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Renin and Distal Tubule Na During Stop Flow in Dogs (38620)

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Several possible mechanisms for the control of renin secretion by the kidney were described by Vander (1). Many experimental observations were consistent with either or both the baroreceptor and the macula densa theories. According to the former, some renal hemodynamic parameter (afferent arteriolar stretch or transmural pressure) is the controlling stimulus. According to the latter, some tubular fluid parameter in the region of macula densa cells controls renin secretion. Macula densa Na concentration and load have been proposed as controlling stimuli (1–5). Although recent research utilizing a nonfiltering kidney model provides support for the baroreceptor theory (6, 7), support for a specific macula densa stimulus is largely inferential. The difficulty of measuring tubular fluid flow and composition while simultaneously measuring renin secretion explains the lack of more direct support.

In the experiments described below, tubular fluid composition in anesthetized dogs was estimated using the stop-flow technique (8–10). Mannitol in NaCl was infused during two stop-flow periods in control dogs. In experimental dogs, mannitol in NaCl was given during the first, and mannitol in Na_2SO_4 during the second stop-flow period. This was expected to produce an increase in the Na concentration of early distal tubule fluid. Arterial (A) and renal venous (RV) plasma renin activities were measured. Changes in renin secretion rate were inferred from changes in RV-A renin.

Methods. Twelve dogs of either sex (10–28 kg body wt) fasted overnight and anesthetized with sodium pentobarbital (30 mg/kg body wt, iv) were used. Incisions were made to expose a femoral artery and vein, a jugular vein, and both ureters. All were catheterized with polyethylene tubing. The femoral arterial catheter was connected to a pressure transducer for monitoring mean arterial blood pressure. Fluids were administered via one venous catheter; the tip of the other was manipulated into the left renal vein for blood sampling (11). Priming doses of inulin and para-aminohippuric acid (PAH) were given and plasma concentrations of both substances were maintained by constant iv administration.

After the prime, all 12 dogs received a constant iv infusion of 10% mannitol in 0.15 *M* NaCl at 0.5 ml/min/kg body wt. After a 30-min equilibration, a sequence of clearance, stop flow, and clearance periods was performed. These periods were of 10-min duration, and each began 10–15 min after completion of the previous period. In five of the dogs (group 1) the iv fluid administration was continued (composition and rate unchanged) and 30 min later, the sequence of clearance, stop flow, and clearance was repeated. In seven dogs (group 2) 0.1 *M* Na_2SO_4 replaced 0.15 *M* NaCl in the infusate, and 30 min later the sequence of clearance, stop flow, and clearance was repeated.

Arterial and renal venous blood samples were drawn into lightly heparinized, ice-cold, plastic syringes at the midpoints of clearance periods and just before release of ureteral occlusion of stop-flow periods. Blood samples were immediately centrifuged at 4°C and duplicate aliquots of each were pipetted into plastic test tubes. EDTA was added (2 mg/ml plasma) and the tubes were frozen. About 25 serial stop-flow samples were collected upon release of ureteral occlusion. Sample volumes were determined by weighing (0.5–1.0 ml range).

Renin was determined using the Renin Activity Radioimmunoassay Kit of Schwarz/Mann (Orangeburg, NY 10962). The plasma samples were thawed, 8-hydroxyquinoline

TABLE I. TYPICAL EXPERIMENT, GROUP 2 DOG.

	10% Mannitol + 0.25 *M* NaCl			10% Mannitol + 0.10 *M* Na_2SO_4		
	C_1	SF_1	C_2	C_3	SF_2	C_4
Mean arterial blood pressure (mmHg)	140		135	125		125
Plasma Na (m*M*)	137		143	142		146
Inulin clearance (ml/min)	23		24	23		22
PAH clearance (ml/min)	128		138	137		139
Urine flow rate (ml/min)	2.8		3.9	4.9		5.2
Na excretion rate (μM/min)	45		88	241		340
RV-A renin (ng A-I/ml/hr)[a]	9.4	23.9	2.2	0.4	6.4	1.0
(Minimum stop flow/plasma) Na[b]		0.07			0.20	

[a] Renal venous minus arterial plasma renin activity, ng Angiotensin I/ml plasma/hr of incubation.
[b] Minimum Na concentration in stop-flow pattern factored by plasma Na. C and SF represent clearance and stop-flow periods of 10-min duration, respectively. Kidney data are unilateral (left) only.

sulfate and dimercaprol were added, and incubated at 37°C for 2 hr. Duplicate aliquots were withdrawn from each at the end of 1 and 2 hr of incubation and the angiotensin I was determined by radioimmunoassay. Renin activity is expressed in ng A-I/ml/hr (ng angiotensin I/ml plasma/hr of incubation).

Potassium (K) and sodium (Na) were determined by flame photometry. Inulin in urine, stop-flow samples, and plasma filtrates was determined using the method of Harrison (12). PAH in the same samples was determined by the method of Smith *et al.* (13).

Results. Data from a typical dog of group 2 are presented in Table I. Plasma Na concentration, urine flow, and Na excretion increased progressively, especially after replacement of NaCl by Na_2SO_4 in the infusate. Ureteral occlusion increased RV-A renin, from 5.8 ng A-I/ml/hr (average of clearance periods 1 and 2) to 23.9 ng A-I/ml/hr during the first stop flow, and from 0.7 ng A-I/ml/hr (average of clearance periods 3 and 4) to 6.4 ng A-I/ml/hr during the second stop flow. As indicated by the ratio (RV-A renin, stop flow 2)/(RV-A renin, stop flow 1) replacing NaCl by Na_2SO_4 in the infusate attenuated the renin response to ureteral occlusion.

The stop-flow pattern of the same dog is presented in Fig. 1. Proximal and distal fluids are identified by maxima in PAH and

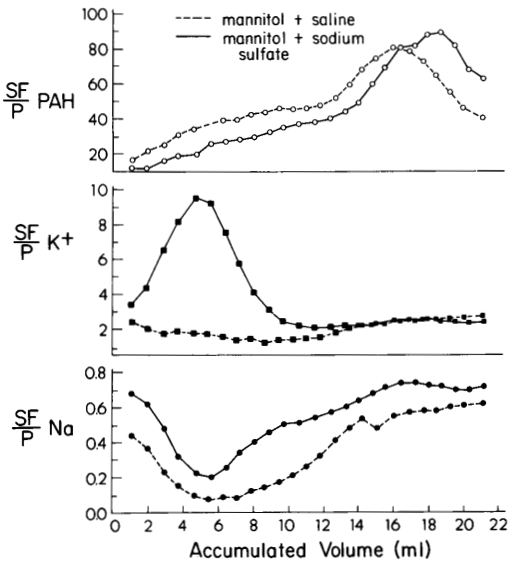

FIG. 1. Stop-flow pattern, group 2 dog. Ordinate: concentration ratio, stop-flow sample/plasma. Abscissa: accumulated volume of serially collected samples. Distal tubular fluid is identifiable by the potassium (K) maximum and the sodium (Na) minimum; proximal tubular fluid by the para-aminohippuric acid (PAH) maximum.

K concentrations, respectively (8–10). The K maximum and Na minimum coincided at about 5.5 ml accumulated volume. At that point, the Na concentration ratio (stop-flow sample/plasma) was 0.07 during the first stop flow and increased to 0.20 during the second. Thus, the increased tubular fluid Na concentration was associated with

TABLE II. CLEARANCE DATA.[a]

	Group 1			Group 2		
	$(C_1 + C_2)/2$	P	$(C_3 + C_4)/2$	$(C_1 + C2)/2$	P	$(C_3 + C_4)/2$
Mean arterial blood pressure (mmHg)	122±4 (10)	NS	129±6 (10)	137±6 (14)	NS	133±5 (14)
Plasma Na (mM)	137±1 (10)	NS	138±1 (10)	141±1 (14)	<0.05	144±1 (13)
Inulin clearance (ml/min)	37±3 (10)	NS	33±3 (10)	25±1 (14)	NS	25±2 (14)
PAH clearance (ml/min)	132±16 (10)	<0.05	109±13 (10)	120±5 (14)	NS	123±6 (13)
Urine flow rate (ml/min)	5.6±0.6 (10)	<0.05	6.7±0.7 (10)	4.6±0.4 (14)	<0.02	6.4±0.6 (14)
Na excretion rate (μM/min)	310±53 (10)	NS	346±54 (10)	246±44 (14)	<0.01	508±72 (14)

[a] C and SF represent clearance and stop flow periods of 10-min duration, respectively. Group 1 dogs received 10% mannitol in 0.15 M NaCl throughout; group 2 dogs received 10% mannitol in 0.15 M NaCl during C_1, SF_1, and C_2 and 10% mannitol in 0.1 M Na_2SO_4 during C_3, SF_2, and C_4. Kidney data are unilateral (left) only. Values in table are means ± SEM with the number of observations in parentheses. Paired t test was used for statistical analysis. NS = $P > 0.05$.

TABLE III. RENIN AND STOP-FLOW NA.

RV-A renin[a]			
10% Mannitol + 0.15 M NaCl		10% Mannitol + 0.1 M Na_2SO_4	
Clearance	Stop flow	Clearance	Stop flow
1.7 ± 0.4 (32)	20.3 ± 5.4 (17)	2.4 ± 0.8 (12)	14.4 ± 5.1 (7)
Stop flow RV-A renin, 2/1[b]		Minimum stop-flow Na/plasma Na, 2/1[c]	
Group 1	Group 2	Group 1	Group 2
1.0 ± 0.2 (5)	0.5 ± 0.1 (7)	1.22 ± 0.32 (5)	2.71 ± 0.29 (7)

[a] RV-A renin, renal venous minus arterial plasma renin activity, ng angiotensin I/ml of plasma/hr of incubation.

[b] (RV-A renin during stop flow 2)/(RV-A renin during stop flow 1).

[c] (Minimum Na concentration in stop flow 2/ plasma Na concentration)/(minimum Na concentration in stop flow 1/ plasma Na concentration). Group 1 dogs received 10% mannitol in 0.15 M NaCl during all clearance and stop-flow periods; Group 2 received 10% mannitol in 0.15 M NaCl during clearances 1 and 2 and stop-flow 1, then during clearances 3 and 4 and stop-flow 2, they received 10% mannitol in 0.1 M Na_2SO_4. Values are means ± SEM with number of observations in parentheses.

a decreased effect of ureteral occlusion on RV-A renin.

The clearance data of all dogs are presented in Table II as averages: (clearances 1 + 2)/2 and (clearances 3 + 4)/2. In both groups there were fluctuations in mean arterial blood pressure, inulin clearance, and PAH clearance, but the only statistically significant change ($P < 0.05$) in these parameters was a small decrease in PAH clearance of group 1. Plasma Na, urine flow rate, and Na excretion rate all increased significantly in group 2 but of these, only urine flow rate increased in group 1.

The renin and stop flow Na data of all dogs are presented in Table III as averages. RV-A renin averaged 1.7 ± 0.4 ng A-I/ml/hr during all clearance periods when mannitol in NaCl was infused (clearances 1–4 in group 1, clearances 1 and 2 in group 2). When mannitol and Na_2SO_4 were infused (clearances 3 and 4 of group 2) RV-A renin averaged 2.4 ± 0.8 ng A-I/ml/hr. The average paired differences, (clearances 3 + 4)/2 minus (clearances 1 + 2)/2 were not significantly different from zero ($P > 0.05$) in either group of dogs, indicating that RV-A renin did not show any tendency to change over time or as a result of replace-

ment of NaCl by Na_2SO_4 in the infusate. Table III also shows that ureteral occlusion during stop flow increased RV-A renin above its value during clearance periods. The ratios (RV-A renin, stop flow 2)/(RV-A renin, stop flow 1) were calculated for the two groups of dogs. In group 1, the ratio was 1.0 ± 0.2 indicating that ureteral occlusion reproducibly increased RV-A renin. In contrast, the ratio was 0.5 ± 0.1 for group 2 indicating that replacement of NaCl by Na_2SO_4 in the infusate reduced by 50% the effect of ureteral occlusion on RV-A renin. The ratios for groups 1 and 2 differed significantly ($P < 0.025$).

The stop-flow patterns of the individual dogs within each of the two groups were nearly identical. In group 1, the second pattern was virtually superimposable on the first. In group 2, samples distal to the PAH maximum always had higher Na during Na_2SO_4 administration than during NaCl. The stop-flow sample in which Na reached its minimum was determined, then the ratios were calculated: (minimum stop-flow Na/plasma Na, stop flow 2)/(minimum stop flow Na/plasma Na, stop flow 1). For group 1 dogs, this ratio was 1.22 ± 0.32 (not significantly different from 1.0) indicating relative stability of tubular fluid Na during continued administration of mannitol and NaCl. For group 2, the ratio was 2.7 ± 0.3 (different from group 1, $P < 0.005$) indicating that replacement of NaCl by Na_2SO_4 resulted in higher tubular fluid Na concentration.

In summary, reproducible stop-flow Na concentrations were associated with reproducible stimulation of RV-A renin by ureteral occlusion in group 1 dogs, whereas increased stop flow Na concentrations were associated with decreased RV-A renin upon ureteral occlusion in group 2 dogs.

Discussion. These experiments demonstrate that RV-A renin increases in response to ureteral occlusion. Changes in RV-A renin reflect changes in renin secretion rate and/or changes in renal plasma flow. Although changes in renal plasma flow in the present experiments cannot be excluded with certainty, many investigators have reported that renal plasma flow does not decrease during ureteral occlusion after diuretic administration (14–16). Moreover, unusually large decreases, on the order of 5- to 10-fold reductions, would be required to account for the observed increases in RV-A renin. Similarly, within group 2 animals, the 50% drop in RV-A renin during the second stop flow probably reflects a large reduction in renin secretion rate rather than a doubling of renal plasma flow.

Both the baroreceptor and the macula densa theories can explain the increased RV-A renin associated with ureteral occlusion that we and others have observed (1, 5, 17). According to the former, renin secretion is stimulated as a result of decreased transmural pressure and stretch of the afferent arteriole. On the other hand, tubular fluid flow and composition are certainly altered by ureteral occlusion, and these are involved in controlling renin secretion accordng to the macula densa theory.

More difficult to reconcile with the baroreceptor theory is the attenuated renin response to ureteral occlusion in group 2 dogs. Since mean arterial pressure remained unchanged between the two stop-flow periods, the reduced RV-A renin could be explained, by the baroreceptor theory, if interstitial pressure was less during stop flow 2 than during stop flow 1. This cannot be entirely ruled out, but a further increase in interstitial pressure is more probable in view of the increased diuresis after beginning the Na_2SO_4 administration.

On the other hand, these observations are consistent with the macula densa theory. Every stop-flow sample distal to the PAH secretory site (thus, tubular fluid in the region of macula densa cells) had a higher Na concentration during the second stop flow, when RV-A renin was reduced.

Our experimental design does not allow discrimination between Na concentration and Na load as the controlling stimulus. To the extent that replacement filtration occurs during stop flow (8), tubular fluid flow rate is nonzero. Assuming no difference in replacement filtration between the two diuretic conditions, load would parallel concentration; both would increase upon

replacement of NaCl by Na_2SO_4. Thus, our results support the hypothesis that renin secretion and macula densa Na concentration and/or load are inversely related (1–3, 5, 18) rather than directly related as has been proposed by others (4, 14, 19).

A discrepancy is apparent. Na load and concentration during clearance periods probably increased in group 2 dogs, yet there was no detectable decrease in RV-A renin. Possibly error and variability inherent in renin determinations prevented detection of a small but real change. Others have noted that in order to detect inhibition of renin secretion, conditions must be used in which secretion is initially high, due to aortic clamping, acute sodium depletion, or chronic sodium deprivation (5, 20, 22). However, alternative explanations cannot be excluded as renin secretion is influenced by many factors (1).

Summary. Using radioimmunoassay techniques, arterial (A) and renal venous (RV) plasma renin activities were measured in sodium pentobarbital-anesthetized dogs during clearance and stop-flow periods. Changes in RV-A renin were used to estimate changes in renin secretion. RV-A renin increased during stop flow from its average value during clearance periods, whether 10% mannitol in 0.15 *M* NaCl or 10% mannitol in 0.1 *M* Na_2SO_4 was being administered intravenously. However, RV-A renin during mannitol + NaCl stop-flow periods was significantly greater than during mannitol + Na_2SO_4 stop-flow periods. Distal tubular fluid Na concentrations as indicated by stop-flow analysis were higher during the latter. These observations suggest that a macula densa stimulus, Na concentration, and/or load, may control renin secretion during ureteral occlusion.

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