Effects of Reopening the Ureter in the Nonfiltering Kidney Model (36283)

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Increased plasma levels of renin activity have been demonstrated in response to a variety of experimental forcings, such as hemorrhage (1, 2), renal artery constriction (3), and sodium depletion (4, 5). Two hypotheses have been advanced to explain these findings. According to one hypothesis, the baroreceptor hypothesis, the signal for renin secretion in these situations is a decreased stretch of baroreceptors located in the kidney; another hypothesis proposes that a change in the sodium load or concentration at the macula densa is the stimulus for altering renin secretion. Recent reviews by Vander (6) and by Davis (7) have presented the details of these hypotheses.

In order to investigate the proposed baroreceptor mechanism, one approach was to develop a renal preparation in which these various forcings would not affect the macula densa. For this purpose Blaine, Davis, and Witty (8) developed a nonfiltering kidney model in the dog by a combination of ureteral ligation and two hours of total ischemia. Evidence that this model was nonfiltering was that a filterable dye, lissamine green, failed to appear in the superficial renal tubules; also, histologic sections of these kidneys showed casts in the proximal tubules which would block the flow of tubular urine. However, additional information was needed regarding the possibility that filtration could be occurring in the deeper nephrons. The present study was designed to test this model for deep-nephron filtration by measuring the renal clearance of creatinine after reopening the ureter. Secondarily, we hoped to obtain information on renin secretion as tubular urine flow and sodium load were restored by reopening the ureter.

Methods. Eight female mongrel dogs, which ranged from 14.2 to 18.7 kg body wt, were used in this study. All dogs were maintained on a standard diet which provided 70 mEq of sodium and 55 mEq of potassium per day; water was available *ad libitum*. In each animal a nonfiltering kidney was produced on the left side under sterile conditions by the technique described by Blaine *et al.* (8). This consisted of ligating the left ureter and placing a serrefine clamp on the renal artery for two hours to produce renal ischemia. Two or three days later the right kidney was removed by a flank incision; no further food was allowed. The experiment was performed on the following day.

At the beginning of the acute study, the dog was anesthetized with sodium pentobarbital, 30 mg/kg body wt. The left kidney was exposed by a flank incision. An electromagnetic flow probe was placed around the renal artery, and renal blood flow was measured with a Carolina square-wave electromagnetic flow meter. A polyvinyl catheter (Fr. 5) was passed through the ovarian vein into the renal vein for obtaining renal venous blood. A polyvinyl catheter (Fr. 8) was placed in the aorta by way of the femoral artery to collect arterial blood samples for renin, creatinine, and electrolytes, and to measure mean arterial blood pressure. A similar catheter was also placed in the femoral vein for infusing a solution of creatinine. A catheter with a clamp on its end was placed in the ureter, proximal to the point of ligation. Arterial pressure was determined with a Sanborn model P23Db pressure transducer. Both renal blood flow and arterial blood pressure were recorded on a Sanborn model 7700 recorder.

After the completion of all surgical procedures, a priming solution of creatinine

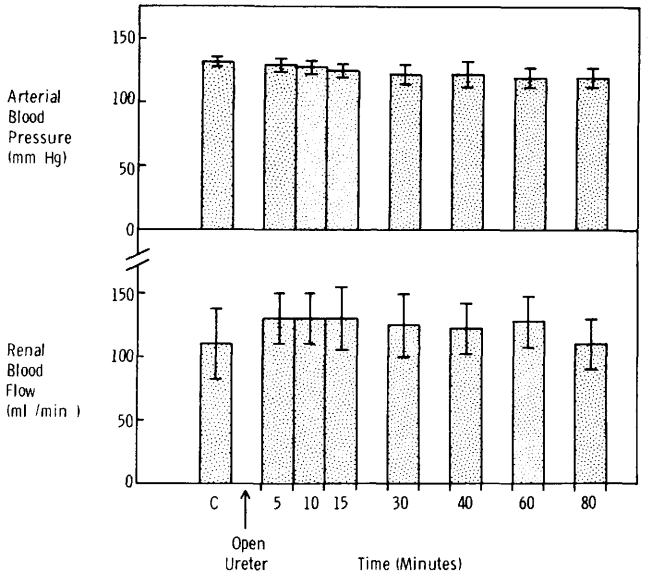

FIG. 1. Mean values ± SEM for arterial blood pressure and renal blood flow. *C* indicates control period.

was administered and the continuous infusion of a creatinine solution (36 mg/ml in saline) was initiated at a rate of 0.59 ml/min. Thirty minutes later control measurements were obtained for renal blood flow and mean arterial blood pressure, and blood samples were obtained from the aorta and the renal vein for the determination of renin secretion. The ureter was then opened by removing the clamp on the ureteral catheter, and the urine was collected every two minutes for 30 min. Measurements of arterial pressure, renal blood flow, and renin secretion were made at 5, 10, 15, 30, and 90 min after reopening the ureter. Three renal clearance periods for creatinine of 20 min each were begun 30 min after reopening the ureter.

The arterial and renal venous blood samples for the determination of plasma renin activity were collected in ethylenediaminetetraacetate (EDTA), 0.1 ml of a 10% solution per 10 ml of blood, and the blood samples were cooled immediately in an ice bath. The plasma was separated by centrifugation in the cold, and the plasma samples were frozen for temporary storage. Later, the plasma samples were prepared for assay by the method of Schneider *et al.* (9). This consisted of dialyzing 2-ml samples against a phosphate buffer (pH 5.3), and adding NaCl and diisopropylfluorophosphate (DFP). The samples were incubated for 3 hr at 37°. Following inactivation of the enzyme in a boiling water bath, each sample was diluted to 4 ml with a phosphate buffer solution. The samples were stirred and centrifuged and the supernatant solution was frozen until assayed. Samples were assayed by a pressor response in the pentobarbital-anesthetized, pentolinium-blocked rat with synthetic angiotension 11 (Hypertensin, Ciba) as the standard. The results were expressed as nanograms of angiotensin formed per ml of plasma. The rate of renin secretion was calculated by subtracting the arterial plasma concentration of renin from the renal venous renin concentration and multiplying this difference by the renal plasma flow. Renin secretion rates were expressed as nanograms of angiotensin per minute. Plasma and urine concentrations of creatinine were measured by standard methods. Plasma and urine concentrations of sodium and potassium were determined by flame photometry. Hematocrit values were obtained by a microhematocrit method.

Results. A slight increase in renal blood flow occurred immediately after reopening the ureter and persisted for the next hour.

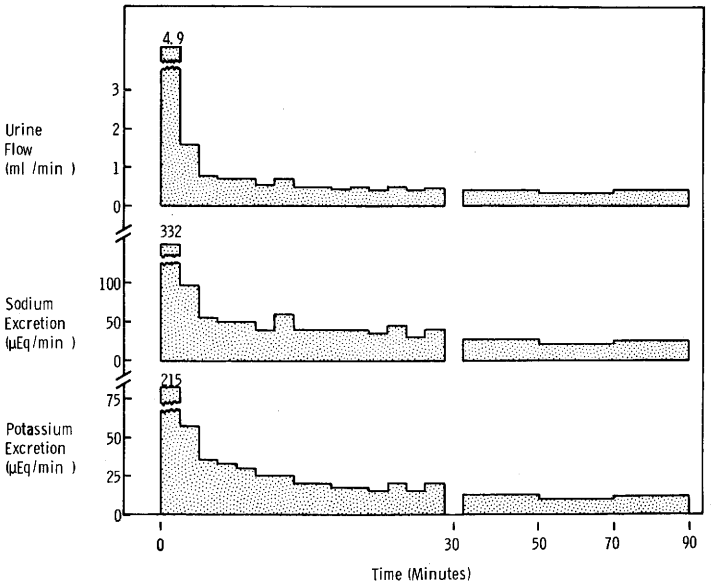

FIG. 2. Mean values for urine flow rate, sodium excretion, and potassium excretion after reopening the ureter ($N = 8$).

Reopening the ureter had no effect on mean arterial blood pressure. These results are presented in Fig. 1. Opening the ureter resulted in a sudden, rapid increase in urine flow which soon declined and stabilized at an average of about 0.4 ml/min within 10 min (Fig. 2). The urinary concentration of sodium ranged from 62 to 83 μEq/ml while that of potassium was between 40 and 50 μEq/ml. The urinary concentrations of these electrolytes were fairly constant after the first 6 min and the rate of electrolyte urinary excretion paralleled the rate of urine flow.

The data on renin secretion for each dog are presented in Table I. The results were variable; renin secretion decreased in three dogs (No. 1, 2, and 3), increased in two dogs (No. 6 and 7), and showed variations but no definite change in two dogs (No. 4 and 5). The control rates of renin secretion were similar to those observed in nonfiltering kidneys in previous studies from this laboratory (8).

The clearance of creatinine was very low and averaged 2.2 ml/min for the three clearance periods for the eight animals after reopening the ureter. These results are summarized in Fig. 3, together with the average clearance of creatinine for nine normal dogs from a previous study (10).

In two dogs a solution of lissamine green dye was injected into the suprarenal aorta at the termination of the experiment. In both instances the dye was observed in superficial renal tubules when the surface of the decapsulated kidney was viewed under a dissecting microscope.

Discussion. An incidental finding is the variable response in renin secretion following reopening the ureter. Other workers have reported that occluding the ureters in dogs with

TABLE I. Renin Secretion in Response to Reopening the Ureter in Dogs with a Nonfiltering Kidney. (Renin secretion given in ng of angiotensin/min.)

		Minutes after reopening the ureter				
Dog	Control	5	10	15	30	90
1	112	173	0	0	—	0
2	127	96	58	32	0	52
3	77	62	75	0	—	—
4	73	55	60	164	83	35
5	32	40	60	49	64	27
6	151	143	234	359	436	332
7	94	116	76	763	1650	782

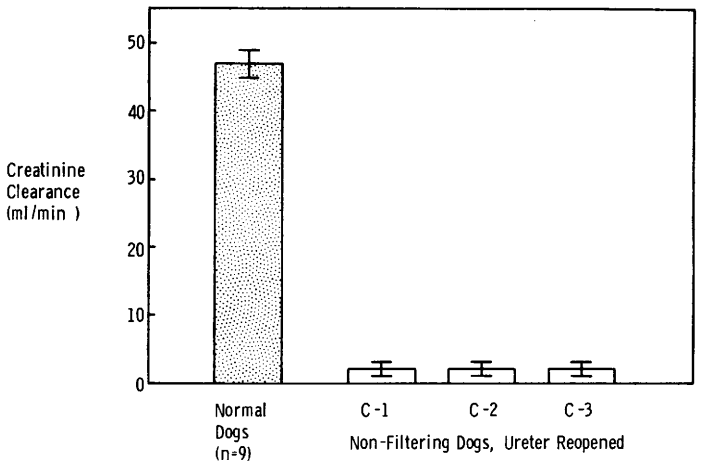

FIG. 3. Mean values ± SEM for creatinine clearance in nine normal, conscious dogs (both kidneys) and for the three clearance periods following reopening the ureter in eight dogs with the nonfiltering renal model.

filtering kidneys consistently resulted in an increase in renin secretion (11); this was explained on the basis of alterations in the sodium load or concentration at the macula densa. However, renal interstitial pressure is increased during ureteral occlusion and the decrease in renal interstitial pressure which occurs secondary to opening the ureter would produce an alteration in the transmural pressure gradient and, consequently, might influence the renal baroreceptor mechanism. Previous studies have suggested that the nonfiltering kidney model has a functional baroreceptor mechanism for the control of renin secretion (8, 12, 13); consequently, it might be expected that reopening the ureter would elicit a baroreceptor signal to inhibit renin release. However, this result was observed in only three of eight dogs. Although Blaine (unpublished observations) found that the macula densa appeared normal in stained histologic sections of nonfiltering kidneys, it is possible that varying degrees of damage occurred and this might account for the varied response of renin secretion following reopening the ureter.

Following reopening the ureter a very low value for creatinine clearance was a consistent finding in the present study. In two dogs lissamine green dye appeared in the superficial nephrons following reopening the ureter. Thus, lissamine dye can be detected in the tubules even when the filtration rate is very low.

In previous experiments with the nonfiltering kidney model we have routinely checked for nonfiltration at the conclusion of each experiment by decapsulating the kidney and injecting a solution of lissamine green dye into the suprarenal aorta; failure to observe the appearance of the dye in the superficial tubules when viewed under a dissecting microscope has been used as evidence of nonfiltration. Employing this method, our degree of success in producing a nonfiltering kidney has been about 95%. This test for filtration by lissamine green injections, however, only gives information as to the nonfiltration of the superficial nephrons, and thus filtration could still be occurring in the deeper nephrons. In the present study the finding of very low values for creatinine clearance after reopening the ureter shows that in the nonfiltering kidney model very few, if any, nephrons are filtering, and strongly suggests that this kidney model is, indeed, nonfiltering.

Summary. The renal clearance of creatinine following reopening of the ureter in eight dogs with a nonfiltering kidney was found to be very low and to average 2.2 ml/min. The secretion of renin was variable. These studies

provided evidence that in the nonfiltering kidney model in dogs there is no glomerular filtration occurring even in the deeper nephrons.

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