Renal Denervation on Free Water Reabsorption in Dog and Rabbit (36054)

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Trueta and associates (1) and subsequently Pomeranz *et al.* (2) postulated that activation of the renal nerves shunted blood flow from the cortical to the medullary region. Reubi (3) suggested the reverse, *vis.*, pharmacologic blockade of the adrenergic nerve supply to the kidney, redistributed blood flow toward the cortex in the anesthetized animal. The possibility that such redistribution toward juxtamedullary or cortical nephrons might be responsible for alterations in sodium excretion during neural activation or ablation has been considered (2, 4–7). In dogs, all nephrons have loops of Henle which enter the medulla; whereas in rabbits, approximately 50% of the cortical nephrons have short loops or none at all (8). Consequently, in rabbits, any regional redistribution of glomerular filtration and blood flow from juxtamedullary to cortical nephrons should limit delivery of sodium to the medullary concentrating site. Sodium turnover rate and, therefore, free water reabsorption ($T^c_{H_2O}$) might be expected to decrease in rabbits but not in dogs. The present studies demonstrate that variations of $T^c_{H_2O}$ and renal tissue electrolyte are consistent with redistribution resulting from alterations in neural activity to the kidneys.

Methods. Details of procedures and methods used in these studies have been previously reported (6, 7). All animals were anesthetized with pentobarbital, 30 mg/kg body weight, and surgically prepared with bilateral ureteral cannulation through an abdominal incision and exposure of the left and right kidneys through separate flank incisions. A mild diuresis was induced by intravenous infusion of a solution containing 0.4% NaCl and 4% mannitol at 0.7 ml/min in rabbits and 4.5 ml/min in dogs.

In a control group of sham operated rabbits with innervation to both kidneys intact, urine collection periods (10 to 15 min duration) were taken 30 min apart, the first commencing approximately 60 min after completion of surgery. Immediately after termination of the second collection period, the kidneys were removed from 5 animals. A second group of animals was similar to the control group except that the left kidney was denervated by nerve section and the first urine collection period commenced 60 to 90 min after completion of denervation. Kidneys were removed from 15 rabbits and 6 dogs.

In order to eliminate possible artifacts arising from loss of blood or other fluids from the cut renal pedicle, the artery, vein, and ureter were simultaneously occluded with a thread prior to removal of the kidney. Both kidneys were removed, right side first, within a span of 15 sec and placed immediately in an acetone–Dry Ice mixture to freeze. Cross-sectional cuts near the middle of the frozen kidney were made and samples (300 to 800 mg) of inner and outer medulla and cortex were obtained. The samples were desiccated under vacuum. The dry tissue was dissolved completely in concentrated nitric acid which was then neutralized with NH_4OH and appropriately diluted for analysis of Na and K on an internal standard flame photometer.

Creatinine clearance was estimated as previously (7) and free water reabsorption ($T^c_{H_2O}$) was calculated as osmolal clearance (C_{osm}) less urine flow. Details of the similar precedures used in dog experiments have also been reported (6).

Results. Denervation on free water reabsorption, $T^c_{H_2O}$. In all control experiments done on rabbits, glomerular filtration rate (GFR) in the left kidney was normally within ±30% of that in the right. Consequently, results from denervation experiments have

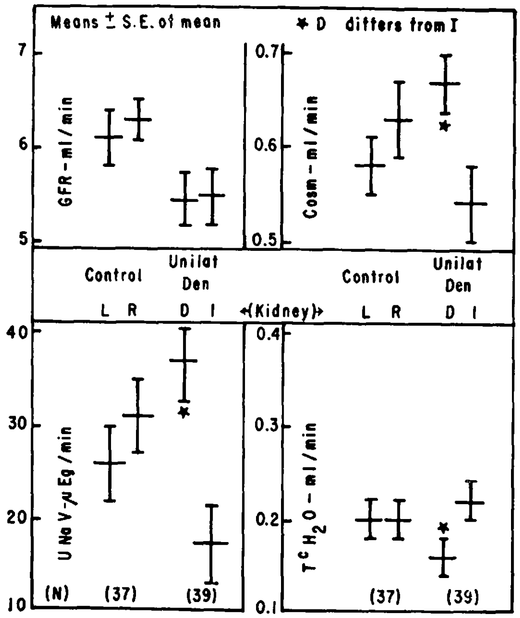

FIG. 1. Comparison of left and right and denervated (D) and intact (I) kidney functions in control and denervation experiments in rabbits: (N) = number of experiments.

been included only when GFR in the denervated (D) kidney was within ±30% of that in the innervated (I). Results from control or denervation experiments were excluded if urine from one or both kidneys was hypotonic. Under these conditions, GFR was equal in D and I kidneys, $T^c_{H_2O}$ was significantly less on the denervated side, and C_{osm} and sodium chloride excretion were greater (Fig. 1). The increased sodium excretion was related to increased sodium concentration in urine as well as to increased urine flow and could account for the increase in C_{osm}. (It should be noted that the difference in $T^c_{H_2O}$ between D and I kidneys was even greater if results of all denervation experiments were included.)

Ratios of D/I kidney function have been used to make comparisons between rabbits and dogs since absolute values were markedly different (Fig. 2). The ratios for GFR and C_{osm} were kept within the same range for both species; results from 2 experiments on dogs had to be excluded. The mean D/I ratio for $T^c_{H_2O}$ significantly exceeded 1.0 in dogs and was significantly less than 1.0 in rabbits.

Tissue analysis experiments. In both control and renal denervation groups, urine flow and GFR changed by less than 10% during the 30 min interval preceding nephrectomy. Hence, it was assumed that a reasonably steady state had been attained in each of the kidneys. During the collection period immediately before nephrectomy, differences between D and I kidneys for all experiments with tissue analyses were similar to those for the total group shown in Fig. 1, *i.e.*, GFR was the same in D and I kidneys and C_{osm} and $U_{Na}V$ were higher and $T^c_{H_2O}$ lower on the denervated side in rabbits and equal in dogs (Table I).

Results of tissue analyses for all experiments are shown in Table II. Because the time from completion of surgery to nephrectomy was 30 to 60 min shorter in the control group and the state of hydration of the animals may contribute to the differences among groups, it is appropriate to compare the two kidneys of the same animal.

The mean water content per gram of wet tissue increased significantly along the corti-

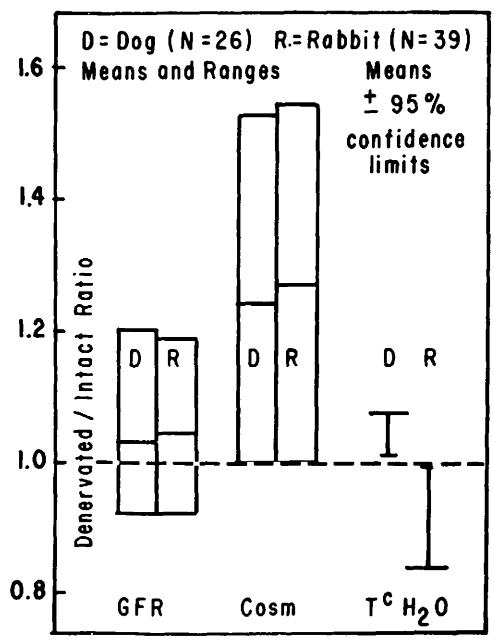

FIG. 2. Comparison of denervated/intact (D/I) kidney function ratios for dogs and rabbits: The horizontal lines further from the 100% line are the mean values for $T^c_{H_2O}$ ratios.

TABLE I. Renal Functions in the Denervated and Intact Kidneys of Rabbits and Dogs with Tissue Analysis.[a]

	Rabbits ($N = 15$)		Dogs ($N = 6$)	
	I	D	I	D
V	7.5 ± 1.0	12.0 ± 2.0	5.9 ± 0.8	8.6 ± 1.1
C_{osm}	11.1 ± 1.3	14.3 ± 1.5	9.1 ± 1.1	11.7 ± 0.9
$T^c_{H_2O}$	3.6 ± 0.7	2.3 ± 0.7	3.2 ± 0.8	3.8 ± 0.8
C_{Na}	3.2 ± 0.6	7.9 ± 1.5	1.1 ± 0.9	3.9 ± 0.8

[a] N = number of experiments; urine flow (V); C_{osm}; $T^c_{H_2O}$; C_{Na} are in milliliter per 100 ml of GFR.

co-papillary radial axis; and denervation produced no significant change at any level (Table II). Potassium concentration profiles in renal tissue were similarly not altered by denervation. A cortico-papillary sodium concentration gradient was present in all groups and only slightly less steep than that found by Cizek and Nocenti (9) in rabbits not subjected to any form of diuresis. Relative to the contralateral control kidney, denervation had no significant effect on medullary or papillary sodium concentration in dogs or rabbits (Table II). The slightly lower sodium concentration in cortical tissue of the denervated rabbit kidney was not found in the dog.

In the control and denervation rabbit groups, $T^c_{H_2O}$ correlated positively ($p < .05$) with inner medullary sodium concentration for both kidneys. Thus, $T^c_{H_2O}$ was related to inner medullary sodium in these animals but inner medullary sodium was no different in I and D kidneys. The difference between D and I kidneys of a pair was significant for urine sodium concentration, C_{osm}, and $T^c_{H_2O}$ but not for GFR or inner medullary sodium concentration.

Discussion. Renal denervation in anesthetized dogs and rabbits evokes a diuresis secondary to increased sodium chloride excretion and independent of any change in glomerular filtration rate (6, 7). In dogs, dener-

TABLE II. Effect of Denervation on Renal Tissue Na, K, and H_2O in Rabbits and Dogs Undergoing Mild Osmotic Diuresis.

	Control group		Denervation group			
	Rabbits		Rabbits		Dogs	
	Right	Left	I (R)	D (L)	I (R)	D (L)
	Na (μEq/g of wet wt; mean ± SE)					
Cortex	64.3 ± 4.2	64.5 ± 3.6	77.3 ± 2.3	71.1 ± 1.7	77.8 ± 2.4	78.9 ± 4.6
Medulla						
outer	90.3 ± 2.7	91.1 ± 4.4	90.1 ± 2.4	87.8 ± 2.7	123.4 ± 4.9	136.8 ± 10.7
inner	142 ± 6.4	141 ± 8.8	131 ± 4.3	125 ± 7.9	141.4 ± 8.9	161.5 ± 13.8
	K (μEq/g of wet wt; mean ± SE)					
Cortex	56.0 ± 2.6	59.1 ± 2.0	57.0 ± 1.8	55.9 ± 1.6	50 ± 1.4	48 ± 1.5
Medulla						
outer	51.5 ± 2.4	57.2 ± 2.1	49.9 ± 1.4	49.7 ± 1.7	36.7 ± 2.2	34.1 ± 2.1
inner	49.6 ± 3.4	49.7 ± 3.8	44.3 ± 1.6	44.0 ± 2.0	32.7 ± 1.8	31.3 ± 1.7
	Percentage H_2O [(g/100 g of wet wt); mean ± SE]					
Cortex	79.3 ± 0.5	78.8 ± 0.4	79.2 ± 0.5	79.8 ± 0.6	82 ± 0.4	81.7 ± 0.4
Medulla						
outer	83.3 ± 0.4	82.5 ± 0.6	83.6 ± 0.5	83.9 ± 0.5	85.9 ± 0.5	86.5 ± 0.3
inner	86.8 ± 0.4	86.2 ± 0.4	86.2 ± 0.5	87.0 ± 0.5	87.6 ± 0.5	88 ± 0.3

vation increased C_{osm} and this was accompanied by an increase in free water reabsorption similar to that seen in dogs infused with NaCl or mannitol solution (10). In rabbits, the same proportional increase in C_{osm} in the denervated kidney was associated with a significant decrease in $T^c_{H_2O}$. Clearly, there was species difference in the renal response to mild diuresis during denervation and this difference was in some way related to structural and/or functional differences which affected the efficient operation of the medullary countercurrent system in the rabbit.

Usual possible causes (11) for the decreased $T^c_{H_2O}$ found in denervated rabbit kidneys can be reasonably excluded. Equal GFR in denervated and innervated kidneys excludes decreased delivery of sodium to the medullary interstitial pool by virtue of decreased total GFR. Equally high sodium concentrations in medullary tissue of D and I kidneys excludes increased washout on the denervated side resulting from massive osmotic diuresis or increased medullary blood flow (12–16). Decreased permeability of the collecting duct to water in the denervated kidney is unlikely in view of the absence of any such effect in dogs and the observation that norepinephrine probably impairs permeability of the collecting duct to water when antidiuretic hormone is present (17).

Another mechanism for impaired operation of the countercurrent system could be partial exclusion of the system, *i.e.*, by selective reduction of blood flow and filtration rate to juxtamedullary nephrons while simultaneously increasing cortical blood flow and filtration. Such a redistribution of flow would be particularly effective in rabbits if there is a limited loop system from cortical nephrons in this species (8). The decreased juxtamedullary blood flow and filtration rate would reduce delivery of sodium to the medullary interstitial pool but would also decrease rate of its removal. Sodium concentration in the medullary pool would remain unchanged and there would be a slower turnover rate of sodium and water at the urinary concentrating site. In dogs, which do not have so-called "cortical nephrons", redistribution would not limit delivery of sodium to the medulla to the same extent nor removal of sodium and water therefrom.

The conclusion that the nervous system controls distribution of flow within the kidney is consistent with the anatomical distribution of adrenergic vasoconstrictor fibers within the kidney (2, 20). Since these fibers are activated by anesthesia (18, 19), the reverse of the redistribution of blood flow observed during neurogenic stimulation (2) would be expected to occur following denervation in the anesthetized animal.

Summary. Renal functions were evaluated in left and right kidneys of pentobarbital-anesthetized dogs and rabbits using the usual clearance techniques and following acute, surgical denervation of one kidney. In dogs, free water reabsorption was greater on the denervated side; in rabbits it was significantly less. The decreased free water reabsorption in the denervated rabbit kidney could not be attributed to decreased filtration rate, osmolal clearance, or sodium delivery distal to the proximal tubule or to increased medullary blood flow.

The conclusion was that denervation, by relaxing cortical arterioles, partially shunted blood flow and glomerular filtration from medulla to cortex. In the rabbits, but not the dogs, such a redistribution should limit sodium turnover rate in the medulla and decrease free water reabsorption because, in rabbits, some cortical nephrons are said to lack medullary loops of Henle.

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