Acute unilateral renal denervation in rats with extracellular volume expansion

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BELLO-REUSS, ELSA, ENRIQUE PASTORIZA-MUNOZ, AND ROMULO E. COLINDRES. Acute unilateral renal denervation in rats with extracellular volume expansion. Am. J. Physiol. 232(1): F26-F32, 1977 or Am. J. Physiol.: Renal Fluid Electrolyte Physiol. 1(1): F26-F32, 1977. Sodium reabsorption along the nephron was studied before and after acute unilateral denervation of the left kidney in anesthetized rats with extracellular volume expansion. Studies were also performed before and after sham denervation. Denervation increased urine volume (V) from 35.2 to 59.2 μl min⁻¹ (P < 0.001) and urinary sodium excretion (U₅₉₋₅₉) from 6.9 to 11.8 μeq min⁻¹ (P < 0.001). The control right kidney showed a simultaneous 45% decrease in V and U₅₉₋₅₉. Inulin clearance (GFR) and renal plasma flow (RPF) remained unchanged after denervation in both kidneys. Left kidney late proximal (F/Pₓ₅₉) decreased from 1.50 to 1.24 (P < 0.001); single-nephron GFR (SNGFR) remained unchanged. (F/Pₓ₅₉) ratios were also decreased in early distal (3.87-2.65, P < 0.005) and late distal (5.48-3.83, P < 0.02) convolutions. Fractional and absolute Na reabsorption in the distal convolution did not decrease. GFR, RPF, V, U₅₉₋₅₉, late proximal (F/Pₓ₅₉), and SNGFR were unchanged in sham-denervated rats. The increases in V and U₅₉₋₅₉ produced by acute renal denervation in the volume-expanded anesthetized animal are thus caused by further depression of proximal tubular salt and water reabsorption.

METHODS

Eighteen male Sprague-Dawley rats, weighing 220-330 g, were studied. The animals were fasted overnight and anesthetized with intraperitoneal sodium pentobarbital, 50 mg/kg body wt. A tracheostomy was performed; the animals were placed on a thermo-regulated heating table and the body temperature was maintained at 37°C. Polyethylene catheters were inserted into an external jugular vein for the infusion of 0.9% NaCl solution, 5% buffered solution of FD & C Green dye (Keystone Aniline & Chemical Co., Chicago), and anesthetic.
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FD & C Green dye.

as required. Arterial blood pressure was measured in a femoral artery by means of a Statham pressure transducer (model P23Db, Statham Instruments, Inc., Oxnard, Calif.) connected to a Beckman RP Dynograph recorder (Beckman Instruments, Inc., Fullerton, Calif.). The left kidney was exposed through an abdominal incision and prepared for micropuncture as previously described (11). The peritoneal reflection covering the kidney was left intact. Both ureters were cannulated near the kidney with PE-50 polyethylene tubing (Clay Adams, Division of Becton, Dickinson & Co., Parsippany, N.J.) for urine collections.

The animals were given 100% body wt of 0.9% NaCl over a period of approximately 30 min. A maintenance infusion of the same solution containing appropriate amounts of [3H]inulin (ICN, Irvine, Calif.), for determination of GFR, was given at a rate of 0.194 ml min⁻¹. In some animals para-aminohippurate (PAH) was also added for determination of RPF. Blood samples were collected at the midpoint of each period from the femoral artery and the left renal vein. An equilibrium period of 1 h was allowed to elapse and whole-kidney and single-nephron (SN) measurements were performed during two 45-min control periods. After this, the animal was subjected to either left renal denervation or to a sham denervation and 30 min later this was followed by two 45-min experimental periods. Only animals having a mean arterial blood pressure greater than 90 mmHg were used.

Denervation was performed by stripping the left renal artery of its adventitia and simultaneously coating it with a solution of 10% phenol in absolute alcohol. We and a late proximal tubular transit time of less than 11 s have previously shown that this procedure produces a complete depletion of catecholamines in the experimental kidney (2). Sham denervation was accomplished by exposing the renal artery while leaving its adventitia intact, and coating it with a 0.9% solution of NaCl.

**Collections of tubular fluid.** In 12 animals, late proximal tubular fluid collections were performed while simultaneously measuring urine volume, sodium excretion, GFR, and in some instances RPF. Late proximal convolutions were selected by injecting small amounts of Nigrosine dye into proximal convolutions with small-tipped pipettes (4 μm external tip diam) and identifying the last loop on the surface.¹ The collections were performed with sharpened micropipettes (external tip diam, 10–12 μm). A column of stained mineral oil, five tubular diameters long, was introduced into the lumen and fluid was collected at a rate such that the oil block was maintained stationary, just distal to the pipette tip, while avoiding changes in tubular diameter; tubular fluid was collected at a rate such that the oil block, approximately five tubular diameters in length, was introduced into the lumen. Tubular fluid was collected for an average of 2 min (range, 1.5–2.5 min) in early distal convolutions and of 4.5 min (range, 3–7 min) in late distal convolutions. The volume of fluid was measured and the samples divided for the determination of inulin and sodium concentrations and for osmolality measurements. The osmolality of the large samples was compared to that of the small ones. Sample having an osmolality difference of more than 10% were assumed to be the result of retrograde or accelerated flow collection (10), and were thus discarded. After denervation fluid was always collected from new tubules, selected by the same method and at the same time as those used during the control periods. Repuncture of the tubules sampled during the latter periods was not done to avoid any possible errors induced by the re-collection micropuncture technique during extracellular volume expansion.

In six animals a sham denervation was performed after the control periods. After the procedure, collections from fresh proximal tubules were performed during at least two 45-min experimental periods. No distal fluid collections were obtained in this group of animals. Whole-kidney measurements were carried out as in the denervated animals.

**Analytical methods.** [3H]inulin was measured in a three-channel liquid scintillation spectrometer (Packard Instrument Co., Downers Grove, Ill.). PAH was measured by the method of Bratton and Marshall (8). Sodium concentration in distal tubular fluid was measured with an Aminco helium-glow flame photometer (American Instrument Co., Travenol Laboratories, Inc., Silver Spring), and in urine and plasma with a Zeiss PMQII flame photometer (Carl Zeiss, NYC). The osmolality of tubular fluid and plasma was measured by the microcryoscopic method of Ramsay and Brown (16).

**Calculations.** The clearance of inulin (Cₚₐₜ) was used as a measurement of GFR. The extraction ratio of PAH (Eₚₐₜ) by the left kidney was calculated from the following formula:

\[ E_{PAH} = \frac{A_{PAH} - V_{PAH}}{A_{PAH}} \]

where \( A_{PAH} \) and \( V_{PAH} \) were the concentrations of PAH in systemic arterial and left renal venous plasma, respectively. RPF in each kidney was estimated from the ratio \( C_{PAH}/E_{PAH} \), where \( C_{PAH} \) was the clearance of PAH in the ipsilateral kidney. The left kidney \( E_{PAH} \) was used to calculate RPF.
in both kidneys. Filtration fraction was calculated from the ratio \( C_n / RPF \) for each kidney.

Single-nephron filtration rate (SNGFR) = the ratio of the concentration of inulin in tubular fluid to that of plasma \( [ \frac{F}{P_{\text{inulin}}} ] \times \text{tubular fluid flow rate (Vf)} \). Absolute
reabsorption of filtrate in proximal tubule = SNGFR - (Vf) in late proximal tubules. Fractional reabsorption in
proximal tubule = 1 - (\( \frac{P}{F} \) in proximal tubules) \( \times 100 \).

The contribution of different nephron segments to the tubular reabsorption of sodium was determined from
proximal and distal micropuncture data. Proximal tu-
bule is arbitrarily defined as the portion of the nephron
between the glomerulus and the last proximal coil ac-
cessible to micropuncture. The loop of Henle is defined
as the portion of the nephron between the last proximal
coil on the surface of the kidney and the earliest distal
convolution accessible to micropuncture. Distal con-
volution is the portion of the nephron between the early
and late distal convolution; collecting duct is the portion
of the nephron between the late distal convolution and
renal pelvis.

The calculations involved in determining fractional
and absolute sodium reabsorption in each nephron seg-
ment have been described previously (2).

Statistical analyses were carried out by the Student t
test for paired or unpaired groups. All the results are
expressed as means \( \pm \) SE. Results are reported as sig-
ificant when \( P \) values are < 0.05.

RESULTS

The mean blood pressure was 126 \( \pm \) 7 mmHg during the
difficult period and 110 \( \pm \) 8 mmHg in the experimen-
tal period in sham-denervated animals; in denervated
animals, it was 120 \( \pm \) 6 mmHg during the control
and 106 \( \pm \) 7 mmHg during the experimental period.
Venous hematocrits were 46 \( \pm \) 1% during the control
period and 46 \( \pm \) 1% in the experimental period in sham-
denervated animals, and 47 \( \pm \) 1% and 46 \( \pm \) 1%,
respectively.

Whole-kidney function. Table 1 shows that GFR and
RPF did not change significantly in either kidney after
unilateral denervation or sham denervation. There
were no differences in GFR or RPF between the right
and left kidneys during control or experimental periods
in the two groups of animals. In denervated animals,
the filtration fraction was similar in both kidneys and
unchanged after denervation, with a mean value of 0.23
\( \pm \) 0.03 before and 0.23 \( \pm \) 0.02 after the procedure. PAH
extraction by the left kidney also remained constant
(control: 84 \( \pm \) 5%; denervation: 87 \( \pm \) 5%). Filtration
fraction and PAH extraction during the control period
in the sham-denervated animals were not different from
those of the denervated animals (0.28 \( \pm \) 0.04 and 82 \( \pm \)
4%, respectively). There was no significant change in
these values after sham denervation.

Table 2 depicts the urine flow rate, urinary sodium excretion, urinary sodium concentration, and fractional
sodium excretion from each kidney during the control
period and during the experimental period (denervation
or sham denervation). During control periods, the val-
ues were similar in both kidneys and the measurements
in the sham animals that were sham denervated were not
different from those obtained during control periods in the
animals that were denervated. Unilateral renal de-
ervation led to a significant 70% increase in urine flow
rate and urinary sodium excretion from the left (dener-
vedated) kidney, while the right (innervated) kidney
showed a significant 45% decrease in these measure-
ments. Fractional excretion of sodium increased signifi-
cantly from the right kidney, while it decreased signifi-
cantly from the right kidney. Urinary sodium concen-
tration remained unchanged in both kidneys after de-
ervation. In contrast to the response seen after den-
ervation, sham denervation produced no changes in ur-
nary volume or sodium excretion from either kidney.

Proximal fluid collections. Figure 1 shows the \( \frac{F}{P_{\text{inulin}}} \)
ratios in late proximal tubules in individual experi-
ments, before and after denervation or sham denerva-
tion. In all but one experiment denervation was fol-
lowed by a fall in the \( \frac{F}{P_{\text{inulin}}} \) ratio, and mean values
changed significantly \( (P < 0.01) \) from 1.50 \( \pm \) 0.06,
before denervation, to 1.24 \( \pm \) 0.04 after denervation.
There were no consistent changes in \( \frac{F}{P_{\text{inulin}}} \) ratios after sham
denervation.

Figure 2 depicts the mean values of SNGFR and absolute
and fractional water reabsorption, obtained from
proximal fluid collections. Acute denervation did not
significantly change SNGFR (38.3 \( \pm \) 2.6 nl min\( ^{-1} \)
before and 37.9 \( \pm \) 1.9 nl min\( ^{-1} \) after denervation). Absol-
ute water reabsorption was reduced from 14.7 \( \pm \) 2.8 nl
min\( ^{-1} \) to 6.4 \( \pm \) 1.0 nl min\( ^{-1} \) \( (P < 0.02) \). This corresponds
to a reduction of fractional water reabsorption from 32 \%
3% to 18 \( \pm \) 3% \( (P < 0.005) \). There were no significant
changes in SNGFR, and absolute or fractional water
reabsorption after sham denervation.

Distal fluid collections. The mean serum sodium con-
centration during these experiments was 143.0 \( \pm \) 1.4
meq/liter before and 143.3 \( \pm \) 1.3 meq/liter after denerva-
tion. Mean plasma osmolalities before and after de-
ervation were 290 \( \pm \) 3 and 291 \( \pm \) 2 mosmol/kg H2O,
respectively.

Table 3 shows the mean values of the micropuncture
data from early and late distal convolutions before and
after left renal denervation. There was a significant reduc-
TABLE 1. Effects of left renal denervation and sham denervation on GFR and RPF in rats with extracellular volume expansion

<table>
<thead>
<tr>
<th>GFR</th>
<th>Left</th>
<th>Right</th>
<th>RPF</th>
<th>Left</th>
<th>Right</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>E</td>
<td>C</td>
<td>E</td>
<td>C</td>
<td>E</td>
</tr>
<tr>
<td>Sham denervation</td>
<td>0.57</td>
<td>0.51</td>
<td>0.57</td>
<td>0.52</td>
<td>1.67</td>
</tr>
<tr>
<td>Plasma</td>
<td>&gt;0.04</td>
<td>&gt;0.03</td>
<td>&gt;0.03</td>
<td>&gt;0.04</td>
<td>&gt;0.18</td>
</tr>
<tr>
<td>P</td>
<td>6</td>
<td>6</td>
<td>4</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Denervation</td>
<td>0.48</td>
<td>0.44</td>
<td>0.44</td>
<td>0.40</td>
<td>1.67</td>
</tr>
<tr>
<td>Plasma</td>
<td>&gt;0.02</td>
<td>&gt;0.03</td>
<td>&gt;0.02</td>
<td>&gt;0.01</td>
<td>&gt;0.18</td>
</tr>
<tr>
<td>n</td>
<td>12</td>
<td>12</td>
<td>4</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td></td>
</tr>
</tbody>
</table>

Values are means \( \pm \) SE; n, number of experiments. C, control period; E, experimental period after denervation or sham denervation. Left, left kidney; right, right kidney. P, comparison of control and experimental values by paired t test.
TABLE 2. Effects of left renal denervation and sham denervation on urinary volume, urinary Na concentration, and absolute and fractional sodium excretion during extracellular volume expansion

<table>
<thead>
<tr>
<th></th>
<th>Urinary Volume</th>
<th>Urinary Na Concentration</th>
<th>Urinary Na Excretion</th>
<th>Fractional Na Excretion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Left</td>
<td>Right</td>
<td>Left</td>
<td>Right</td>
</tr>
<tr>
<td>Denervation</td>
<td>μl min⁻¹</td>
<td>meq liter⁻¹</td>
<td>μg min⁻¹</td>
<td>%</td>
</tr>
<tr>
<td>Sham dener-</td>
<td>35.6</td>
<td>32.4</td>
<td>32.9</td>
<td>33.1</td>
</tr>
<tr>
<td>+3.8</td>
<td>24.6</td>
<td>23.9</td>
<td>28.3</td>
<td>20.7</td>
</tr>
<tr>
<td>n</td>
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<td>6</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>P</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Denervation</td>
<td>35.2</td>
<td>59.2</td>
<td>35.3</td>
<td>19.0</td>
</tr>
<tr>
<td>+4.2</td>
<td>+7.9</td>
<td>+4.1</td>
<td>-2.1</td>
<td>+0.7</td>
</tr>
<tr>
<td>n</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, number of experiments.

FIG. 1. Late proximal fluid (F/P)$_{\text{H}_2}$ values observed in individual experiments before and after denervation or sham denervation. Each circle represents mean value per experiment. Closed circles represent values after denervation.

FIG. 2. Fractional and absolute water reabsorption and SNGFR measured from late proximal fluid samples before and after denervation or sham denervation. Results are calculated from means of individual experiments.

convolutions after denervation. The SNGFR did not change significantly. Early and late distal (F/P)$_{\text{Na}}$ and (F/P)$_{\text{osmol}}$ ratios remained unchanged during the experimental period. The tubular fluid osmolality and (F/P)$_{\text{osmol}}$ ratio of samples collected from early distal convolutions were not significantly different from that of samples collected from late distal convolutions. This lack of osmotic equilibration along the distal convolution was observed during the control and experimental periods. The load of sodium delivered to the early and late distal convolutions was significantly increased after denervation.

Figure 3 illustrates that the decreases in the (F/P)$_{\text{Na}}$ ratio in early and late distal convolutions after denervation were seen in all animals. The changes in (F/P)$_{\text{Na}}$ ratios were variable and the mean values were unchanged after denervation.

The upper panel of Fig. 4 depicts the percent reabsorption of sodium in proximal tubules, loops of Henle, distal convolutions and cortical collecting ducts, as a fraction of the load presented to each of these segments. The fractional reabsorption of sodium fell significantly in proximal tubules after denervation, but remained unchanged in other segments. The fractional reabsorption of sodium fell significantly in proximal tubules after denervation, but remained unchanged in other segments. The lower panel of Fig. 4 shows the absolute reabsorption of sodium along the nephron before and after the experimental intervention. There was a significant decrease in reabsorption in proximal tubules after denervation. The increase in
DISCUSSION

These experiments demonstrate that a marked increase in sodium and water excretion occurs after acute unilateral renal denervation in the anesthetized rat with extracellular volume expansion. This effect was due to a decrease in fractional and absolute reabsorption of sodium from the proximal tubule in the absence of changes in GFR, RPF, or filtered load of sodium. We have previously demonstrated that the application of phenol to the renal artery leads to a complete ipsilateral depletion of renal catecholamines (2). Since the response in the present experiments was very reproducible and the morphologic appearance of the tubules normal, it is likely that the diuresis and natriuresis were due to the denervation and not to tubular damage secondary to phenol or to other artifactual changes. Furthermore, in other experiments involving rats with volume expansion, we have shown that crushing the splanchic nerve leads to an ipsilateral diuresis and natriuresis due to an inhibition of proximal salt and water reabsorption and that stimulation of the distal end of this nerve leads to the opposite response (3). Our results, therefore, show that a tonic nerve influence on the kidney persists during volume expansion in anesthetized rats subjected to surgery, and that suppression of this influence leads to changes in salt and water reabsorption. These studies are consistent with observations in the anesthetized rabbit which suggest that volume expansion decreases, but does not abolish efferent neural traffic to the kidney (9).

The results presented here are qualitatively similar to those reported previously in hydropenic anesthetized rats after acute renal denervation (2); however, the absolute magnitude of the response was greater in the present experiments. The marked additive effects of volume expansion and renal denervation might indicate that these two experimental interventions decrease sodium reabsorption in different ways. However, it is equally possible that both maneuvers inhibit sodium reabsorption in a similar fashion and that total suppression of neural impulses to the kidneys simply leads to a more complete expression of the effects of volume expan-
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Although it has been claimed that the acutely denervated kidney responds to volume expansion with an unimpaired diuresis and natriuresis (5), it is conceivable that at least part of the diuretic and natriuretic effect of volume loading is related to a decrease in neural traffic to the kidneys. Measurements of neural impulses during progressive extracellular volume expansion will be needed to evaluate this question.

One can only guess about the mechanisms involved in the diuretic and natriuretic response to denervation. The tubular effects could have been direct, due only to the lack of release of renal catecholamines, or indirect, via the release of a humoral factor. If a humoral factor was involved, one would have to postulate that it was produced in the left kidney and inactivated locally or during its passage through the vascular system since it failed to produce natriuresis in the right kidney. Intrarenal hydrostatic and oncotic pressures were not measured in these experiments so that any role played by these factors in the observed response cannot be evaluated. In hydropenic rats, however, the natriuretic response after denervation occurs in the absence of measurable changes in intrarenal physical factors (2).

After denervation, absolute reabsorption of sodium beyond the proximal tubule increased significantly (if one considers the effects of the three nephron segments combined), as did the fraction of filtered sodium reabsorbed in these segments. The increase in sodium reabsorption, however, was not enough to fully compensate for the excess sodium leaving the proximal tubule so that there was still a significant increase in sodium excretion. Absolute sodium reabsorption in the distal convolution was relatively small during both control and experimental conditions (Fig. 4). This makes difficult an evaluation of any possible changes induced by the denervation. Even with these limitations in mind, it is of note that sodium reabsorption in the distal convolution, although tending to increase, did not change significantly. The important feature here is that a fall in reabsorption could not be demonstrated and that it was therefore impossible to prove any effect of the renal nerves on distal tubular function. Even if an increase in absolute reabsorption of sodium had been shown, it would have been impossible to exclude an effect of the renal nerves because of the difficulties involved in evaluating the appropriateness of the compensatory response. An approach to investigate this problem might be to compare distal sodium reabsorption in denervated and innervated kidneys while delivering varying loads of sodium to this nephron segment during free flow micropuncture studies or preferably during microperfusion of distal convolutions.

There was a significant decrease in urine volume and urinary sodium excretion from the right kidney that occurred simultaneously with the increase in urine volume and sodium excretion from the left kidney. This was due to altered tubular reabsorption since there were no changes in GFR or RPF. Similar contralateral effects have been reported in the dog (7) and rabbit (6) after acute unilateral renal denervation. As has been suggested previously (6), we suspect that this effect is due to a progressive increase in sympathetic tone to the intact kidney. If such is the case, the afferent limb of this response is presumably extrarenal in origin unless one postulates that inhibitory afferent nerve fibers, influencing the right kidney, were interrupted by the denervation procedure.

An unanticipated finding in this study was the lack of osmotic equilibration of tubular fluid along the distal convolution before and after denervation. These results were different from those reported previously from this laboratory in Wistar rats, in which osmotic equilibration occurred even under conditions of osmotic diuresis (12). A possible explanation for the discrepancy in these findings may lie in strain differences between Sprague-Dawley and Wistar rats with respect to the water permeability of the cells of the distal convolution as suggested by Woodhall and Tisher (18).

In summary, these studies demonstrate that acute unilateral renal denervation in the anesthetized rat with extracellular volume expansion leads to increased diuresis and natriuresis in the absence of renal circulatory effects. This response was due to a further decrease in proximal, fractional and absolute sodium reabsorption. The results show that a “tonic” nerve influence on the kidney persists during extracellular volume expansion in anesthetized rats.

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2 The longer transit times and the higher F/P insulin ratios from late distal convolutions suggest that the lack of osmotic equilibration was real and not due to selection of only relatively early distal convolutions.

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