nephrectomy, NS = not significant, BP = blood pressure, ECF = extracellular fluid.

<table>
<thead>
<tr>
<th>GROUP</th>
<th>n</th>
<th>DAYS</th>
<th>FINAL BP</th>
<th>BODY WT</th>
<th>SERUM Na</th>
<th>ECF VOL</th>
<th>Na_e</th>
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<tbody>
<tr>
<td>PN + Na</td>
<td>6</td>
<td>48±10</td>
<td>172±10</td>
<td>259±24</td>
<td>149±3</td>
<td>47±8</td>
<td>68±4</td>
</tr>
<tr>
<td>PN + H₂O</td>
<td>6</td>
<td>53±4</td>
<td>117±6</td>
<td>322±14</td>
<td>146±4</td>
<td>27±1</td>
<td>49±5</td>
</tr>
<tr>
<td>p</td>
<td>&gt;0.4</td>
<td>&lt;0.005</td>
<td>&gt;0.05</td>
<td>&gt;0.6</td>
<td>&lt;0.01</td>
<td>&lt;0.002</td>
<td></td>
</tr>
</tbody>
</table>

Group II

As can be seen in figure 4, there was a slight weight loss after the partial nephrectomy procedure, lasting about 7 days. Thereafter, there was a steady weight gain, averaging 2.5 g per day. The fluid intake while on distilled water averaged 20 ml per day. As soon as the 1% NaCl was commenced, intake increased to nearly 80 ml/day and remained near this level throughout the 30 days or so of the experiment. This indicated that the daily intake of Na was about 13-14 mEq. The feces contained no Na (determined as previously described). Since the animals did not become overtly edematous, this meant that the bulk of the NaCl load traversed the remaining renal tissue and was excreted in the urine.

As soon as the 1% NaCl was begun as drinking fluid, the arterial pressure became elevated, changing from nearly 120 mm Hg to nearly 150 mm Hg within 3 days. It remained between 155 and 160 mm Hg until Day 28 when it rose to 170-180 mm Hg.

Light Microscopy

In Group II, which had an initial 75% renal ablation, the glomeruli revealed the deposition of fibrin-like material. Of the glomeruli studied, 35% were so involved (fig. 5A). An occasional glomerulus appeared completely disrupted as a result of having its loops filled with fibrin. A proteinaceous precipitate was present in Bowman's space. Scattered groups of tubules were dilated and filled with proteinaceous material. Most of the tubules, however, appeared intact. The small arteries and arterioles often displayed either fibrinoid necrosis of the entire wall (fig. 5D) or disruption plus replacement of the wall by a concentric accumulation of connective tissue-like material (fig. 5C).

The control animals on distilled water revealed no change in glomeruli, small arteries, and arterioles. The animals of Group III (66% initial renal ablation) also revealed no changes by light microscopy.

Electron Microscopy

Group II showed the following electron microscopic features:

1. Cortex. The presence of fibrin within the capillary loops of the salt-receiving animals of Group II was identified by its periodicity (fig. 5B). In addition, some dense deposits were noted in the mesangial area. We suspect these also consisted of fibrin. Focal foot process fusion was present.

2. Medulla. Fibrin was also noted in the vasa recta. We suggest that this represented fibrin that passed through the glomerular capillary wall in a manner previously suggested.
3. Renomedullary Interstitial Cells. The RIC were distinctly abnormal. Of special note was the decrease of osmiophilic granules, the disappearance of the cisternal system and organelles, and transformation of the cytoplasm into a granular structure (figs. 6 and 7).

Morphometric Study (table 1)

The number of RIC was significantly decreased in the PN-SH rats of Group II when compared to either the normotensive partial nephrectomy-water group or the normal group on a regular diet. The RIC of the PN-SH group also contained fewer granules in the cytoplasm.

Group III

The arterial pressure of the animals sacrificed at Days 7, 12, 18, and 25 averaged 150–170 mm Hg (fig. 8).

Sequential Morphometric Study

On Day 7, when the arterial pressure was already elevated, the number of RIC per grid was the same as

<table>
<thead>
<tr>
<th>Group</th>
<th>No. RIC/grid</th>
<th>No. granules/RIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) PN-salt*</td>
<td>5.2 ± 0.3</td>
<td>2.3 ± 0.14</td>
</tr>
<tr>
<td>(n = 10)</td>
<td></td>
<td>p &lt; 0.005</td>
</tr>
<tr>
<td>2) PN-H2O†</td>
<td>7.76 ± 0.4</td>
<td>5.0 ± 0.2</td>
</tr>
<tr>
<td>(n = 5)</td>
<td></td>
<td>p &gt; 0.05</td>
</tr>
<tr>
<td>3) Normal‡</td>
<td>11.04 ± 1.5</td>
<td>4.48 ± 0.4</td>
</tr>
<tr>
<td>(n = 5)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*PN-salt = partial nephrectomy-salt.
†PN-H2O = partial nephrectomy-water.
‡Normal = intact animals on regular diet.

The number of renomedullary interstitial cells (RIC) per grid and the number of granules per RIC of the morphometric study of animals in Group II are tabulated. Note that there was no significant difference in these parameters between the partial nephrectomy—no salt (on water) group and the normal (intact) group on a regular diet. Both values were significantly suppressed in the partial nephrectomy-salt group (p < 0.005).
in controls having PN and drinking water (7.0 ± 0.05 vs 7.8 ± 0.4, p > 0.25) but the number of granules per RIC was significantly lower (4.8 ± 0.2 vs 2.7 ± 0.2, p < 0.005). Thereafter, at 12, 18 and 25 days, the number of cells per grid and granules per cell continued to decrease significantly, to a minimum of 3.7 ± 0.05 and 1.1 ± 0.08, respectively (fig. 8). Moreover, as with Group II, the cytoplasm of the RIC underwent marked regressive changes, especially the disappearance of the normal granule/cisternal relationship.

Group IV

The preliminary experiment is illustrated in figure 9. The degree of the antihypertensive effect produced by transplanting normal rat renal papillae into a one-kidney, one clip Goldblatt hypertensive rat was a function of the number of papillae transplanted. A definite antihypertensive effect was produced by two normal papillae so that, in further experiments, two papillae from PN-SH animals and two papillae from normal controls were transplanted into hypertensive recipients.
Transplants of papillae from PN-SH animals at 25-30 days, at which time the number of RIC and the number of granules were both markedly decreased, failed to lower the blood pressure of one-kidney, one clip Goldblatt hypertensive rats, whereas normal controls caused a distinct drop in pressure (fig. 10). Transplants performed earlier in the hypertensive state at 14 days were also ineffective in producing an antihypertensive effect, but at 7 days a partial effect was observed (fig. 11).
FIGURE 9. Results of the preliminary study for Group IV. Normal fragmented papillae were transplanted into one-kidney, one clip hypertensive recipients of the same colony (one-kidney Goldblatt hypertensive rats). The arterial pressure was followed for 3 days and the experiment was terminated. Transplantation of one papilla was ineffective against the hypertensive state. Transplantation of four to 20 papillae lowered the mean arterial pressure by 45 mm Hg. Transplantation of two papillae gave intermediate but significant results (minus 20 mm Hg). We chose to use two papillae in the experiment in order to conserve animals. By $O = 168 \pm 2.4$, etc., is meant the mean arterial pressure in mm Hg as measured over the week before the transplantation occurred, then averaged. Data are mean values $\pm$ SEM.

Discussion

A 66% to 75% partial nephrectomy in the rat (Chanutin-Ferris maneuver) gives rise to hypertension when a salt load is added (PN-SH). A slight elevation of blood pressure has been noted by some, following partial nephrectomy alone, but not by others. We did not observe an elevation of blood pressure following partial nephrectomy alone while the rats remained on a low sodium intake. This form of hypertension may be associated with either nephrosclerosis or fibrinoid necrosis of arteries and arterioles plus glomerulitis. We observed the necrotizing vascular lesion in the animals with the greater degrees of partial renal ablation (75%) that had established hypertension for 28-36 days, but not in the animals with only 66% partial nephrectomy that were sacrificed at 7-25 days. The difference does not seem to be due to the degree of hypertension developed alone, as this colony of rats had withstood higher blood pressures for longer periods without vascular necrosis. In this respect, the animals with 75% partial nephrectomy appeared

FIGURE 10. The effect of renal papillary transplants from partial nephrectomy-salt hypertensive (PN-SH) rats of 25-30 days' duration into hypertensive recipients was compared to the effect of renal papillary transplants from normal kidneys into similar recipients. The difference between the two groups was highly significant beginning with the first day. The papillae of PN-SH rats of this duration lost their antihypertensive action, thus correlating with the decrease and damage of the renomedullary interstitial cells. Data are mean values $\pm$ SEM.
similar to renoprivival dogs. The overlap between these two models included the acute arterial-arteriolar damage in the face of a modest elevation of arterial pressure, similar hemodynamic sequences, and a significant response to renomedullary transplants.

In the rat, PN-SH causes progressive morphologic changes in the RIC of the remaining renal papilla. These changes occurred both after 66% nephrectomy (with fibrinoid vascular necrosis and glomerulitis). The number of cells was decreased, the number of lipid granules was decreased, and the cisternal system became less well developed with loss of the lipid granule-cisternal relationship. The mechanism for these morphologic alterations is not clear. It does not seem related to overt acute vascular disease. The other papillary structures were essentially intact so that severe ischemia appears unlikely. Exhaustion atrophy, possibly due to the sodium overload, might be invoked as a possible explanation, since partial nephrectomy without the added salt load did not disturb the RIC.

The failure of the morphologically damaged cells to lower blood pressure when transplanted to one-kidney, one clip Goldblatt hypertensive recipient rats demonstrates the functional importance of these morphologic changes and re-emphasizes the significance of the lipid granule-cisternal relationship in the RIC. We have noted previously a relationship to capillaries. In addition, a tissue culture of RIC derived from transplants of renal medulla when retransplanted into hypertensive recipients has also been shown to have an antihypertensive effect. Transplants of fragmented renal medulla in a variety of experimental models of hypertension have been shown to have an antihypertensive effect. The main viable cells in these transplants are the RIC, and these develop a close relationship to capillaries. In addition, a tissue culture of RIC derived from transplants of renal medulla when retransplanted into hypertensive recipients has also been shown to have an antihypertensive effect in several models of hypertension (Du Charme, personal communication). The substance(s) from the RIC that mediate this effect remains unknown. The RIC make prostaglandins. These compounds not only have local effects within the kidney, but the newest class of prostaglandin (prostacyclin or PGI2) is not inactivated by the lungs and could act as a circulating hormone. Other nonprostanoid lipid substances have been extracted from the renal medulla and cultured RIC, and these may be mediators of the antihypertensive action.

In conclusion, we have demonstrated a combination of morphologic and functional derangements of the RIC in the remaining renal nubbin of PN-SH of the rat. These changes seem to be related to the high salt intake and thus, to the handling of this salt load by the renal remnant. The alterations of the RIC did not appear due to vascular disease, as they were encountered in the absence as well as in the presence of involvement of renal arteries, arterioles, and glomeruli. We propose that the alterations of the RIC contributed to the pathogenesis of this hypertensive state in the sense that the Na volume load was under improper control. This proposal is supported by the prior demonstration of a powerful antihypertensive action of normal RIC in PN-SH.

FIGURE 11. The effect of renal papillary transplants from partial nephrectomy-salt hypertensive (PN-SH) rats of 7 and 14 days' duration is compared with that of the rats of 25-30 days' duration. At 14 days, the transplants were ineffective. At 7 days, the results were intermediate between those of normal papillae and papillae after 14-30 days of PN-S. Thus, it appears that the insufficiency of the antihypertensive action of the renal papillae begins during the first week. Data are mean values ± SEM.
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