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Specificity of Neural Effect on Renal Tubular Sodium Reabsorption (39254)

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A number of studies have provided evidence that increased renal sympathetic nerve activity participates in the regulation of renal tubular sodium reabsorption in normal and pathologic states. Studies from this laboratory 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 (1, 2). The reversal of the antinatriuresis could not be explained by alterations in systemic or intrarenal hemodynamic variables. These studies provided suggestive evidence for a direct effect of the renal sympathetic nerves on tubular sodium transport. Light and electron microscopic studies of monkey renal cortex have provided firm anatomic evidence for the existence of adrenergic nerve fibers in direct contact with the basement membrane of tubular epithelial cells (3). Subsequent experiments have shown that there exists a low level of direct renal nerve stimulation which enhances tubular sodium reabsorption in the absence of changes in glomerular filtration rate, renal blood flow, or intrarenal distribution of blood flow (4).

These studies were performed to test the adrenergic specificity of this response.

Methods. All studies were performed on female mongrel dogs, 15-20 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 iv 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 arteries, 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 transected 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. An external flow probe was placed on the left renal artery and led to an electromagnetic flow meter (Carolina); this system was calibrated *in vivo* at the end of each experiment. A 25 gauge curved needle attached to polyethylene tubing was placed in the left renal artery against the direction of flow. At the conclusion of surgery a priming dose of inulin and *p*-aminohippurate (PAH) was 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/min. A steady state saline diuresis was achieved by the constant iv infusion of 0.9% NaCl at 5 ml/min. Approximately 2 hr prior to collection of urine samples, the animal was given desoxycorticosterone acetate (DOCA) 10 mg im. A minimum of 60 min was allowed for equilibration and stabilization of solutions before collection of urine samples.

Control Period (C) consisted of two consecutive 15-min urine collection periods with midpoint arterial and renal venous blood samples. Following the Control Pe-

riod, continuous direct electrical stimulation of the left renal nerves was performed using a Grass S9 stimulator at 10 V, 0.5–1.0 msec, 1.25 mA, and 0.5–2.0 Hz. Stimulation Period (S) samples were collected beginning 10 min after onset of nerve stimulation. Nerve stimulation was stopped and following stabilization, Recovery Period (R) samples were collected. During the above three periods, the left renal artery infusion was 0.9% NaCl at 0.5 ml/min. At the conclusion of the Recovery Period (R), the left renal artery infusion was changed to phenoxybenzamine (POB); POB was administered at a dose of 0.2 μg/kg/min in 0.9% NaCl at 0.5 ml/min for the remainder of the study. Following stabilization of at least 60 min the sequence of Control (C), Stimulation (S, same level), and Recovery (R) Periods was again made. Intrarenal distribution of blood flow was measured in the Control (C) and Stimulation (S) Periods following phenoxybenzamine administration with radioactive microspheres of 15-μm diameter. At the midpoint of the Control Period (C) and Stimulation Period (S) 1–2 × 10^5 microspheres (^{141}Ce, ^{85}Sr) in a volume of 0.1–0.2 ml were injected into the arch of the aorta. Studies in this (5) and other (6–9) laboratories indicate that this procedure provides adequate mixing of the tracer and gives results similar to left ventricular injection. The adequacy of blockade was evaluated by observing the renal vasoconstrictor response to the renal arterial administration of 10–40 μg of phenylephrine.

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.* (10).

Blood specimens were collected in chilled test tubes, centrifuged immediately, and the plasma separated off within 5 min. Plasma and urine samples were analyzed for inulin by an anthrone method (11) and for PAH by the method of Smith *et al.* (12). Plasma and urine sodium concentrations were measured by flame photometry with a lithium internal standard. Hematocrit (Hct) was measured with a microhematocrit centrifuge.

Glomerular filtration rate was taken to be the clearance of inulin (C_{in}). Total renal plasma flow (TRPF) was calculated as C_{PAH}/E_{PAH} where C_{PAH} is the clearance of PAH and E_{PAH} is the extraction of PAH. Total renal blood flow (TRBF) was calculated as TRPF/1–0.95 Hct. TRBF calculated in this manner agreed with flowmeter values within 10%.

The data in the text, tables and figures are expressed as the mean ± SE. Student's *t* test was used for statistical analysis of paired data within each group (13).

Results. The results ($N = 10$) are shown in Figs. 1–4 and Table I. C_{in} and TRBF exhibited minor random changes which were not significant. When 0.9% NaCl was infused into the left renal artery, low level direct renal nerve stimulation elicited a reversible 21% decrease ($P < 0.001$) in $U_{Na}V$. When the left renal artery infusion was changed to phenoxybenzamine (POB), no change in $U_{Na}V$ occurred in response to an identical level of stimulation. The intrarenal distribution of blood flow was not altered in either kidney; the lower values for C_i in the left as compared to the right kidney probably reflect the surgical manipulation involved. Right kidney $U_{Na}V$, C_{in}, and TRBF were constant throughout.

We have previously shown that this low level of direct renal nerve stimulation has no effect on intrarenal distribution of blood flow (4).

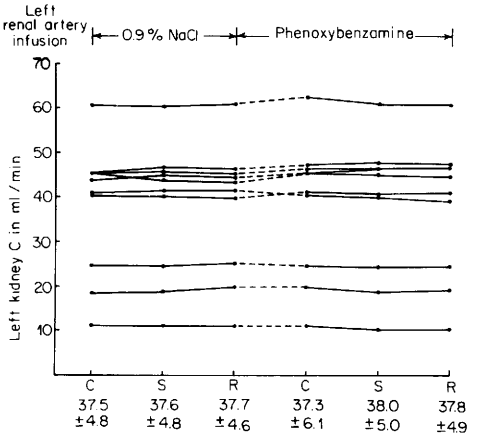

FIG. 1. Left kidney inulin clearance (C_{in}) data. In this and subsequent figures: C = Control, S = Stimulation, and R = Recovery. Numerical data are mean ± SE.

Discussion. This study, employing a low level of direct renal nerve stimulation, shows that renal tubular sodium reabsorption could be increased in a reversible fashion in the absence of changes in glomerular filtration rate, renal blood flow, or intrarenal distribution of blood flow. In addition, since this effect was blocked by renal alpha-adrenergic receptor blockade with phenoxybenzamine, these results suggest that this effect is mediated by catecholamines. Taken together with our previous studies wherein this effect was blocked by renal adrenergic blockade with guanethidine (4), these findings suggest that this effect is mediated by direct adrenergic innervation of the renal tubule.

The cross-circulation studies of Gill and Casper (14) 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 (15) 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 fil-

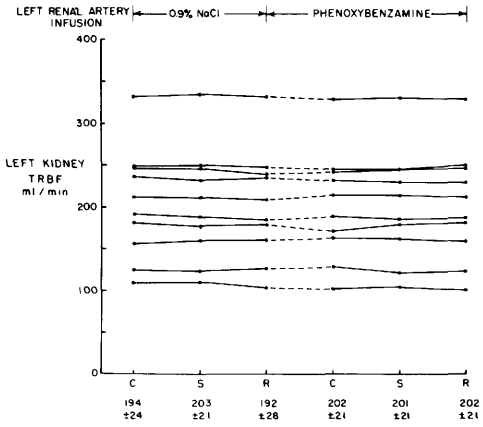

FIG. 2. Left kidney total renal blood flow (TRBF).

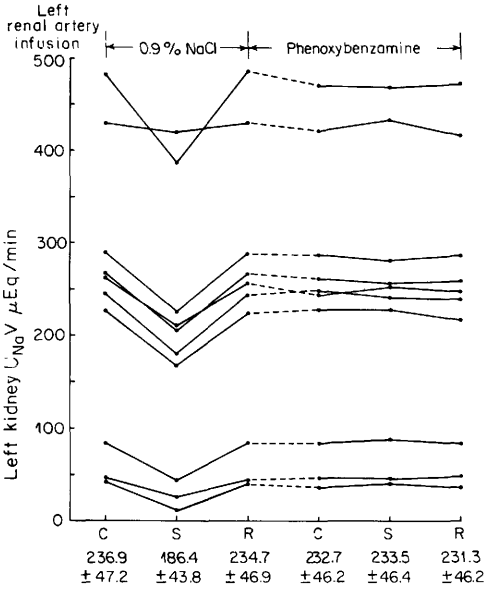

FIG. 3. Left kidney urinary sodium excretion ($U_{Na}V$).

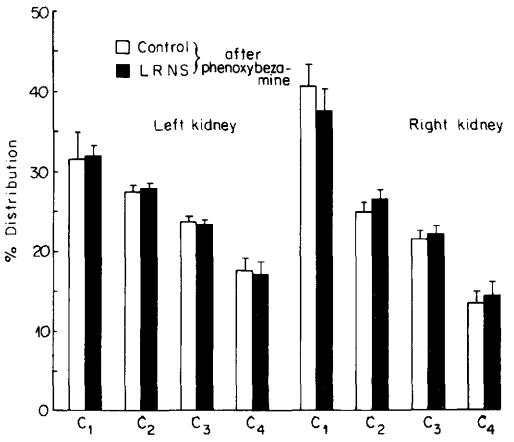

FIG. 4. The percentage distribution of renal cortical blood flow during Control and Stimulation (LRNS = left renal nerve stimulation) Periods after left renal arterial administration of phenoxybenzamine. C_1 is the outer cortical zone, C_4 is the inner cortical zone, and C_2 and C_3 are intermediate cortical zones.

TABLE I. SUMMARY OF E_{PAH} AND MAP DATA[a]

	NaCl			POB		
	C	S	R	C	S	R
E_{PAH}	0.73 ± 0.02	0.75 ± 0.03	0.77 ± 0.03	0.73 ± 0.05	0.71 ± 0.06	0.73 ± 0.05
MAP (mm Hg)	126 ± 7	127 ± 8	127 ± 8	127 ± 7	128 ± 7	126 ± 7

[a] Entries are mean ± SE for Control (C), Stimulation (S), and Recovery (R) Periods during renal arterial infusion of 0.9% NaCl (NaCl) or phenoxybenzamine (POB). $N = 10$.

tration 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 phenoxybenzamine, 0.09 μg/kg/min, resulted in a 67% increase in $U_{Na}V$ while C_{in} and TRBF were unchanged (16). Proximal tubular fractional reabsorption, as assessed by micropuncture technique, was unaltered and the natriuresis was ascribed to depression of sodium reabsorption at some more distal nephron segment. Similarly, intrarenal infusion of guanethidine resulted in an increase in sodium excretion without a change in glomerular filtration rate or effective renal plasma flow (17). 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 (3), referred to earlier.

Summary. Low level direct renal nerve stimulation increases renal tubular sodium reabsorption in the absence of changes in glomerular filtration rate, renal blood flow, or intrarenal distribution of blood flow. Blockade of this response with phenoxybenzamine (or guanethidine) supports the interpretation that it is mediated by direct adrenergic innervation of the renal tubule.

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