Renal neuroadrenergic transmission

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1975.—To study the role of the renal sympathetic nerves in the regulation of sodium excretion, we examined the renal functional response to left renal nerve stimulation before (group I) and after (group II) left renal adrenergic blockade with guanethidine. In group I dogs, absolute sodium excretion from the left kidney fell markedly after left renal nerve stimulation; the decreases in glomerular filtration rate and renal blood flow were of a similar magnitude. Using the radiolabeled microsphere technique, distribution of renal blood flow to the outer cortex was diminished after left renal nerve stimulation. In group II dogs, guanethidine blocked all of these effects of left renal nerve stimulation. In group III studies, a low level of left renal nerve stimulation was used which resulted in a decrease in sodium excretion in the absence of changes in glomerular filtration rate, renal blood flow, or intrarenal distribution of blood flow; this effect was blocked by renal adrenergic blockade with guanethidine in group IV studies. These data support a role for the renal sympathetic nerves to directly influence renal tubular sodium transport in the absence of alterations in renal hemodynamics.

renal sympathetic nerves; guanethidine; adrenergic blockade

A number of studies have provided evidence that increased renal sympathetic nerve activity participates in the sodium retention of thoracic inferior vena cava (TIVC) constriction (summarized in ref. 15). It was noted that the kidney transplanted to the neck failed to exhibit the same degree of sodium retention as the intact kidney in response to caval constriction (19). Autonomic blockade with pentolitium resulted in a natriuresis in dogs with chronic caval constriction (6). Studies from this laboratory have shown that acute surgical renal denervation partially reverses the antinatriuresis of acute caval constriction (1). Studies employing renal clearance and micropuncture techniques showed that acute surgical or pharmacological renal denervation with phenoxybenzamine or guanethidine resulted in a restoration of the normally observed decrease in proximal tubule fractional reabsorption after saline loading and partial reversal of the antinatriuresis in dogs with acute caval constriction (15). These changes were seen despite persistent reductions in mean arterial pressure, cardiac output, glomerular filtration rate, and total renal blood flow. Measurement of intrarenal distribution of blood flow by radiolabeled microspheres and of intrarenal distribution of glomerular filtrate by comparison of superficial nephron and whole-kidney glomerular filtration rates showed that the reversal of the antinatriuresis was not related to alteration of these variables. These studies provided evidence for a direct effect of the renal sympathetic nerves on tubular sodium transport. Recent light and electron microscopic studies of monkey renal cortex firmly establish the existence of adrenergic nerve fibers in direct contact with the basement membrane of both proximal and distal tubules (11).

Because the antinatriuresis of acute TIVC constriction could be reversed without changes in total renal blood flow and glomerular filtration rate, it may be presumed that the level of renal sympathetic nerve activity present in acute TIVC constriction is sufficient to increase tubular sodium reabsorption directly. This is less than that more intense degree of renal sympathetic nerve stimulation, which results in a fall in total renal blood flow and glomerular filtration rate. By analogy, therefore, if guanethidine is capable of blocking this more intense degree of nerve stimulation this would provide support for the argument that guanethidine mediates its natriuretic effect in acute TIVC constriction by inhibiting renal sympathetic nerve activity.

METHODS

All studies were performed on female mongrel dogs, 15-18 kg in weight, fed a standard kennel ration. On the day prior to the study, all dogs were deprived of food but water was permitted ad libitum. On the day of the study the animal was anesthetized with intravenous sodium pentobarbital 30 mg/kg, and supplemental doses were added throughout the experiment to maintain anesthesia. The animal was intubated with an endotracheal tube and mechanically ventilated to maintain arterial pH between 7.35 and 7.45.

Catheters were inserted into both femoral arterial, inferior vena cava via right femoral vein and left jugular vein to permit sampling of blood, pressure measurement, and infusion of fluids. An indwelling catheter was placed in the urinary bladder. The left kidney was exposed via a left subcostal incision, cleared of perirenal tissue, and supported in a Lucite cup ring. The renal nerves were dissected free and platinum electrodes were placed on the transsected distal portion. A small catheter was placed in the left ureter. A small catheter was placed in the left renal vein via the left ovarian vein. At the conclusion of surgery a priming dose of inulin and p-aminohippurate...
(PAH) were given followed by a constant infusion of these substances in 0.9% NaCl at 1.0 ml/min to maintain plasma concentrations of 0.2 and 0.02 mg/ml. Aqueous vasopressin was added to the infusion in an amount calculated to deliver 0.5 mU/kg per min. Approximately 2 h prior to collection of urine samples, the animal was given deoxycorticosterone acetate (DOCA), 10 mg, int. A minimum of 60 min was allowed for equilibration and stabilization of solutions before collection of urine samples.

Group I studies began with a control period (period I or C) consisting of two consecutive 15-min urine collection periods with midpoint arterial and renal venous blood samples. At the conclusion of period I 0.9% NaCl was infused intravenously at 0.5 ml/kg per min for the remainder of the experiment. After 60 min, period II (NaCl) samples were collected as in period I. Following period II continuous direct electrical stimulation of the left renal nerves was performed using a Grass S9 stimulator (Grass Instrument Co., Quincy, Mass.) at 20 V, 1.5 ms, 2.5 mA, and 20 Hz. Period III (LRNS) samples were collected as in period I beginning 10 min after the onset of nerve stimulation. Intrarenal distribution of blood flow was measured during periods I and III using radioactive microspheres of 15 μm diameter. At the midpoints of periods I (46C and III (85Sr), 1–2 × 105 microspheres in a volume of 0.1–0.2 ml were injected into the arch of the aorta. Studies by ourselves (10) and others (3, 12, 13, 16) indicate that this procedure provides adequate mixing of the tracer and gives results similar to left ventricular injection.

Group II studies were similar to group I studies, except that following period II guanethidine was infused into the left renal artery beginning 20 min prior to initiation of left renal nerve stimulation. Guanethidine was infused intravenously via a 25-gauge curved needle at a dose of 0.5 mg/min in 0.9% NaCl at a rate of 0.5 ml/min for the remainder of the experiment. Intrarenal distribution of blood flow was measured in periods I and III.

Group III studies were designed to examine the effect of low-level renal nerve stimulation on renal hemodynamics and urinary sodium excretion. Surgical preparation was similar to group I and II studies, except that an external flow probe was placed on the left renal artery and led to an electromagnetic flowmeter (Carolina Biological Supply); this system was calibrated in vivo at the end of each experiment. In addition a steady-state saline diuresis was achieved by the constant intravenous infusion of 0.9% NaCl at 5 ml/min. After obtaining control period (C) urine and blood samples, the left renal nerves were stimulated at a level just below the threshold level that caused a reduction in renal blood flow as reflected by the flowmeter recording. These settings were: 10 V, 0.5–1.0 ms, 1.25 mA, and 0.5–2.0 Hz. Following stabilization, stimulation period (S) urine and blood samples were collected. Nerve stimulation was stopped and following stabilization, recovery period (R) urine and blood samples were collected. Intrarenal distribution of blood flow was measured in the control and stimulation periods.

Group IV (n = 9) studies were designed to test the specificity of the effect of low-level renal nerve stimulation. The first part of the study was conducted similar to group III with the following exceptions: 0.9% NaCl was infused into the left renal artery via a 25-gauge curved needle at 0.5 ml/min; intrarenal distribution of blood flow was not measured. At the conclusion of the recovery period, guanethidine was added to the left renal artery infusion so as to deliver 0.5 mg/min for the remainder of the study. Following stabilization of at least 60 min, the sequence of control, stimulation (same level), and recovery periods was again made. Intrarenal distribution of blood flow was measured in the control and stimulation periods following guanethidine administration.

Mean arterial pressure (MAP) was measured with a pressure transducer and recorded on a direct-writing recorder. For the distribution of renal blood flow studies, slicing and weighing of both kidneys, isotopic counting, and calculations were performed according to the method of Stein et al. (18).

Blood specimens were collected in chilled test tubes, centrifuged immediately, and the plasma was separated off within 5 min. Plasma and urine samples were analyzed for inulin by an anthrone method (5) and for PAH by the method of Smith et al. (17). Plasma and urine sodium concentrations were measured by flame photometry with a lithium internal standard. Hematocrit (Hct) was measured with a microhematocrit centrifuge. Plasma proteins were estimated by refractometry.

Glomerular filtration rate was taken to be the clearance of inulin (ClIN). Total renal plasma flow (TRPF) was calculated as CIN/Hct where CIN is the clearance of PAH and Hct is the extraction of PAH. Total renal blood flow (TRBF) was calculated as TRPF/1–0.95 Hct.

The data in the text, tables, and figures are expressed as the mean ± SE. The Student t test was used for statistical analysis of paired data within each group (9).

RESULTS

Data are presented in Tables 1 and 3 and Figs. 1–11.

Absolute sodium excretion (UNaV, Fig. 1) increased significantly (P < .01) from both kidneys in groups I and II following saline loading. After left renal nerve stimulation, sodium excretion from the left kidney in group I was significantly reduced (P < .01), whereas sodium excretion from the left kidney in group II continued to increase. Sodium excretion from the right kidney in groups I and II continued to rise during the left renal nerve stimulation period showing that there was no important spread of stimulation to the right kidney.

CIN (Fig. 2) of both kidneys was not significantly altered in groups I and II following saline loading. After left renal nerve stimulation, CIN of the left kidney in group I was significantly reduced (P < .001), whereas CIN of the left kidney in group II was unchanged. CIN of the right kidney in both groups I and II was unchanged during the left renal nerve stimulation period.

TRPF (Fig. 3) of the left kidney was not significantly altered in groups I and II following saline loading. CIN of the right kidney was not significantly altered in groups I and II following saline loading. CIN of the right kidney in group I was significantly reduced (P < .001), whereas TRPF of the left kidney in group II was unchanged. CIN of the right kidney in groups
FIG. 1. Urinary sodium excretion (U_{NaV}) in each experiment for left and right kidneys in control and guanethidine groups. C = control period, NaCl = saline period, LRNS = left renal nerve stimulation period. Mean ± 1 SE is shown for each period.

FIG. 2. Inulin clearance (C_{in}) data displayed as in Fig. 1.

FIG. 3. Total renal plasma flow (TRPF) and PAH clearance (CPAH) data displayed as in Fig. 1.

FIG. 4. Intrarenal distribution of blood flow in control and left renal nerve stimulation (LRNS) periods in group I (control) studies. C_1 = outer cortical zone; C_2 = inner cortical zone; C_3, C_4 = intermediate cortical zones. Mean ± 1 SE is shown for each zone.

FIG. 5. Intrarenal distribution of blood flow in group II (guanethidine) studies.

FIG. 6. Inulin clearance (C_{in}) data from left kidney for control, stimulation, and recovery periods in group III studies.

FIG. 7. Total renal blood flow (TRBF) in group III studies. Open square symbol represents 1 experiment (no. 7) in which E_{PAH} was not measured.

FIG. 8. Urinary sodium excretion (U_{NaV}) in group III studies.


I and II was unchanged during the left renal nerve stimulation period. Left kidney E_{PAH} data for both groups I and II are shown in Table 1. Saline loading resulted in a slight but significant (P < .001 for both) decrease in left kidney E_{PAH} in groups I and II: there was no change in the left renal nerve stimulation period.

In group I (Fig. 4) the percentage distribution of intrarenal blood flow to C_1 and C_2 in the left kidney was sig-
significantly reduced ($P < .01$ and $P < .05$, respectively) during the left renal nerve stimulation period as compared to the control period. No changes in intrarenal distribution of blood flow were seen in the right kidney.

In group II (Fig. 5) no changes in intrarenal distribution of blood flow were seen in the left or right kidney during the left renal nerve stimulation period as compared to the control period. No changes in intrarenal distribution and hematocrit were similar in direction and magnitude, reflecting hemodilution.

The increase in MAP (Table 2) during the left renal nerve stimulation period appeared greater in group II than in group I; however, this was not statistically significant ($P < .1$). The changes in plasma protein concentration and hematocrit were similar in direction and magnitude, reflecting hemodilution.

In group III studies the level of left renal nerve stimulation was selected so as to produce minimal if any change in hemodynamics of the left kidney. Left kidney $C_{In}$ (Fig. 6) showed no significant changes between control, stimulation, and recovery periods. Left kidney $TRBF$ (Fig. 7) was similarly unchanged. Left kidney $EPAH$ data are shown in Table 3; the lower values as compared with groups I and II reflect the greater degree of saline loading in the group III studies. No significant changes were seen. Left kidney $UNaV$ (Fig. 8) fell significantly ($P < .001$) from control to stimulation periods; the mean decrease was 36.0 ± 6.1 μeq/min or 17.3%. Following cessation of stimulation, $UNaV$ returned to control period levels with the mean decrease being 32.1 ± 5.0 μeq/min. Right kidney $UNaV$, $C_{In}$, and $TRBF$ were constant throughout control, stimulation, and recovery periods. No changes in intrarenal distribution of blood flow (Fig. 9) were seen in the left or right kidney during the left renal nerve stimulation period as compared to the control period.

### Table 1. Summary of $EPAH$ data in groups I and II

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### Table 2. Summary of data

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Values are mean ± SE.

In group IV studies, the pregnant women data confirm the results of the group III studies in that left kidney $UNaV$ (Fig. 10) fell significantly ($P < .005$) from control to stimulation periods; the mean decrease was 66.4 ± 26.5 μeq/min or 36.7%. Following cessation of stimulation, $UNaV$ returned to control period levels with the mean increase being 60.6 ± 26.1 μeq/min. During control, stimulation, and recovery periods left kidney $C_{In}$ was 34.3 ± 2.7, 34.4 ± 2.7, and 33.9 ± 2.6 ml/min; left kidney $EPAH$ was .647 ± .023, .665 ± .026, and .654 ± .021. Right kidney $UNaV$, $C_{In}$, and $TRBF$ were constant throughout control, stimulation, and recovery periods.

Following left renal adrenergic blockade with guanethidine, the identical level of left renal nerve stimulation previously shown to be effective in the same dog now had no effect on left kidney $UNaV$; the control, stimulation, and recovery period values were 181.1 ± 25.6, 180.9 ± 24.8, and 182.2 ± 24.9 μeq/min, respectively. During control, stimulation, and recovery periods left kidney $C_{In}$ was 34.0 ± 2.7, 34.4 ± 2.6, and 34.3 ± 2.7 ml/min; left kidney $EPAH$ was .647 ± .023, .665 ± .021, and .654 ± .021. Right kidney $UNaV$, $C_{In}$, and $TRBF$ were constant throughout control, stimulation, and recovery periods. No changes in intrarenal distribution of blood flow (Fig. 11) were seen in the left or right kidney during the left renal nerve stimulation period as compared to the control period after guanethidine administration.

### DISCUSSION

This study shows that renal adrenergic blockade with guanethidine effectively antagonizes the effects of direct renal nerve stimulation on glomerular filtration rate, sodium excretion, total renal plasma flow, and the intrarenal distribution of blood flow. In addition, employing a low level of direct renal nerve stimulation, it was shown that renal tubular sodium reabsorption could be increased in a reversible fashion in the absence of any changes in glomerular...
filtration rate, renal blood flow, or intrarenal distribution of blood flow; this effect was completely blocked by renal adrenergic blockade with guanethidine.

No significant effects of renal nerve stimulation on $E_{\text{PAH}}$ were noted. Others have observed no significant change in $E_{\text{PAH}}$ following 25 min of renal nerve stimulation (8) or a return of $E_{\text{PAH}}$ to control (contralateral kidney) values after 15 min of renal nerve stimulation (2). The contralateral unstimulated kidney shows no change in $E_{\text{PAH}}$ (2, 8).

Utilizing the microsphere method, Rector et al. (14) did not observe any effect on intrarenal distribution of blood flow when norepinephrine was infused into the renal artery at 4 $\mu g$/min. This may possibly indicate that the locus and/or mechanism of action of exogenously administered norepinephrine differs from that released from nerve endings in response to direct electrical nerve stimulation.

Our previous studies in which acute surgical or pharmacological renal denervation with phenoxybenzamine or guanethidine partially reversed the antinatriuresis of acute caval constriction suggested that the renal sympathetic nerves participated in the mediation of the renal functional alterations. If this were so then the characteristic alterations in renal function produced by direct stimulation of the renal nerves should be blocked by guanethidine; these studies clearly show this.

However, the reversal by guanethidine of the antinatriuresis in the acute caval dog occurred without any significant changes in glomerular filtration rate, total renal blood flow, mean arterial pressure, cardiac output, filtration fraction, or intrarenal distribution of blood flow or glomerular filtrate. It was therefore concluded that the renal sympathetic nerves had a direct influence on tubular sodium transport and that guanethidine was inhibiting this action. In the current studies, following renal nerve stimulation, the differences in sodium excretion between the control and guanethidine-treated kidneys were accompanied by changes in glomerular filtration rate, total renal blood flow, and intrarenal blood flow distribution. These hemodynamic alterations could explain the observed changes in sodium excretion without consideration of the rate of net tubular reabsorption of sodium. One possible explanation would be that acute caval constriction results in a level of renal sympathetic nerve stimulation which directly increases tubular sodium transport but does not affect glomerular filtration rate or total renal blood flow. In this situation interruption of renal sympathetic nerve traffic would be expected to increase sodium excretion without altering glomerular filtration rate or total renal blood flow, precisely what was observed in the acute caval dog studies.

The $E_{\text{PAH}}$ III data clearly show that there is a level of direct renal nerve stimulation which can increase tubular sodium reabsorption without affecting glomerular filtration rate or renal blood flow. The cross-circulation studies of Gill and Casper (7) also showed that there is a level of renal nerve activity which can increase tubular sodium reabsorption but is not sufficient to decrease glomerular filtration rate. Cant and Vander (4) infused norepinephrine, the neurotransmitter released on sympathetic nerve stimulation, into kidneys at a dose that reduced total renal blood flow by less than 10% and did not alter intrarenal distribution of blood flow or glomerular filtration rate. A significant reduction in urinary sodium excretion was observed; it was suggested that norepinephrine directly increases tubular sodium transport. In the anesthetized dog, in which a certain degree of renal sympathetic nerve activity might be presumed to be present, intrarenal infusion of guanethidine resulted in an increase in sodium excretion without a change in glomerular filtration rate or effective renal plasma flow (20). This effect was attributed to a direct effect of renal sympathetic nerves on tubular sodium transport which was blocked by guanethidine. These views receive strong anatomic support from the studies of Muller and Barajas (11), referred to earlier.

Guanethidine blocked the effects of both high-level and low-level direct renal nerve stimulation. Moreover, guanethidine partially reversed the antinatriuresis in the acute caval dog without altering renal hemodynamics. Therefore, these studies support the view that acute caval constriction is characterized by a level of renal sympathetic nerve activity which enhances tubular sodium reabsorption without affecting glomerular filtration rate or renal blood flow.
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