

Effects of Acute Unilateral Renal Denervation in the Rat

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ABSTRACT Studies were undertaken to characterize the renal responses to acute unilateral renal denervation and the mechanisms involved in these responses. Denervation was produced in anesthetized nondiuretic rats by application of phenol to the left renal artery. Studies were also performed in sham-denervated nondiuretic rats. Whole kidney and individual nephron studies were performed before and after denervation or sham denervation. Denervation increased urine volume from the left kidney to about twice its control value ($P < 0.001$) and increased urinary sodium excretion from 332 neq min^{-1} to 1,887 neq min^{-1} ($P < 0.001$). Glomerular filtration rate (GFR) and renal plasma flow (RPF) remained unchanged in both kidneys after the procedure. The innervated right kidney showed no changes in urine volume or in sodium excretion. After denervation, late proximal ratio of tubular fluid inulin concentration to that of plasma $[(F/P)_{\text{in}}]$ decreased from 2.23 to 1.50 ($P < 0.001$) while single nephron GFR remained unchanged. Absolute reabsorption decreased from 16.5 to 9.9 nl min^{-1} ($P < 0.001$). $(F/P)_{\text{in}}$ ratios were also decreased in early distal (from 6.21 to 3.18, $P < 0.001$) and late distal convolutions (from 16.41 to 8.33, $P < 0.001$) during the experimental period. $(F/P)_{\text{Na}}$ ratios remained unchanged in the early distal convolutions, but increased from 0.18 to 0.38 ($P < 0.01$) in late distal convolutions after denervation. Absolute Na reabsorption after denervation increased in the loop of Henle, distal convolution, and collecting ducts. Any changes in intrarenal hydrostatic pressures

after denervation were always small. There were no changes in GFR, RPF, urine volume, urinary sodium excretion, or late proximal $(F/P)_{\text{in}}$ after sham denervation. We conclude that the diuresis and natriuresis seen after acute renal denervation were caused by a marked depression of sodium and water reabsorption in the proximal tubule with partial compensation in more distal nephron segments. These responses appeared to be unrelated to systemic or intrarenal hemodynamic changes. The results demonstrate an effect of the renal nerves on proximal tubular function.

INTRODUCTION

The influence of the renal nerves on the regulation of sodium and water excretion by the kidney has been a subject of controversy yet to be resolved. It has long been known that renal denervation leads to diuresis and natriuresis in several mammalian species (1-6). Recent studies suggest that this "denervation diuresis" is mainly the result of an effect of the renal nerves on tubular function (1-3). There are, however, contradictory results and it has been suggested by some (4, 5) that the responses are due to an increased filtered load of sodium, secondary to increases in renal plasma flow (RPF)¹ and glomerular filtration rate (GFR).

The purpose of this study was to further characterize the changes in salt and water reabsorption by the kidney after acute unilateral denervation. We were particularly interested in determining the sites of the nephron involved in the response and in gaining some insight into the mechanisms that might explain this response.

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¹Abbreviations used in this paper: $(F/P)_{\text{in}}$, ratio of tubular fluid inulin concentration to that of plasma; $(F/P)_{\text{Na}}$, ratio of tubular fluid sodium concentration to that of plasma; GFR, glomerular filtration rate; PAH, para-aminohippurate; RPF, renal plasma flow; SN, single nephron.

Our results show that acute unilateral renal denervation in the rat produced increases in urinary sodium and water excretion in the absence of changes in RPF and GFR. A marked depression of salt and water reabsorption in the proximal tubule after denervation was partially compensated for by increased salt and water reabsorption in the loop of Henle, distal convolution, and collecting duct, so that only a small fraction of the load which escaped reabsorption by the proximal tubule was excreted in the urine.

METHODS

Observations are reported on 62 male Sprague-Dawley rats, weighing 210–330 g. The animals were fasted overnight and anesthetized with intraperitoneal sodium pentobarbital, 50 mg/kg body wt. Intermittent i.v. doses of the drug were used as needed throughout the experiment. A tracheostomy was performed, the animals were placed on a heated board, and the body temperature was maintained at 37°C. Catheters were placed into an external jugular vein for the infusion of 0.9% NaCl solution at a rate of 2 ml/h, and for other infusions. Arterial blood pressure was monitored from a femoral artery by means of a Statham pressure transducer (Model P23 Db, 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 (7). The peritoneal reflection covering the kidney and the perirenal fat were left intact. Both ureters were cannulated near the kidney with PE 10 polyethylene tubing (Clay Adams, Div. of Becton, Dickinson & Co., Parsippany, N. J.), for urine collections.

After the surgical procedure was completed, appropriate amounts of [³H]inulin (ICN, Irvine, Calif.) or nonradioactive inulin² and [¹⁴C]para-aminohippurate (PAH) (New England Nuclear, Boston) were added to the saline infusion for determination of GFR and RPF. Blood samples were collected at the midpoint of each period from the femoral artery. When necessary, blood was collected from the renal vein with a 27-gauge needle. An equilibration 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 at least two 45-min experimental periods.

Denervation was performed by stripping the left renal artery of its adventitia by coating it with a solution of 10% phenol in absolute alcohol during a period of 20–30 min. During the application of phenol, the left kidney and adjacent tissues were carefully protected from exposure to the chemical; disruption of the major lymphatic vessels in the area was avoided. Less than 10% of the animals showed spasm of the renal artery, and they were discarded. Sham denervation was accomplished by exposing the renal artery while leaving its adventitia intact, and coating it with a 0.9% solution of NaCl.

Only animals having a mean arterial blood pressure

² Observations in our laboratory have yielded similar results with radioactive and nonradioactive inulin for the measurement of GFR or single nephron GFR.

higher than 90 mm Hg and, when measured, a late proximal tubular transit time less than 11 s were used.

Collections of tubular fluid. In a group of 21 animals, late proximal tubular fluid collections were performed while simultaneously urine volume, sodium excretion, GFR, and in some instances RPF were measured. Late proximal convolutions were selected by injecting small amounts of nigrosin dye into proximal convolutions with small tipped pipettes (external tip diameter, 4 μ m) and identifying the last loops on the surface. In some animals, late proximal convolutions were identified with the use of a 5% buffered solution of FD & C green dye (Keystone Aniline & Chemical Co., Chicago, Ill.).³ The collections were performed with sharpened micropipettes having an external tip diameter of 9–12 μ m. A column of stained mineral oil, 3–4 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 samples were collected from three or more convolutions for an average of 2.5 min (range 1.5–3 min). The volume of fluid obtained was measured in calibrated capillary tubing and its inulin concentration determined. After the control period, denervation was performed and collections were obtained from the same or from new convolutions.

In 11 animals, distal tubular fluid was also collected. After denervation, new distal tubules were always selected in these experiments. The tubules were identified as early or late by the intravenous injection of FD & C green dye. For this purpose, “early distal convolutions” were identified as those where the dye first appeared on the surface of the kidney, after its transit through the loop of Henle. “Late distal convolutions” were identified as those where the dye last appeared on the surface. In previous studies from our laboratory, convolutions so selected always corresponded to puncture sites within the first and second half of the distal convolution, respectively, as determined by microdissection.⁴ Before denervation, the transit time to early distal convolutions averaged 39 ± 2 s; after denervation it averaged 32 ± 2 s ($P < 0.02$). The transit times to late distal convolutions averaged 70 ± 5 s and 67 ± 4 s, respectively (NS). The selected tubules were punctured with sharpened pipettes having tip diameters of 4–5 μ m. A droplet of oil was introduced into the lumen to ascertain the direction of tubular flow and a small volume (less than 0.1 nl) of tubular fluid was collected for osmolality determination (8). The same convolutions were reentered with a 6–7- μ m tipped pipette, and an oil block, approximately 5 tubular diameters in length, was introduced into the lumen. Tubular fluid was collected for an average of 7 min (range 4–20 min) with care being taken to avoid retrograde flow, changes in tubular diameter, or wide oscillations of the oil block. 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. Samples having an osmolality difference of more than 10% were assumed to have been contaminated by retrograde or accelerated flow (8), and were thus discarded.

³ No difference in ratio of tubular fluid inulin concentration to that of plasma $[(F/P)_{in}]$ or SNGFR determinations was found between samples collected from late proximal tubules selected by either method.

⁴ Colindres, R. E., C. W. Gottschalk, and J. R. Oliver. Unpublished observations.

In seven animals a sham denervation was performed after a control period. After the procedure, collections from new proximal tubules and recollections were performed during at least two 45-min experimental periods. Whole kidney measurements were carried out as in the denervated animals.

Measurement of hydrostatic pressures. In a separate group of animals, the left kidney was bathed with a solution of isotonic saline at 37°C and hydrostatic pressures were measured in proximal and distal convolutions and in post-glomerular vessels with a servo-nulling device, before and after denervation. The vessels were classified as previously described from this laboratory (9). Stop-flow pressures were also measured and glomerular capillary pressures were estimated from the sum of the stop-flow pressures and the systemic plasma oncotic pressure (9).

Microinjections. In five animals, tracer amounts of ^{22}Na (ICN) and [^3H]inulin were injected into late distal convolutions before and after denervation. For this purpose, aliquots (2–3 nl) of a solution containing ^{22}Na and [^3H]inulin, stained with FD & C green dye, were prepared for injection. The sodium concentration of this solution was less than 3 mEq/liter to avoid any alterations in sodium transport by the collecting ducts related to the injection of carrier sodium (10). After injection, urine was collected for several 5-min periods. A control 5-min urine collection containing 2–3 nl of injectate was used as a standard for estimation of fractional recovery. Radioactivity was measured in a three-channel liquid scintillation spectrometer, as previously described (11). Appropriate corrections were made for the crossover of ^{22}Na into the tritium channel.

Determination of kidney content of norepinephrine. A group of 12 animals was subjected to denervation; 6 animals were killed 10 min–3 h after the procedure and the other 6 were killed 3–6 days after the denervation. A group of six sham-denervated rats was used as a control for the chronic studies.

The kidneys were removed immediately after death, chilled in ice, and weighed, and their catecholamine content was determined by the method of Anton and Sayre (12). The method consists of the selective absorption of the catecholamines onto aluminum oxide, elution with perchloric acid, and measurement by the formation of a fluorescent trihydroxyindole derivative. This highly sensitive assay can detect as little as 3 ng norepinephrine/g of tissue.

Analytical methods. Plasma and urine inulin concentrations were determined by the anthrone method (13). Protein concentration was measured with a micro-Lowry method (14) with rat plasma as a standard. Inulin concentration in tubular fluid samples was measured with the use of a microfluorometer by the method of Vurek and Pegram (15). Sodium concentration in distal tubular fluid was measured with an Aminco helium glow flame photometer (American Instrument Co., Travenol Laboratories Inc., Silver Spring, Md.), and in urine and plasma with a Zeiss PMQ II flame photometer (Carl Zeiss, New York). The osmolality of tubular fluid and plasma was measured by the microcryoscopic method of Ramsay and Brown (16). [^3H]Inulin, ^{22}Na , and [^{14}C]PAH were measured in a three-channel liquid scintillation spectrometer (Packard Instrument Co., Downers Grove, Ill.).

Calculations.

$$\text{SNGFR} = (\text{F/P})_{\text{in}} \times \text{tubular fluid flow rate (V}_t\text{)} \\ \text{in nanoliters per minute} \quad (1)$$

$$\text{Absolute reabsorption of filtrate in proximal tubule} \\ \text{in nanoliters per minute} = \text{SNGFR} - \text{V}_t \quad (2)$$

Fractional reabsorption in proximal tubule

$$(\text{Prox.}) = 1 - (\text{F/P})_{\text{in Prox.}} \times 100. \quad (3)$$

The contribution of different nephron segments to the tubular reabsorption of Na was obtained from proximal and distal micropuncture data.⁵ The data used for these calculations were: the (F/P)_{in} and the (F/P)_{Na} measured in late proximal, early distal, and late distal convolutions, and the fractional excretion of Na.

The following equations were used:

Fractional reabsorption of sodium in the loop of Henle

$$= \left(1 - \frac{(\text{F/P})_{\text{Na ED}} \cdot (\text{P/F})_{\text{in ED}}}{\text{P/F}_{\text{in Prox.}}} \right) \times 100 \quad (4)$$

Fractional reabsorption of sodium in distal convolution

$$= \left(1 - \frac{(\text{F/P})_{\text{Na LD}} \cdot (\text{P/F})_{\text{in LD}}}{(\text{F/P})_{\text{Na ED}} \cdot (\text{P/F})_{\text{in ED}}} \right) \times 100 \quad (5)$$

Fractional reabsorption of sodium in collecting duct

$$= \left(1 - \frac{\text{fractional excretion (FE)}_{\text{Na}}}{(\text{F/P})_{\text{Na LD}} \cdot (\text{P/F})_{\text{in LD}}} \right) \times 100 \quad (6)$$

ED, early distal tubule; LD, late distal tubule.

The absolute reabsorption of Na in each nephron segment was calculated from the load presented to the segment and the fractional reabsorption.

Statistical analyses were carried out by the Student *t* test for paired or nonpaired groups according to the experimental design. Results are referred to as "significant" when their *P* value is < 0.01. All the results are expressed as means ± SE.

RESULTS

The mean blood pressure during the control period was 110 ± 3 mm Hg, after sham denervation 109 ± 2 mm Hg, and after denervation 103 ± 3 mm Hg. Arterial hematocrits were 50 ± 2%, 50.9 ± 0.8%, and 51.0 ± 3%, respectively.

The adequacy of the denervation procedure was tested in some animals by measuring kidney norepinephrine content in presumed denervated kidneys and comparing their content with that of sham-denervated and contralateral untouched kidneys (Table I). The content in left and right kidneys, 10 min–3 h after the presumed denervation of the left, was similar to that reported by Anton and Sayre (12) in normal kidneys. After 72 h, none of the examined left kidneys had detectable levels

⁵ In this study "proximal tubule" is defined as the portion of the nephron between the glomerulus and the last convolution of the proximal tubule accessible to micropuncture. The "loop of Henle" is defined as the portion of the nephron between the last proximal convolution on the surface of the kidney and the earliest distal convolution available for micropuncture. "Distal tubule" is the portion of the nephron between the early and late distal convolution and "collecting duct" is the portion of the nephron between the late distal convolution and the ureter.

of norepinephrine, while the content in the right kidney was not significantly different from normal. After left sham denervation, kidney norepinephrine content was similar and normal in both kidneys.

Whole kidney function. The results from whole kidney measurements in denervated and sham-denervated rats are summarized in Table II. The urine flow rate of the denervated kidneys increased significantly to about twice its control value, while no changes were observed on the right. There was a fivefold increase in sodium excretion after denervation, without significant changes by innervated kidneys. The sodium concentration increased significantly in the urine after denervation and remained unchanged in the urine from contralateral kidneys. Whole kidney GFR and RPF did not change significantly on either side during the experiment; the filtration fractions remained similar and unchanged in both kidneys, with a mean value of 0.34 ± 0.02 before and 0.34 ± 0.02 after denervation. PAH extraction also remained constant (control: $82 \pm 2\%$, after denervation: $81 \pm 1\%$).

Although the base-line measurements in the second series of denervated animals (given nonradioactive inulin and green dye) were higher than those in the first series, presumably related to an osmotic effect of the inulin and dye, the differences were not significant. Furthermore, the magnitude of the response to denervation by the left kidney was similar to that seen in the first series; the function of the innervated kidney remained unchanged.

The measurements during the control period in the sham-denervated animals were not different from those obtained in the control periods in the denervated animals. However, in contrast to the response seen after denervation, sham denervation produced no changes in urinary volume, sodium concentration, or sodium excretion in either kidney.

Proximal fluid collections. Fig. 1 shows the $(F/P)_{in}$ ratios in late proximal tubules in individual experiments, before and after denervation or sham denervation. In every instance denervation was followed by a fall in the $(F/P)_{in}$ ratio, and mean values changed significantly, from 2.23 ± 0.09 before denervation to 1.50 ± 0.03 after denervation. There were no consistent changes in $(F/P)_{in}$ ratios after sham denervation.

Fig. 2 shows the mean values of SNGFR, and absolute and fractional water reabsorption before and after left renal denervation and sham denervation. Acute denervation did not significantly change SNGFR (31.51 ± 1.61 nl min⁻¹ before and 31.07 ± 1.73 nl min⁻¹ after denervation). Absolute water reabsorption was significantly reduced from 16.5 ± 0.9 to 9.9 ± 0.8 nl min⁻¹ ($P < 0.001$). This corresponds to a reduction of fractional water reabsorption from $54 \pm 2\%$ to $33 \pm 2\%$ ($P < 0.001$). There

TABLE I
Results of Kidney Norepinephrine Measurements after Left Renal Denervation and Sham Denervation

Experimental conditions	Kidney	Norepinephrine ng/g kidney
Denervation		
10 min-3 h	Right	78 ± 33
$n = 6$	Left	86 ± 22
P		NS
3-6 days	Right	69 ± 7
$n = 6$	Left	*
Sham denervation		
3-6 days	Right	89 ± 20
$n = 6$	Left	71 ± 10
P		NS

Values are means \pm SEM. P , comparison of right and left kidney content by paired t test. Times given refer to the interval between the denervation or sham denervation and the measurements.

* Undetectable levels.

were no significant changes in SNGFR, and absolute or fractional water reabsorption after sham denervation.

Fig. 3 shows the results of late proximal $(F/P)_{in}$ values of recollection samples after denervation or sham denervation. In every instance there was a fall after denervation, while after sham denervation the results distributed randomly around the line of identity. The values obtained in recollection samples, in both groups, did not differ from those obtained in samples collected from new tubules.

Distal fluid collections. Table III presents the mean values of the micropuncture data from early and late distal convolutions before and after left renal denervation. There was a significant reduction of the $(F/P)_{in}$ ratio in both early and late distal tubules after denervation. The SNGFR did not change significantly. The $(F/P)_{sa}$ and F/P osmolality ratios also remained unchanged in the early distal segments. After denervation, in late distal convolutions, the $(F/P)_{sa}$ increased significantly from 0.18 ± 0.02 to 0.38 ± 0.04 ($P < 0.01$), while the F/P osmolality ratio remained unchanged. Fig. 4 shows that the changes in $(F/P)_{in}$ values after denervation were seen in all animals. The changes in $(F/P)_{sa}$ values were seen in all but one experiment.

The mean serum sodium concentration during these experiments was 146 ± 2 meq/liter before and 148 ± 2 meq/liter after denervation. The serum osmolality was 296 ± 5 mosm/kg H₂O and 300 ± 6 mosm/kg H₂O, respectively.

Fig. 5 shows the absolute and fractional reabsorption of sodium in each nephron segment before and after denervation. Absolute sodium reabsorption increased

TABLE II
Effects of Left Renal Denervation and Sham

	GFR				RPF				Urinary volume			
	Left		Right		Left		Right		Left		Right	
	C	E	C	E	C	E	C	E	C	E	C	E
	<i>ml min⁻¹/100 g body wt</i>				<i>ml min⁻¹/100 g body wt</i>				<i>μl min⁻¹</i>			
Sham denervation*	0.36 ±0.02	0.35 ±0.02	0.38 ±0.02	0.35 ±0.02	1.16 ±0.06	1.12 ±0.06	1.17 ±0.09	1.11 ±0.07	3.6 ±0.2	3.6 ±0.4	3.7 ±0.3	3.5 ±0.2
<i>n</i>	7				5				7			
<i>P</i>	NS		NS		NS		NS		NS		NS	
Denervation, First series*	0.37 ±0.02	0.35 ±0.02	0.37 ±0.02	0.33 ±0.02	1.01 ±0.07	1.07 ±0.08	1.06 ±0.15	0.96 ±0.16	3.5 ±0.5	6.8 ±0.6	3.8 ±0.5	3.6 ±0.6
<i>n</i>	10				5				10			
<i>P</i>	NS		NS		NS		NS		<0.001		NS	
Second series†	0.46 ±0.02	0.48 ±0.02	0.46 ±0.02	0.45 ±0.02	1.38 ±0.10	1.44 ±0.08	1.39 ±0.07	1.36 ±0.07	5.1 ±0.4	10.8 ±1.3	5.4 ±0.6	4.3 ±0.5
<i>n</i>	11				11				11			
<i>P</i>	NS		NS		NS		NS		<0.001		NS	

C, control; E, experimental.

* [³H]inulin in saline infusion.

† Nonradioactive inulin in saline infusion and FD & C green used for localization of puncture sites.

significantly in the loop of Henle, distal convoluted, and collecting ducts after denervation. Fractional sodium reabsorption, calculated as percentage of load presented to the segments, remained unchanged in the loop of Henle while it decreased significantly, from 82.5±2.7% before to 58.2±7.3% after denervation in the distal convoluted, and from 90.3±1.9% to 79.3±3.2% in the collecting ducts.

Microinjections. 16 microinjections were made into late distal convolutions before and 12 microinjections after denervation. The [³H]inulin recovery was 99.0±1.0% before and 98.0±1.7% after denervation. ²²Na recovery was 32.5±2.3% before denervation. After denervation the recovery was 41.2±4.2% (*P* < 0.02).

Hydrostatic pressures. The results of hydrostatic pressure measurements are shown in Fig. 6. Free-flow proximal pressure increased significantly from 11.9±0.1

mm Hg in control conditions to 13.9±0.3 mm Hg after denervation. Mean distal pressures before and after denervation were 6.2±0.2 and 8.6±0.4 mm Hg, respectively (*P* < 0.001). No changes were observed in the pressure in either the efferent arterioles (14.5±0.5 and 14.6±0.4 mm Hg) or the intermediate vessels. Peritubular capillary pressure increased from 9.8±0.2 to 10.7±0.2 mm Hg. This change, although small, was significant (*P* < 0.01). Estimated glomerular capillary

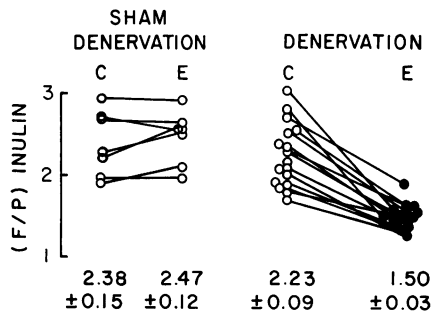


FIGURE 1 Late proximal fluid (F/P)_{in} values shown in individual experiments before and after denervation (*P* < 0.01) or sham denervation (NS). Each circle represents the mean value per experiment. Closed circles represent values after denervation.

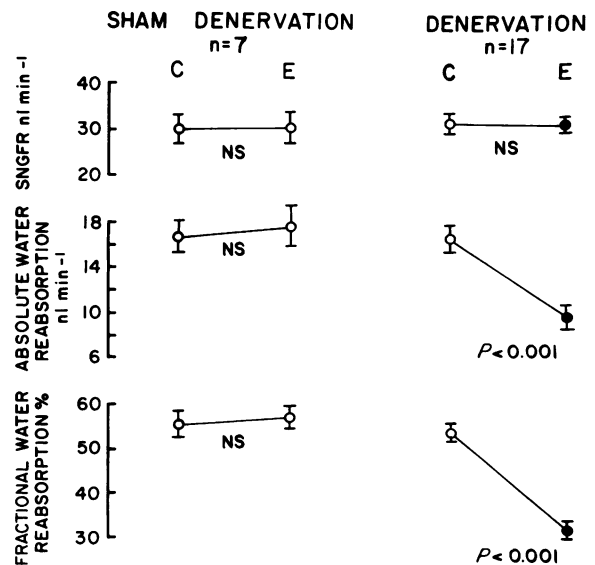


FIGURE 2 Fractional and absolute water reabsorption and SNGFR measured from late proximal fluid samples before and after denervation or sham denervation. The results are calculated from the means of individual experiments.

Urinary Na concentration				Urinary Na excretion				Fractional Na excretion			
Left		Right		Left		Right		Left		Right	
C	E	C	E	C	E	C	E	C	E	C	E
<i>meq liter⁻¹</i>				<i>meq min⁻¹</i>				<i>%</i>			
33 ±3	34 ±2	33 ±3	31 ±2	121 ±17	112 ±11	117 ±13	111 ±12	0.07 ±0.008	0.07 ±0.004	0.07 ±0.008	0.07 ±0.007
NS		NS		NS		NS		NS		NS	
35 ±8	95 ±20	31 ±8	42 ±9	147 ±47	710 ±182	155 ±55	170 ±45	0.10 ±0.03	0.54 ±0.13	0.10 ±0.03	0.12 ±0.03
<0.02		NS		<0.001		NS		<0.01		NS	
52 ±18	163 ±17	50 ±18	58 ±14	332 ±159	1,887 ±325	350 ±179	291 ±88	0.19 ±0.09	1.05 ±0.18	0.21 ±0.10	0.18 ±0.06
<0.001		NS		<0.001		NS		<0.001		NS	

hydrostatic pressure remained unchanged with a mean value of 47.1 ± 1.0 mm Hg before and 46.0 ± 1.1 mm Hg after denervation.

DISCUSSION

The rat kidney is abundantly supplied with nerves derived mainly from the celiac plexus and from the thoracic and lumbar splanchnic nerves. These nerves converge into the hilum of the kidney and follow the renal artery, especially in the last part of its course (17). In preliminary experiments, we made detailed anatomical dissections of the renal nerves and it was obvious that minor variations in their origin and distribution were frequent and that care was needed to avoid a partial denervation or interruption of fibers to the contralateral kidney. To minimize these possibilities we chose, therefore, to block neural conduction in the hilum by

stripping the adventitia of the renal artery while exposing it to phenol for at least 20 min. Phenol is known to produce an irreversible blockage of nerve conduction if exposure of neural tissues to the agent is sufficiently prolonged (18). It was found that this procedure rarely produced arterial spasm and was associated with a predictable and reproducible diuretic and natriuretic response limited to the left kidney. Although the adequacy of the denervation could not be determined by independent means during the experiment, renal catecholamines were always undetectable 72 h later in other kidneys subjected to the same procedure. It is likely, therefore, that the denervation procedure either inter-

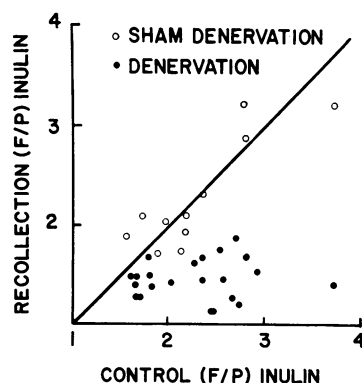


FIGURE 3 Late proximal $(F/P)_{in}$ values in recollection samples after denervation or sham denervation.

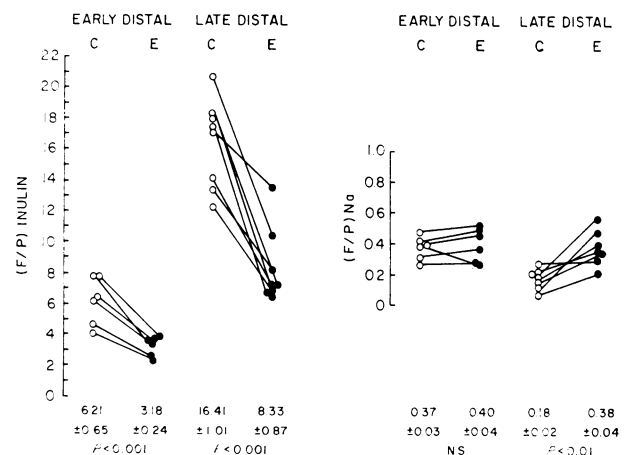


FIGURE 4 Early and late distal convolution $(F/P)_{in}$ and $(F/P)_{Na}$ ratios in individual experiments before and after acute renal denervation. Each circle represents the mean value of at least two measurements.

TABLE III
Results of Distal Fluid Collections before and after Left Renal Denervation

	Early distal n = 6		Late distal n = 8	
	C (13)	E (17)	C (16)	E (16)
(F/P) _{In}	6.21±0.65	3.18±0.24	16.41±1.01	8.33±0.87
	<i>P</i> < 0.001		<i>P</i> < 0.001	
SNGFR, nl min ⁻¹	35.5±1.8	31.7±1.9	35.3±1.6	33.9±1.2
	NS		NS	
(F/P) osmolality	0.51±0.02	0.51±0.01	0.82±0.04	0.81±0.05
	NS		NS	
(F/P) Na*	0.37±0.03	0.40±0.04	0.18±0.02	0.38±0.04
	NS		<i>P</i> < 0.01	
(F/P) _{Na} /(F/P) _{In} × 100	6.4±0.8	12.3±1.4	1.0±0.2	4.9±0.9
	<i>P</i> < 0.01		<i>P</i> < 0.005	

C, control; E, experimental.

*Tubular fluid sodium concentration was measured in seven of the eight late distal experiments (14 collections). () = number of collections.

rupted neural traffic completely or that those branches of the renal nerves not exposed to the phenol were few and/or of relatively minor importance.

It is unlikely that the observed diuresis and natriuresis were due to changes other than the denervation. Tubular collapse was not seen, so one cannot attribute the response to a diuretic phase after a period of transient ischemia. It is possible that interruption of lymphatic

channels during the denervation, with subsequent obstruction, might have led to changes in salt and water reabsorption by the kidney. However, in most instances it was possible to demonstrate that the main lymphatic channels were intact after denervation. Furthermore, there is evidence that increased resistance to lymph flow may under certain circumstances lead to antinatriuresis rather than to natriuresis (19).

One might also question whether phenol itself might have been directly responsible for the observed effects. Although the left kidney was carefully protected from exposure to the chemical, it is possible that some absorption into the renal artery might have taken place, leading to an effect limited to that kidney. We consider this to be unlikely since the intima and media of the renal artery, whenever examined histologically, were always normal. Furthermore, it is difficult to conceive a predictable response from the accidental absorption of phenol from the renal artery. Phenol is known to be a poison leading to coagulation necrosis of tissue protein and we have observed that injecting even minimal amounts of this drug into the renal artery results in immediate collapse of the surface tubules, an effect never seen after the denervation procedure. Also any systemic effect related to the duration of the experiment, such as extracellular fluid volume expansion leading to diuresis and natriuresis, can be excluded since there were no changes in salt and water excretion by the innervated kidney or in the sham-denervated animals.

The results of this study are therefore best interpreted as demonstrating a consistent effect secondary to acute renal denervation. This effect consists of a twofold increase in urinary volume and a fivefold increase in sodium excretion. Contrary to the results reported by

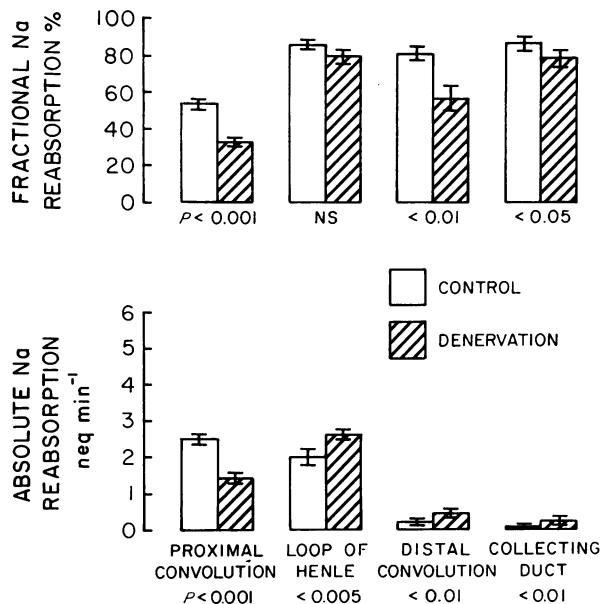


FIGURE 5 Fractional and absolute sodium reabsorption before and after acute renal denervation, in proximal convoluted, loop of Henle, distal convoluted, and collecting duct. The results are calculated from the mean values of individual experiments.

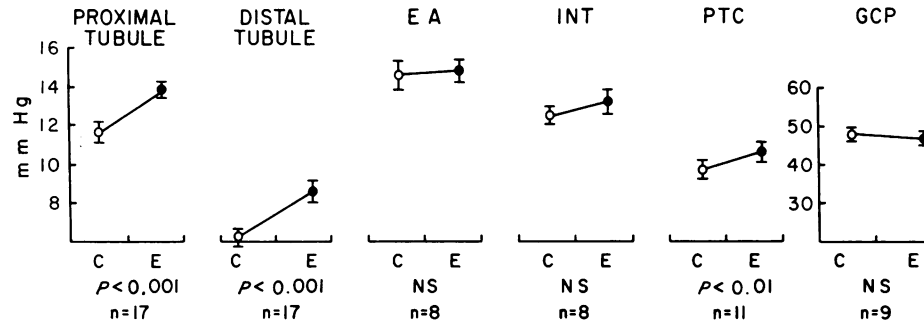


FIGURE 6 Results of hydrostatic pressure measurements in proximal tubules, distal tubules, glomerular capillaries, and postglomerular capillaries, before and after denervation. The results are calculated from the means of individual experiments.

others in the dog (4, 5) we found no significant increases in GFR, RPF, or in the filtered load of sodium after the denervation procedure. Furthermore, in some instances the natriuretic and diuretic response persisted even when there were decreases in blood pressure and/or GFR. It is well known that the renal vasculature is abundantly supplied with nerves (20) and the absence of an effect of denervation on RPF and GFR is somewhat surprising and unexplained. It is possible that the renal nerves have little tonic influence on the renal vasculature under these circumstances or that the suppression of neural tone is followed by compensatory responses such that vasodilatation was not seen. Whatever the explanation, the natriuresis and diuresis were due to a direct or indirect effect of the renal innervation on the tubular reabsorption of Na and water. A similar tubular effect has been observed after chemical or surgical denervation in the dog (2), rat (3), and rabbit (1). In addition, an increase in tubular reabsorption of sodium in the absence of changes in RPF and GFR has been reported in the dog after renal nerve stimulation (21). Barajas and Müller have presented electron microscopic and histochemical evidence of a direct innervation of tubular cells of proximal and distal convolutions in the monkey and in the rat (22, 23). These studies provide an anatomical correlate to the results obtained in our experiments.

Acute denervation was accompanied by a marked depression of salt and water reabsorption in the proximal convolution. These results are similar to those reported by Bencsáth, Bonvalet, and deRouffignac (3) and are in agreement with the indirect studies of Gill and Casper (24, 25), who suggested that alpha and beta adrenergic drugs led to changes in proximal tubular reabsorption of sodium and water. The results depicted in Fig. 5 show that the augmented sodium excretion after denervation was caused by decreased proximal reabsorption of sodium with partial compensation by more distal nephron segments, so that only a small fraction of the load that escaped proximal reabsorption was excreted

in the urine. Fractional reabsorption of sodium, expressed as percent of the load reaching the segment, was unchanged in the loop of Henle, but significantly decreased in the distal convolution and in the collecting ducts after the denervation procedure. Absolute reabsorption of sodium, however, was significantly increased in these three nephron segments. The microinjections from late distal convolutions after denervation showed decreases in fractional sodium reabsorption along the collecting ducts that were similar to those measured by comparing sodium and inulin concentrations in fluid from late distal convolutions with those in the urine. The base-line fractional reabsorption measured by the microinjection technique was lower than that calculated by conventional methods, presumably reflecting technical artifacts or differences in function between superficial and deep nephrons.

Changes in fractional and absolute water reabsorption paralleled and presumably were caused by changes in net sodium transport in all parts of the nephron.

The impressive degree of compensation that occurred beyond the proximal convolution should not be construed as evidence for or against an effect of the renal nerves on distal and collecting duct function. It is known from micropertusion (26) and other micropuncture studies in the rat (27, 28) and in the dog (29) that increasing the delivery of sodium to the loop of Henle, the distal convolution, and the collecting ducts leads through unknown mechanisms to an increase in the absolute reabsorption of sodium in these nephron segments. It is thus possible that an effect of the renal nerves on distal tubular or collecting duct function was obscured by the normal response of these nephron segments to an increased delivery of sodium. If this were the case, it is conceivable that such an effect might be uncovered under conditions where these compensatory mechanisms are impaired, such as after volume expansion. Moreover, it is known from other studies (28) that the distal tubule and collecting duct have the capacity to reabsorb a load of sodium in excess of that delivered to

these segments in the present experiments. Thus the lack of complete compensation may be an abnormal response. Based on these considerations, and since the distal tubules have an innervation similar to that of the proximal tubules (22, 23), one cannot exclude an effect of the renal nerves on distal tubular function. Our results, however, show that in hydropenia, acute renal denervation predominantly affects proximal tubular function. Further studies under different physiological conditions will be needed to evaluate any role of the renal nerves on the function of the more distal nephron segments.

It is impossible from the results of this study to identify the mechanisms responsible for the decreased proximal tubular reabsorption of sodium and water or to determine whether the effects were direct or indirect. The observed changes, however, appear to be unrelated to alterations in physical factors or intrarenal hemodynamics. The plasma protein concentration did not change after denervation and the SNGFR and whole kidney filtration fraction remained unchanged. Although single superficial nephron filtration fractions were not measured, it is unlikely that they were decreased. These findings suggest that there were no decreases in peritubular oncotic pressure to explain the decrease in proximal sodium reabsorption. The observed increases in hydrostatic pressure after denervation in proximal tubules and peritubular capillaries were always small. Since intratubular pressures increased more than peritubular capillary pressures, it is likely that the increase in intratubular pressure was a result of the increased flow rate caused by the reduction of tubular reabsorption and not its cause. Although our results do not exclude pressure changes in a critical portion of the interstitium inaccessible to measurement, we do not believe that alterations in hydrostatic pressure of the magnitude seen provide an explanation for the diuretic and the natriuretic response. Furthermore, one might expect that the increased tubular-capillary pressure gradient would tend to increase rather than decrease proximal fluid reabsorption.

The release or activation of systemic natriuretic factors as a cause for the responses was excluded by the absence of an effect on the right kidney. One cannot, however, exclude the possibility of an intrarenal humoral factor that might be metabolized or inactivated in the kidney or during its passage through the venous system. Such an agent might inhibit sodium reabsorption in the left kidney, yet fail to reach the right kidney. It is also conceivable that the denervation might have produced an inhibition of a local antinatriuretic factor.

The possibility of redistribution of glomerular filtrate to the superficial cortical nephrons seems to be excluded by the constancy of the ratio SNGFR/GFR after denervation.

Bencsáth and associates (3), using the Hanssen technique, were also unable to show a redistribution of glomerular filtrate after acute denervation in rats. It has been suggested by Pomeranz, Birtch, and Barger (30) that renal nerve stimulation can lead to a decreased outer cortical and an increased medullary blood flow. Although we have not measured distribution of blood flow in our study, we consider a redistribution in the opposite direction unlikely in view of the lack of changes in SNGFR. Furthermore, Stein, Boonjarern, Mauk, and Ferris (31), using radioactive microspheres, were unable to show such a redistribution.

We cannot distinguish from our results whether the denervation exerted its effects by increasing the passive backflux of sodium across the proximal tubule or by decreasing active sodium reabsorption. In this regard, it is of interest that norepinephrine has been shown to stimulate active sodium transport across several isolated tight (32, 33) and leaky epithelia (34).

Finally although our results have shown an effect of the renal nerves on tubular function after acute denervation, these experiments do not relate to any role that the renal nerves may have in the long-term physiologic regulation of salt and water reabsorption. Although it has been claimed that chronic renal denervation does not lead to any significant changes in salt and water reabsorption (35), especially in the unanesthetized animal (36), there is recent evidence that under certain abnormal conditions, increased renal nerve activity may play a significant role in the long-term retention of sodium by the kidneys (37). These studies suggest the desirability of evaluating the function of the chronically denervated kidney in more detail.

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