Chronic Reduction in Renal Mass:

Micropuncture Studies of Response to Volume Expansion and Furosemide

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Chronic nephron loss is compensated by functional adaptations which preserve electrolyte homeostasis. The response to volume expansion and diuretics was tested in dogs. Three phase recollection micropuncture studies were performed to assess the response of the remnant kidney in various stages of renal failure to furosemide administration (10 mg/Kg) and graded volume expansion (3 percent and 10 percent body weight). After the diuretic maneuvers, mean fractional excretion of sodium, potassium and water rose progressively in normal dogs (Stage I), with a greater increase in the remnant kidneys in the presence (Stage II) and absence (Stage III) of the contralateral kidney. Proximal and distal TF/P $_{Inulin}$ ratios were depressed after 3 percent volume expansion. However, proximal TF/P $_{Inulin}$ was not further lowered after 10 percent volume expansion and furosemide administration, while distal TF/P $_{Inulin}$ ratios were progressively depressed. The distal TF/P $_{Inulin}$ ratios in Stage III were significantly lower than in Stage II under analogous conditions. Our results suggest that the adaptive increase in the response of sodium transport by the remnant kidney to the diuretic maneuvers occurs in the loop of Henle, both in the azotemic and the non-azotemic stage. Adaptation of potassium excretion, as revealed by distal micropuncture, took place in the azotemic Stage III. Chronic functional adaptation for electrolyte transport occurs even before azotemia in the distal nephron and includes the proximal tubule with azotemia.

INTRODUCTION

It is well known that the renal transport of sodium and potassium undergoes changes as the functioning renal mass is progressively reduced, so that the homeostasis of these electrolytes is rather well maintained even in the face of greatly reduced nephron population [1,2]. The mechanism of this functional adaptation is not known. It is known, however, that such adaptation can occur either in the presence or absence of azotemia [3,4,5]. The purpose of the present study was to explore proximal and distal tubular function in the dog kidney with reduced renal mass in response to the challenge with graded volume expansion and the loop diuretics, in order to unmask more clearly the changes in segmental tubular reabsorption.

METHODS

Effect of Graded Volume Expansion

Micropuncture studies were performed in 28 mongrel dogs weighing 8–20 kg. The remnant kidney model with its three experimental stages was used. Ten normal dogs without any surgical manipulation before the study were used for Stage I experiments. Stage II experiments were carried out in eleven animals which segmental infarction of one kidney was induced by ligating three-fourths to five-sixths of the

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main branches of the renal artery, while the opposite kidney remained intact. Micropuncture experiments were done two weeks after surgery. Stage III was established in seven animals by removal of the contralateral kidney, two weeks after stage II surgery. An additional week was then allowed for azotemia to develop, with an average serum creatinine of 2.5 mg% and a BUN of 46 mg% prior to the experiments. Animals were prepared for micropuncture experiments in a manner described previously [6]. In each experiment, the late proximal tubule segments were sampled by micropuncture as identified by F.D. and C. dye injections into the renal artery. The distal tubule samples were obtained from accessible segments identified on the renal surface by dye injections.

The experimental protocol consisted of a control phase of hydropenia followed by isotonic Ringer's infusion to 3 percent body weight, with a third phase of isotonic Ringer's infusion to 10 percent body weight. Urinary losses were balanced with equal volumes of Ringer's infusion. The dogs studied were not placed on constant salt intake prior to experiments but were all considered well hydrated at the time of the experiments.

Effect of a Diuretic Agent

Micropuncture studies were performed on 15 Stage II dogs and 11 Stage III dogs. The experimental protocol consisted of a hydropenic phase followed by a second phase of 3 percent extracellular volume expansion and a third phase of furosemide administration (10 mg/kg). The rate of Ringer's infusion was increased during furosemide diuresis to maintain fluid balance.

RESULTS

The Effect of Graded Volume Expansion

The mean clearance data from the first group are tabulated in Table 1. In ten Stage I dogs, having comparable glomerular filtration rate in all three phases, there was a greater diuretic response to 10 percent than to 3 percent expansion with the mean fractional excretion of water and sodium increasing progressively with increased expansion. In seven Stage III dogs the GFR of the remnant kidney averaged 6.9 ml/min and increased progressively to 8.5 and then to 9.3 ml/min during the three phases. After ECV expansion to 3 percent and 10 percent body weight, the mean FE_{Na} rose to 9.9 and 20.4 percent, and mean FE_{water} to 19.2 and 31.8 percent. These values appeared to be higher than the corresponding values in the remnant kidney of Stage II, but the differences were not statistically significant. However, these values were significantly higher than those in Stage I and those of the contralateral kidney in Stage II, indicating an enhanced response of the remnant kidney to graded ECV expansion in both Stage II and Stage III (Table 1).

Micropuncture data from Stage I kidneys and from the remnant kidney in Stages II and III are summarized in Table 2.

The mean proximal TF/P_{Inulin} in hydropenia was 1.67 in Stage I, which is not significantly different from 1.82 obtained in Stage II. However, in Stage III the mean TF/P_{Inulin} was highly significantly reduced to 1.49 which reflects a 10 percent reduction in proximal fluid reabsorption when nephron mass is reduced to the point of azotemia. In each stage, proximal TF/P_{Inulin} ratios were significantly depressed by 3 percent body weight Ringer's infusion to 1.49 in Stage I, 1.41 in Stage II and to 1.23 in Stage III, but no further significant decrease occurred with additional volume expansion to 10 percent body weight. These data indicate that proximal tubular

TABLE 1

Summary of Clearance Data in Remnant Kidney Dogs Following Graded Volume Expansion

Expt. Stage	Kidney	Expt. Phase	GFR (ml/min)	V (ml/min)	FE_{H_2O} (%)	$U_{Na}V$ (ueq/min)	FE_{Na} (%)
I (10 dogs)	Mean	H	32.0 ± 2.1	0.6 ± 0.2	1.8 ± 0.4	83.5 ± 16.4	1.6 ± 0.3
		3% E	31.1 ± 2.2	1.7 ± 0.3*	5.7 ± 0.9*	158.7 ± 35.2*	3.6 ± 0.6*
		10% E	30.2 ± 2.1	3.9 ± 0.4*	12.9 ± 1.0*	411.9 ± 71.0*	9.6 ± 1.3*
II (11 dogs)	CK	H	38.9 ± 3.2	1.0 ± 0.3	2.4 ± 0.7	96.6 ± 22.6	1.6 ± 0.4
		3% E	37.5 ± 2.8	3.0 ± 0.9*	8.7 ± 2.3*	251.6 ± 73.7*	5.0 ± 1.7*
		10% E	33.6 ± 1.1	4.9 ± 1.1*	16.7 ± 2.8*	505.4 ± 136.3*	11.8 ± 2.8*
	RK	H	7.1 ± 0.6‡	0.2 ± 0.03‡	3.1 ± 0.8	18.1 ± 3.7‡	1.9 ± 0.3
		3% E	7.1 ± 0.8‡	0.9 ± 0.2*‡	12.6 ± 2.3*‡	77.6 ± 21.1*‡	7.5 ± 1.6*‡
		10% E	7.9 ± 8*‡	1.9 ± 0.3*‡	24.2 ± 2.5*‡	202.1 ± 46.1*‡	17.3 ± 2.5*‡
III (7 dogs)	RK	H	6.9 ± 1.3	0.6 ± 0.1§	9.0 ± 1.2§	74.5 ± 27.9	5.3 ± 1.0§
		3% E	8.5 ± 1.9*	1.4 ± 0.3*	19.2 ± 2.5*	131.1 ± 36.7*	9.9 ± 1.8*
		10% E	9.3 ± 2.0*	2.5 ± 0.5*	31.8 ± 3.5*	264.9 ± 67.1*	20.4 ± 4.2*

Abbreviations: Expt. stage, experimental stage; Expt. phase, experimental phase; V, urine flow; U_{Na}, urine Na excretion; Fe, fractional excretion; H, hydropenic control phase; 3% E, after expansion of ECF by 3 percent of body weight; 10% E, after expansion of ECF by 10 percent of body weight; CK, control kidney; RK, remnant kidney. Values are mean ± SEM.

*Significantly different from preceding phase.

‡Significantly different from corresponding value in the contralateral kidney.

§Significantly different from corresponding Stage II value of the remnant kidney.

GFR values in Stage I dogs represent only those of the micropunctured kidney.

reabsorption is reduced by volume expansion, the maximal response being attained at an expansion of 3 percent body weight.

In the distal tubule (Table 2), there was no change in baseline distal water reabsorption in hydropenia between Stages I and II with TF/P_{Inulin} values of 3.94 and 4.26, respectively. However, a significant reduction to 2.77 occurred in the azotemic Stage III dogs. In the distal tubules, there was a progressive reduction of TF/P_{Inulin}, and a progressive increase in TF/P_{sodium} with progressive volume

TABLE 2

Summary of Micropuncture Data in Proximal and Distal Tubule

Expt. Stage	Expt. Phase	Proximal Tubule		Distal Tubule	
		TF/P_{In}	TF/P_{Na}	TF/P_{In}	TF/P_{Na}
I (10 dogs)	H	1.67 ± 0.09	1.00 ± 0.03	3.94 ± 0.30	0.19 ± 0.02
	3% E	1.49 ± 0.09*	1.03 ± 0.03	2.93 ± 0.16*	0.26 ± 0.03*
	10% E	1.40 ± 0.10	1.03 ± 0.04	2.41 ± 0.14*	0.34 ± 0.03*
II (11 dogs)	H	1.82 ± 0.11	0.97 ± 0.02	4.26 ± 0.55	0.23 ± 0.04
	3% E	1.41 ± 0.09*	0.99 ± 0.02	2.74 ± 0.21*	0.32 ± 0.04*
	10% E	1.36 ± 0.7	1.00 ± 0.02	2.28 ± 0.22*	0.52 ± 0.06*‡
III (7 dogs)	H	1.49 ± 0.09‡	0.99 ± 0.02	2.77 ± 0.25‡	0.25 ± 0.05
	3% E	1.23 ± 0.06*	0.98 ± 0.03	2.08 ± 0.18*‡	0.30 ± 0.03
	10% E	1.22 ± 0.08	0.97 ± 0.03	1.86 ± 0.10*	0.53 ± 0.03*

Abbreviations as in Table 1.

Values are mean ± SEM.

*Significantly different from preceding phase.

‡Significantly different from corresponding value in preceding stage.

TABLE 3
Summary of Clearance Data in Remnant Kidney Dogs with Diuretic Administration

Experimental Stage	Kidney	Experimental Phase	GFR (ml/min)	FE_{H_2O} (%)	$U_{Na}V$ (ueq/min)	FE_{Na} (%)	$U_K V$ (ueq/min)	FE_K (%)
I	CK	Hydropenia	36 ± 4.5	1.3 ± 0.3	45 ± 13	0.8 ± 0.2	29 ± 2	24 ± 2.0
		3% ECF volume expansion	33 ± 4.6	3.8 ± 0.6*	105 ± 24*	1.9 ± 0.3*	46 ± 8*	37 ± 4.9*
		Furosemide	21 ± 2.7*	29.6 ± 2.4*	804 ± 103*	25.2 ± 2.0*	90 ± 12*	118 ± 8.6*
II (15 dogs)	RK	Hydropenia	7 ± 1.3‡	2.3 ± 0.4‡	12 ± 3	1.3 ± 0.3‡	7 ± 1	28 ± 2.3
		3% ECF volume expansion	7 ± 1.6‡	6.6 ± 1.1*‡	36 ± 7	3.4 ± 0.6*‡	12 ± 3*	42 ± 4.4*
		Furosemide	7 ± 0.8‡	49.5 ± 4.0*‡	487 ± 71*	40.7 ± 5.0*‡	31 ± 4*	121 ± 7.8*
III (11 dogs)	RK	Hydropenia	10 ± 0.8	2.8 ± 0.6	30 ± 9	2.0 ± 0.6	14 ± 2	41 ± 3.5
		3% ECF volume expansion	10 ± 1.3	7.5 ± 1.7*	96 ± 28*	5.8 ± 1.6*	22 ± 6*	58 ± 8.7*
		Furosemide	9 ± 0.9	48 ± 1.4*	576 ± 63*	42.7 ± 1.5*	41 ± 7*	153 ± 11.3*

Abbreviations as in Table 1.
*Significantly different from preceding phase. $p < 0.05$, $p < 0.01$ in reference to the Stage II remnant kidney.
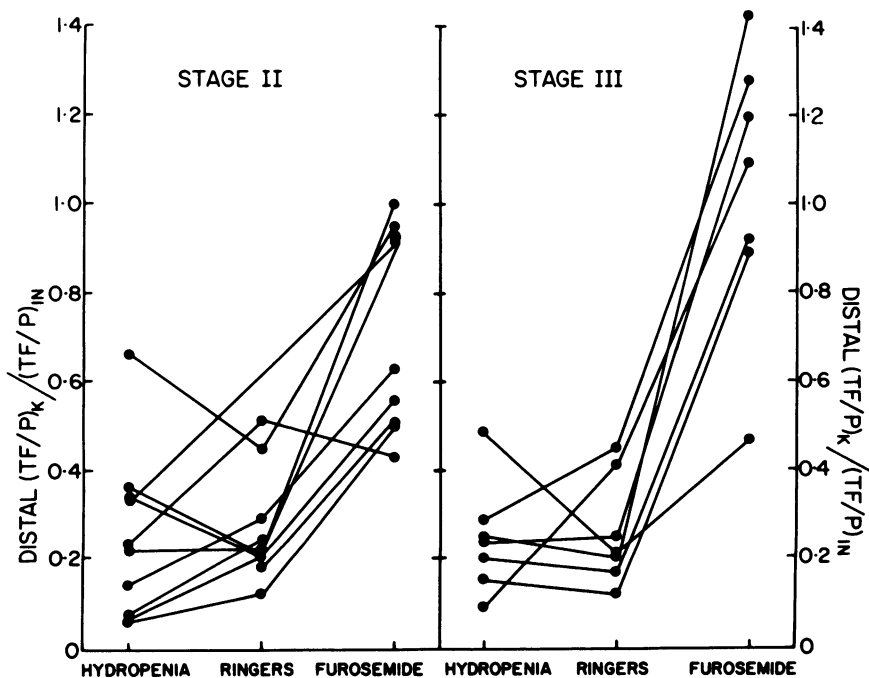$p < 0.01$ in reference to the corresponding contralateral kidney.

FIG. 1. Comparison of fractions of filtered potassium remaining in distal tubule in Stage II and III remnant kidneys before and after diuretic manoeuvres. Points represent mean values for each experiment, and lines connect those of 3 phases in same experiment. Reproduced by permission from [7].

expansion. The mean distal TF/P_{sodium} ratios in Stage I in these three phases were 0.19, 0.26 and 0.34, respectively. In both Stage II and Stage III, slightly higher TF/P_{sodium} values of 0.25 and 0.23 occurred in hydropenia, with significantly greater elevation to 0.32 and 0.30 with 3 percent expansion and values of 0.52 and 0.53, respectively, with 10 percent expansion. The development of higher TF/P_{sodium} values during volume expansion in Stage II suggests that distal tubular sodium reabsorption is reduced in the remnant kidney prior to azotemia, and that this reduced rate is maintained when azotemia ensues.

The Effect of Furosemide

Clearance data from furosemide experiments in Stages II and III are summarized in Table 3.

In Stage III, the values for fractional potassium excretion at each of the experimental phases were significantly higher with values of 41 and 58 percent in the controls and 3 percent expansion and values as high as 153 percent of the filtered load following furosemide. Thus, the remnant kidney has an increased ability to excrete potassium in the Stage III situation.

In Fig. 1, the effects of furosemide on distal tubule potassium transport in Stages II and III are shown. Following Ringer's infusion, the fraction of filtered potassium found at the distal site remained unchanged, in both Stage II and III animals. However, after furosemide, the non-reabsorbed fraction of potassium rose dramatically in both groups, and the change in Stage III was far more prominent than in Stage II. These data indicate that net potassium reabsorption in the distal tubule was inhibited to a greater degree in Stage III by furosemide than in Stage II.

DISCUSSION

Alterations in the renal transport of sodium and potassium have been known to occur in the diseased human kidney [1,3]. The manner by which such changes in renal handling of sodium and potassium occur has not been elucidated. Earlier studies suggest that reduction in nephron mass rather than an intrinsic adaptive change in function is responsible for the adaptation [3]. This is supported by the observation that functional patterns of the diseased kidney in unilateral disease models in the dog are relatively normal. It is only after removal of the contralateral normal kidney and development of uremia that the observed adaptation takes place.

In our studies, unilateral remnant kidney models were used to examine the functional adaptation of sodium and potassium excretion with and without contralateral nephrectomy. Our results suggest that the baseline fractional proximal reabsorption is reduced in Stage III dogs. We also noted that ECF expansion beyond 3 percent body weight in Stage I, II or III dogs did not result in progressive further reduction in fractional proximal tubular water or sodium reabsorption. The greater natriuresis observed after 10 percent ECF expansion in all stages was largely due to an additional effect on reabsorption between the proximal and the distal nephron. In azotemic animals a lower baseline reabsorption contributed to a greater loop delivery. Our results suggest that an additional reduction in fractional sodium and water reabsorption from Henle's loops occurs when the ECF volume expansion increases from 3 percent to 10 percent of body weight. The "distal tubular" response of the remnant kidney in both Stages II and III was greater than in Stage I, i.e., both before and after uremia. These data indicate that although the enhanced sodium excretion per nephron in chronic renal failure may be related to uremia, its exaggerated response to ECF expansion is due, at least in part, to certain as yet unidentified intrarenal changes induced by reduction in functioning renal mass. One could visualize an early depression of reabsorption from the loop as a primary consequence of chronically reduced nephron mass. With the advent of uremia, an additional increase of proximal delivery would further enhance the ability to excrete sodium. The role of the collecting ducts in various stages of reduction of renal mass still needs to be investigated.

The functional changes in potassium transport by the remnant kidney differed from those of sodium transport. The fractional excretion of potassium by the remnant kidney in Stage II was only slightly higher than that by the contralateral kidney during hydropenia, while after infusion of Ringer's solution, and following furosemide administration, no difference was observed between the two kidneys. Fractional sodium excretion, on the other hand, was consistently greater in the Stage II remnant kidney than in its contralateral partner. Fractional potassium excretion in Stage III was also significantly greater than in Stages I and II during hydropenia and after furosemide. An increased potassium secretion into the later distal tubule and/or collecting duct was presumably the major change responsible for the increased fractional potassium excretion. The presence of an exaggerated sodium response to furosemide by the remnant kidney in both Stages II and III, but the absence of a greater potassium response in Stage II and its exclusive occurrence in Stage III suggest that independent factors may be responsible for these adaptations when functioning renal mass is reduced.

REFERENCES

1. Schultze RG, Taggart DD, Shapiro H, Pennell JP, Cagler S, Bricker NS: On the adaptation in potassium excretion associated with nephron reduction in the dog. J Clin Invest 50:1061–1068, 1971

2. Slatopolsky E, Elkan IO, Weerts C, Bricker NS: Studies on the characteristics of the control system governing sodium excretion in uremic man. J Clin Invest 47:521–530, 1968

3. Bricker NS, Klahr S, Lubowitz H, Rieselback RE: Renal function in chronic renal disease. Medicine 44:263–288, 1965

4. Gutmann FD, Rieselbach RE: Disproportionate inhibition of sodium reabsorption in the unilaterally diseased kidney of dog and man after an acute saline load. J Clin Invest 50:422–431, 1971

5. Gutmann FD, Rieselbach RE: Altered effect of furosemide upon the unilateral experimentally diseased dog kidney. J Lab Clin Med 77:14–22, 1971

6. Dirks JH, Cirksena WJ, Berliner RW: The effect of saline infusion on sodium reabsorption by the proximal tubule of the dog. J Clin Invest 44:1160–1170, 1965

7. Wen SF, Wong NLM, Evanson RL, Lockhart EA, Dirks JH: Electrolyte transport in remnant kidney of the dog: effect of furosemide. Amer J Physiology 230:1231–1238, 1976

8. Bank N, Aynedjian HS: A micropuncture study of potassium excretion by the remnant kidney. J Clin Invest 52:1480–1490, 1973