Depression of Proximal Tubular Sodium Reabsorption in the Dog in Response to Renal Beta Adrenergic Stimulation by Isoproterenol

JOHN R. GILL, JR., and ALFRED G. T. CASPER

*From the Endocrinology Branch of the National Heart and Lung Institute,
National Institutes of Health, Bethesda, Maryland 20014*

A B S T R A C T Water diuresis was produced in anesthetized hypophysectomized, cortisone-treated dogs by infusion of 2.5% dextrose. Alpha adrenergic blockade of the left kidney produced by infusion of phenoxybenzamine in the left renal artery was associated with a significantly ($P < 0.05$) greater rate of urine flow (V) and free water excretion (C_{H2O}) in the left kidney than in the right despite similar glomerular filtration rates (GFR) (17 ±1.3 ml/min, left; 18 ±0.9 ml/min, right). Sodium excretion ($U_{Na}V$) was similar in the two kidneys (3 and 5 μEq/min).

When beta adrenergic stimulation of the left kidney was superimposed on alpha blockade by the addition of isoproterenol to the left renal artery infusate, GFR remained unchanged and similar in the two kidneys, as V and C_{H2O} increased significantly ($P < 0.01$) in the left kidney but not in the right. When isoproterenol was discontinued, V and C_{H2O} returned towards control in the left kidney and remained unchanged in the right. The ratios of the left kidney to the right during control, isoproterenol, and postcontrol were 1.22, 1.65, and 1.35, respectively, for V and 1.36, 1.90, and 1.44, respectively, for C_{H2O}. Sodium excretion remained unchanged and similar in the two kidneys throughout the study.

The results indicate that blockade of alpha adrenergic activity inhibits the increased proximal tubular sodium reabsorption which anesthesia induces in the dog.

Beta adrenergic stimulation appears to decrease proximal tubular sodium reabsorption but does not prevent virtually complete reabsorption of the increased quantity of delivered sodium by the ascending limb of the loop

of Henle and the distal tubule. These changes in sodium reabsorption presumably are not associated with changes in colloid osmotic pressure or hydrostatic pressure in the peritubular capillary inasmuch as cortical and noncortical plasma flow, filtration fraction, and mean arterial pressure in the left kidney were unchanged. Thus, isoproterenol probably produced its effects through a direct action on the renal tubule, possibly through the mediation of the adenyl cyclase system.

INTRODUCTION

An increase in renal blood flow in response to a renal arterial injection of isoproterenol has been reported by a number of investigators (1–3), and this observation was the basis for the assumption that beta adrenergic receptors are present in the kidney (1). Recent findings that beta adrenergic blockade, but not alpha adrenergic blockade, prevented an increase in renal blood flow when isoproterenol was injected in a renal artery indicate that renal beta adrenergic receptors do, indeed, mediate the increase in renal blood flow produced by isoproterenol (2, 3).

In another study, a brief infusion of isoproterenol into a renal artery of a saline-loaded dog increased renal blood flow and sodium excretion ($U_{Na}V$) ipsilaterally but did not appear to alter glomerular filtration rate (GFR) (4).

The present experiments were designed to explore in greater detail the effects of isoproterenol on renal tubular function in the dog and to determine if these effects are related to changes in renal hemodynamics. During a stable water diuresis and blockade of alpha adrenergic receptors in the left kidney by phenoxybenzamine, a dose of isoproterenol sufficient to produce a change in urine flow was infused in the left renal artery. Urine flow (V) and free water clearance (C_{H2O}) were
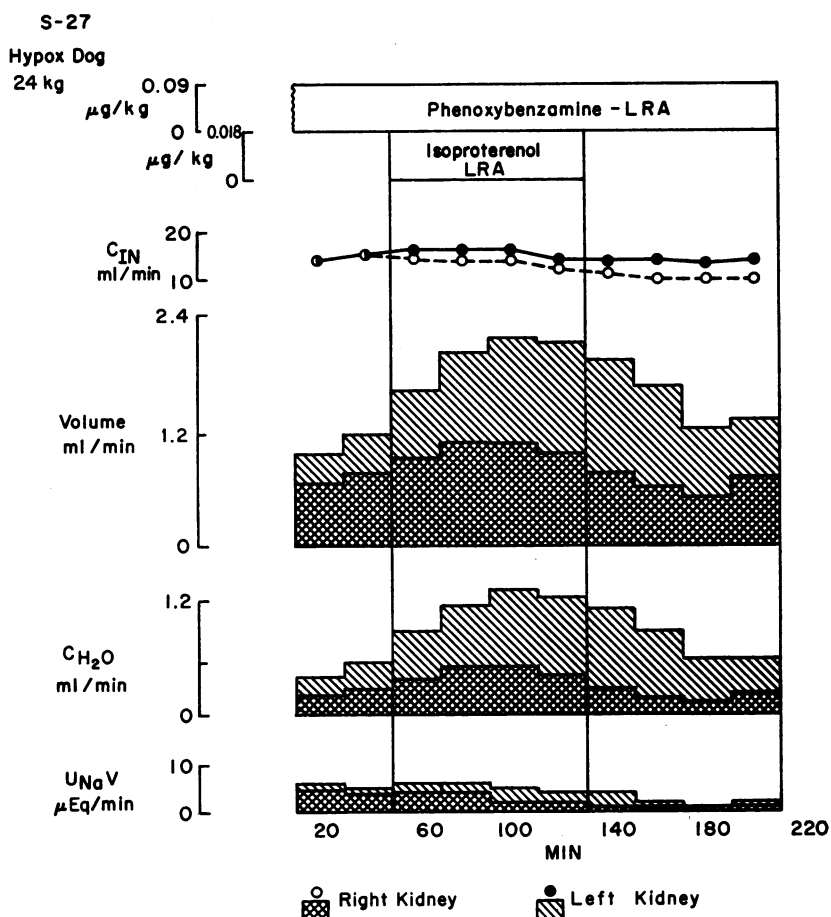
FIGURE 1 The effect of infusion of phenoxybenzamine and of phenoxybenzamine and isoproterenol in the left renal artery (LRA) of a water-loaded hypophysectomized (hypox) dog on C_{IN} (clearance of inulin), urine volume, C_{H_2O} (clearance of solute-free water), and $U_{Na}V$ (sodium excretion) in the right and left kidney.

assumed to reflect delivery of glomerular filtrate out of the proximal tubule and reabsorption of sodium at a distal diluting site (5). The pituitary was removed before the study to ensure against the possibility of an increase in water permeability of the distal nephron as a result of an enhancement of antidiuretic hormone action by isoproterenol such as has been observed in the toad bladder (6).

METHODS

Mongrel dogs weighing 18–27 kg (mean 22.2 kg) were fed a synthetic diet which contained 50 mEq of sodium per day. On the day of study, the dogs were anaesthetized with pentobarbital, hypophysectomized with a dental drill through a buccal approach, and given 12.5 mg of cortisone acetate, intramuscularly. Catheters were placed in both ureters, the left renal artery, the left renal vein by way of the spermatic or ovarian vein, and the aorta to measure mean arterial pressure (7). Normal saline containing inulin and p-aminohippurate was infused in a femoral vein at 0.5 ml/min by

a constant infusion pump. Normal saline containing 0.18 $\mu g/kg$ per ml of phenoxybenzamine (Smith, Kline & French Laboratories, Philadelphia, Pa.) was infused in the left renal artery at 0.5 ml/min by a constant infusion pump throughout the study. Water diuresis was produced by intravenous infusion of 2.5% dextrose solution first, 1000 ml rapidly, then at 8 ml/min. When urine flow was stable, approximately 1 hr after the operative procedure, clearance measurements were started. After three control periods, 0.036 $\mu g/kg$ per ml of isoproterenol (Winthrop Laboratories, New York) was added to the solution infused in the left renal artery for four to six periods. The study was continued for three or four periods after isoproterenol was stopped. The postcontrol data for experiments S-24, S-26, and S-29 were omitted because of decreases in the clearance of inulin and in urine flow in the right kidney during the postcontrol observations. All clearance periods were 20 min in duration. The results of control periods from clearance studies in which phenoxybenzamine was omitted from the left renal artery infusate are included for comparison.

The clearances of inulin (C_{IN}) and p-aminohippurate (C_{PAH}) were determined as previously described (7). Serum

and urinary sodium were determined by internal standard flame photometry; serum and urinary osmolality were measured by a Precision Systems osmometer (Precision Systems Co., Inc., Somerville, N. J.).

C_{H_2O} was calculated by the conventional formula and expressed as milliliters per minute. For purposes of comparison, the data on urine flow and C_{H_2O} for the right and left kidneys were also expressed as ml per 100 ml of glomerular filtrate to correct for small variations in C_{IN}. Extraction of PAH (E_{PAH}) was calculated from the formula $E_{PAH} = (RA_{[PAH]} - RV_{[PAH]})/RA_{[PAH]}$, where $RA_{[PAH]}$ and $RV_{[PAH]}$ are the concentrations of PAH in renal arterial and venous samples, respectively. Renal plasma flow (RPF) then was calculated by the formula $RPF = C_{PAH}/E_{PAH}$. Noncortical plasma flow (NCPF) was calculated as $RPF - C_{PAH}$ and taken as an estimate of medullary plasma flow; C_{PAH} was assumed to approximate cortical plasma flow (8). The significance of the data was determined by paired analysis.

RESULTS

Fig. 1 shows the design of the study and the results of an experiment. An infusion of 2.5% dextrose solution in an hypophysectomized dog during an infusion (0.5 ml/min) of a solution of 0.18 μg/kg per ml of phenoxybenzamine in the left renal artery was associated with a mean V of 0.7 ml/min and a mean C_{H_2O} of 0.24 ml/min from the right kidney and a mean V of 1.05 ml/min and a mean C_{H_2O} of 0.48 ml/min from the left kidney. Mean C_{IN} and mean $U_{Na}V$ in each of the two kidneys were similar. When 0.036 μg/kg per ml of isoproterenol was added to the solution infused in the left renal artery, V and C_{H_2O} increased in the right kidney (to 1.0 and 0.46 ml/min, respectively) and to a greater extent in the left kidney (to 2.12 and 1.25 ml/min, respectively). When isoproterenol was discontinued V and C_{H_2O} decreased in the right kidney (to 0.60 and 0.18 ml/min, respectively) and to a greater extent in the left kidney (to 1.25 and 0.57 ml/min, respectively). C_{IN} and $U_{Na}V$ remained essentially unchanged from the control period throughout the study.

TABLE I

Effects of Infusion of Isoproterenol in the Left Renal Artery on the Function of the Right and Left Kidneys

Experiment	Regimen	Right kidney				Left kidney			
		C_{IN}	V	C_{H_2O}	$U_{Na}V$	C_{IN}	V	C_{H_2O}	$U_{Na}V$
		ml/min			*$\mu Eq/min$*	*ml/min*			*$\mu Eq/min$*
S–24	C*	17	0.87	0.58	2	19	0.93	0.63	3
	I‡	16	1.08	0.67	3	18	1.32	0.91	3
S–25	C	20	0.72	0.24	1	20	0.92	0.39	1
	I	14	0.96	0.32	0	17	2.00	1.01	2
	PC§	11	0.80	0.24	0	16	1.78	0.85	2
S–26	C	19	1.45	0.62	3	20	2.00	1.12	10
	I	16	0.82	0.31	1	18	2.48	1.48	14
S–27	C	14	0.70	0.24	4	14	1.05	0.48	6
	I	13	1.00	0.46	2	15	2.12	1.25	4
	PC	10	0.60	0.18	1	14	1.25	0.57	2
S–29	C	20	1.50	1.00	7	16	1.15	0.80	5
	I	17	1.30	0.80	5	16	2.20	1.62	2
S–30	C	16	1.92	1.16	6	12	2.02	1.28	5
	I	17	1.70	0.97	3	13	2.15	1.32	5
	PC	16	1.65	0.88	3	15	1.95	1.02	3
S–32	C	20	1.42	1.12	1	21	2.08	1.74	2
	I	20	1.48	1.18	2	22	2.32	1.94	4
	PC	18	1.35	1.09	2	20	1.95	1.60	4
	C	18 ±0.9	1.23 ±0.17	0.71 ±0.13	3	17 ±1.3	1.45 ±0.17	0.92 ±0.19	5
Mean	I	16 ±1.0	1.19 ±0.13	0.67 ±0.12	2	17 ±1.3	2.08 ±0.17	1.36 ±0.15	5
±SEM	PC	14 ±1.1	1.10 ±0.26	0.60 ±0.23	2	16 ±1.5	1.73 ±0.18	1.01 ±0.26	3
P	C vs. I	>0.1	>0.7	>0.4		>0.4	<0.01	<0.01	

C_{IN} = mean clearance of inulin; V = mean urine flow; C_{H_2O} = mean free water clearance; $U_{Na}V$ = mean sodium excretion.
* C = control; infusion of phenoxybenzamine in left renal artery.
‡ I = infusion of isoproterenol and phenoxybenzamine in left renal artery.
§ PC = postcontrol; infusion of phenoxybenzamine in left renal artery.

The results for all the studies are presented in Table I. During the control period, mean V and C_{H_2O} were 1.23 ml/min ±0.17 SE and 0.71 ±0.13, respectively, for the right kidney and 1.45 ±0.17 ml/min and 0.92 ±0.19, respectively, for the left kidney. Mean C_{IN} and $U_{Na}V$ in each of the two kidneys were similar (18 ±0.9 ml/min and 3 μEq/min, respectively, for the right, 17 ±1.3 ml/min and 5 μEq/min, respectively, for the left). When isoproterenol was infused in the left renal artery, mean V and C_{H_2O} remained unchanged in the right kidney (1.19 ±0.13 and 0.67 ±0.12 ml/min, respectively; $P > 0.7$ and > 0.4, respectively) but increased significantly in the left kidney (2.08 ±0.17 and 1.36 ±0.15 ml/min, respectively; $P < 0.01$ for both. Mean mean C_{IN} in each of the two kidneys were similar (16 ±1 ml/min, right; 17 ±1.3 ml/min, left) and not significantly different from the corresponding control value ($P > 0.1$, right; $P > 0.4$, left). Mean $U_{Na}V$ was also essentially similar in the two kidneys and unchanged from control values. When isoproterenol was discontinued, mean V and C_{H_2O} continued essentially unchanged in the right kidney (1.1 ±0.26 and 0.6 ±0.23 ml/min, respectively) and decreased toward control values in the left kidney (1.73 ±0.18 and 1.01 ±0.26 ml/min, respectively). Mean C_{IN} and $U_{Na}V$ remained essentially unchanged.

FIGURE 3 The effect of infusion of isoproterenol in the left renal artery (LRA) of hypophysectomized (hypox) dogs on mean urine volume in the right and left kidneys and on the ratio of volume in the left kidney to that in the right. The increase in the ratio is significant ($P < 0.01$).

In Fig. 2, V and C_{H_2O} in ml/min per 100 ml GFR for the right and left kidneys of dogs in which only normal saline was infused in the left renal artery are compared to the values for the right and left kidneys of dogs in which phenoxybenzamine in saline was infused in the left renal artery. Whereas V and C_{H_2O} were similar in the right and left kidneys when only saline was infused, these two variables were significantly ($P < .05$) higher in the left kidney when phenoxybenzamine was present in the left renal artery infusate.

In Figs. 3 and 4, the changes in V and in C_{H_2O} in ml/min per 100 ml GFR are presented for the left and right kidneys and these changes are also expressed as ratios of the left to the right kidney.

For V, the ratio of left to right was 1.22 during control, increased to 1.65 during infusion of isoproterenol, and decreased to 1.35 when isoproterenol was discontinued. For C_{H_2O}, the ratio of left to right was 1.36 during control, increased to 1.90 during isoproterenol, and decreased to 1.44 when isoproterenol was discontinued.

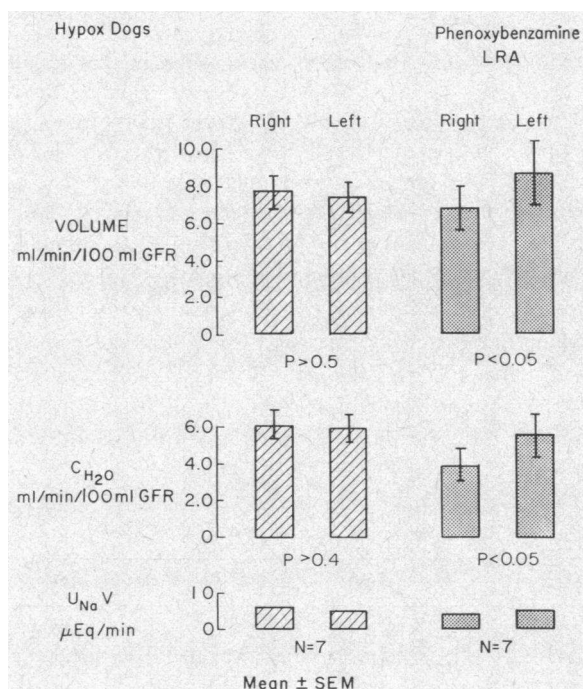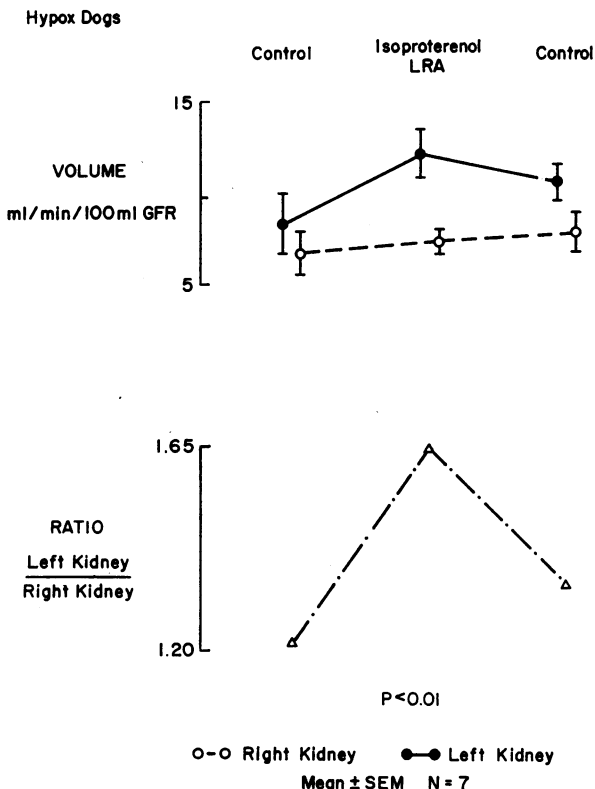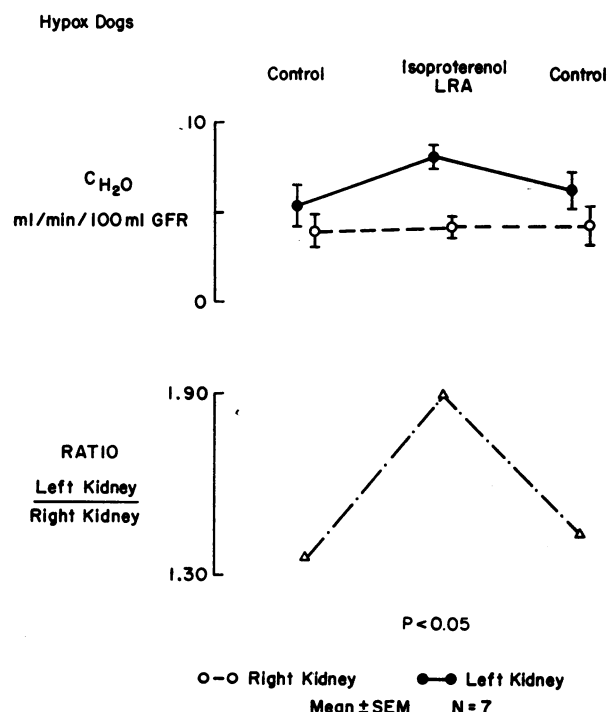The effect of infusion of isoproterenol in the left renal

FIGURE 2 The effect of an infusion of normal saline alone and of normal saline which contained phenoxybenzamine in the left renal artery (LRA) on mean urine volume and mean C_{H_2O} (free water clearance) in the right and left kidneys of hypophysectomized (hypox) dogs.

FIGURE 4 The effect of infusion of isoproterenol in the left renal artery (LRA) of hypophysectomized (hypox) dogs on mean C_{H_2O} (free water clearance) in the right and left kidney and on the ratio of C_{H_2O} in the left kidney to that in the right. The increase in the ratio is significant ($P < 0.05$).

artery on the hemodynamic function of the left kidney is shown in Table II. C_{PAH}, noncortical plasma flow, and total renal plasma flow were 49 ±10.7, 43 ±9, and 92 ±19 ml/min, respectively, during control and did not change significantly with isoproterenol. Mean arterial pressure was 106 ±6 mm Hg during control and did not change significantly ($P > 0.05$) with isoproterenol.

DISCUSSION

Infusion of 2.5% dextrose produced an increase in urine flow and free water clearance in each of the two kidneys

of hypophysectomized dogs treated with cortisone. When urine flow was stable, the rates of urine flow and of free water clearance were significantly ($P < 0.05$) greater in the phenoxybenzamine-treated left kidney than in the untreated right kidney (Table I, Figs. 1–4). No difference between the right and left kidneys is observed when the left renal artery infusate does not contain phenoxybenzamine (Fig. 2). The clearance of inulin (18 ±0.9 ml/min, right; 17 ±1.3 ml/min, left) and the excretion of sodium were similar in the two kidneys; this suggests that renal alpha adrenergic blockade in the anesthetized dog decreased the proximal tubular reabsorption of sodium and water and increased the delivery of proximal tubular fluid distally to the diluting site where the sodium was reabsorbed. In the anesthetized dog, a surgically denervated kidney excretes urine at rates of flow significantly greater than those in the intact contralateral kidney, so-called denervation diuresis (9). The present results suggest that total surgical denervation increases urine flow by interruption of increased renal alpha adrenergic stimuli associated with anesthesia. A corollary to these results is the observation that an increase in adrenergic stimuli to a kidney can increase the tubular reabsorption of sodium and water (10).

When isoproterenol was added to the left renal artery infusate, urine flow and C_{H_2O} increased significantly ($P < 0.01$) without a change in the clearance of inulin ($P > 0.4$) or in sodium excretion in that kidney; urine flow, C_{H_2O}, C_{IN} and $U_{Na}V$ were unchanged in the right kidney (Fig. 1, Table I). When isoproterenol was stopped, left renal urine flow and C_{H_2O} returned toward control values during the continued infusion of phenoxybenzamine into the left renal artery.

The increase in urine flow and in C_{H_2O} without a change in GFR provides indirect evidence that isoproterenol decreased the proximal tubular reabsorption of sodium and water and increased the volume of proximal fluid delivered distally to the diluting site. Reabsorption of the increased load of sodium at a diluting site (presumably the ascending limb of the loop of Henle) and

TABLE II
Effect of Infusion of Isoproterenol in the Left Renal Artery on the Hemodynamic Function of the Left Kidney

	C_{PAH}	E_{PAH}	NCPF	RPF	FF	MAP
	ml/min		*ml/min*	*ml/min*		*mm Hg*
Mean Control ±SEM	49 ±10.7	0.54 ±0.05	43 ±9	92 ±19	0.41 ±0.06	106 ±6
Mean change with isoproterenol ±SEM	−3.6 ±5.3	−0.01 ±0.053	−0.6 ±12.1	−4.4 ±15.4	0.01 ±0.06	11.3 ±5.6
P	>0.5		−	>0.7		>0.05

C_{PAH} = clearance of p-aminohippurate; E_{PAH} = renal extraction of p-aminohippurate; RPF = total renal plasma flow; NCPF = noncortical plasma flow; FF = filtration fraction; MAP = mean arterial pressure.

in the distal tubule increased the excretion of C_{H_2O} and prevented an increase in sodium excretion. This explanation for a decrease in proximal tubular sodium reabsorption without an increase in sodium excretion requires that the increase in V be similar in magnitude to the increase in C_{H_2O}. The magnitude of the change in the ratio of the left kidney to the right for V (Fig. 3) as compared with the magnitude of the change in the ratio of the left kidney to the right for C_{H_2O} (Fig. 4) indicates that this is indeed the case.

There are at least the following three possible mechanisms by which one could explain a decreased tubular reabsorption of sodium and water: (a) renal vasodilatation with a decrease in peritubular capillary colloid osmotic pressure (decreased filtration fraction) (11) or with an increase in peritubular hydrostatic pressure (increased transmission of mean arterial pressure to the peritubular capillary) (12) or both; (b) a redistribution of renal blood flow so that a greater proportion perfuses cortical nephrons with shorter loops of Henle and, presumably, with less capacity to reabsorb sodium (13); and (c) direct effect of an agent on the tubule with inhibition of reabsorptive processes. Infusion of isoproterenol in the left renal artery did not increase total renal plasma flow, decrease filtration fraction, or increase mean arterial pressure (Table II); therefore, presumably, it did not alter either peritubular capillary colloid osmotic pressure or hydrostatic pressure. Isoproterenol also did not alter C_{PAH} which has been equated to cortical plasma flow (8) nor did it alter noncortical plasma flow (Table II); and therefore, presumably, it did not cause a redistribution of renal blood flow. Thus, isoproterenol probably decreased the tubular reabsorption of sodium through a direct effect on the proximal tubule by means of a series of intermediary events as yet unknown.

The presence of cyclic 3',5'-adenosine monophosphate in renal cortical cells (14), the ability of this nucleotide to inhibit the renal tubular reabsorption of another ion, phosphate, (15), and the potent activation of its synthesis by beta adrenergic stimulation in other tissues (16) all suggest that the adenyl cyclase system could serve as a mediator of the tubular effects of isoproterenol.

Depression of tubular sodium reabsorption by expansion of extracellular fluid volume is usually associated with one or more changes in renal hemodynamics (renal vasodilatation, decreased filtration fraction, or increased perfusion pressure) (17) which are thought to decrease colloid osmotic pressure, increase hydrostatic pressure, or both in the peritubular capillary. Whereas such changes in Starling forces in the peritubular capillary are capable of indirectly depressing proximal tubular

sodium reabsorption (11, 12), this does not exclude the possibility that another mechanism(s) for inhibition of tubular sodium reabsorption is also operative (11). Indeed, expansion of blood volume has been observed to decrease proximal tubular reabsorption of sodium despite an increase in renal vascular resistance and an increase in oncotic pressure in the peritubular capillaries (18). If expansion of extracellular fluid volume leads to renal beta adrenergic stimulation in addition to alteration of Starling forces in the peritubular capillary, it could thus inhibit proximal tubular sodium reabsorption directly as well as indirectly.

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