

Effect of Medullary Tonicity on Urinary Sodium Excretion in the Rat

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ABSTRACT In previous reports from this laboratory we have suggested that a reduction in medullary tonicity decreases the thin ascending loop of Henle sodium reabsorption and is in part responsible for the magnitude for the natriuresis accompanying 10% body weight Ringer loading. According to this postulate, one would expect that the medullary washout associated with water diuresis would also result in a natriuresis, but this does not occur. It is possible, however, that increased delivery from the proximal tubule is necessary to demonstrate an effect of medullary tonicity on urinary sodium excretion. Micropuncture studies were designed to test that possibility by increasing distal delivery by 2% Ringer loading in animals with and without reduced medullary tonicity. In an initial series of experiments the α -adrenergic agonist clonidine was used to induce a water diuresis. When given alone, this agent caused a marked decrease in urine osmolality and an increase in urine flow rate but had no effect on proximal reabsorption in either superficial or juxtamedullary nephrons, and did not alter urinary sodium excretion. Volume expansion with 2% body weight Ringer solution resulted in a significant fall in proximal reabsorption and a trivial increment in sodium excretion. When this same degree of volume expansion was conferred on animals undergoing a water diuresis, a marked increase in absolute and fractional sodium excretion occurred. In a second group of studies medullary tonicity was reduced in the left kidney only by removal of the left ureter 1 h before micropuncture. When these animals were infused with 2% body weight Ringer solution, proximal reabsorption was decreased in juxtamedullary nephrons, and a marked increase in sodium excretion was observed only from the left kidney. Finally, the effect of water

diuresis on fractional sodium delivery to the early and late distal tubule of superficial nephrons during 2% Ringer loading was evaluated. Delivery to both of these sites was comparable after 2% Ringer loading alone and during 2% Ringer loading plus water diuresis.

From these data, we conclude that medullary tonicity does influence renal sodium handling but that this effect is manifest in the final urine only under conditions in which proximal reabsorption is decreased. The data also suggest that this effect is limited to juxtamedullary nephrons and is probably localized to the thin ascending limb of the loop of Henle.

INTRODUCTION

The factors responsible for the kidney's ability to excrete an acutely administered saline load remain incompletely understood despite several decades of research and considerable insight into the factors controlling proximal reabsorption. Numerous studies have demonstrated that alterations in tubular sodium reabsorption beyond the proximal tubule are operative in the natriuretic response to a salt load (1-4). Studies by Brenner and Berliner (1) and Knight and Weinman (4) have clearly shown that in the rat a mild degree of saline loading (<4% body weight) is associated with a marked and significant decrease in proximal reabsorption in superficial nephrons but very little or no increment in urinary sodium excretion. Assuming that juxtamedullary nephrons respond similarly to this degree of volume expansion, these findings clearly indicate that more distal nephron segments must in some way participate in the natriuretic response to more massive volume expansion.

In 1951, Ladd (5) reported that prior water ingestion enhanced the ability of normal individuals to excrete an acutely administered saline load. In 1964, Earley and Friedler (6) suggested that medullary tonicity influenced the loop of Henle sodium reabsorption, and, later, Humphreys et al. (7) presented additional data

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supporting this contention. Our studies (8) and those of others (9) have indicated that 10% body weight Ringer loading preferentially inhibits sodium reabsorption in juxtamedullary nephrons. More recently, we (10) suggested that this phenomenon was at least partially due to washout of the hypertonic medullary interstitium. Nevertheless, direct support for an important role of medullary tonicity in the control of urinary sodium excretion has been lacking. Further, the absence of a natriuretic response to water diuresis casts considerable doubt on the validity of this theory. It is possible, however, that enhanced delivery from the proximal tubule is necessary to demonstrate an effect of medullary tonicity on urinary sodium excretion. The present micropuncture studies were therefore undertaken to examine a possible interaction between proximal reabsorption and medullary tonicity in the determination of urinary sodium excretion. To this end, proximal reabsorption was decreased by mild volume expansion, and segmental sodium delivery was determined in animals with and without reduced medullary tonicity.

METHODS

Studies were performed on male and female Munich-Wistar rats weighing 150–220 g. All rats were fed standard rat chow until the morning of the study, when they were anesthetized with Inactin (Promonta, Hamburg, W. Germany), 100 mg/kg, and placed on a thermoregulated heating board. Polyethylene catheters were inserted into the jugular veins for infusion of the various solutions, and another catheter was placed in the femoral artery for monitoring blood pressure and blood withdrawal. A tracheostomy was performed, and a PE-50 catheter was inserted in the bladder. A small left subcostal incision was made, and the left kidney was gently separated from the adrenal gland and the surrounding perirenal fat. The kidney was then placed in a Plexiglas cup, bathed in mineral oil warmed to 37°C, and illuminated with a fiberoptic light. In some studies the extrarenal papilla was exposed at this point, whereas in others this procedure immediately preceded the obtaining of micropuncture samples (see below). Late proximal tubular transit time was then determined with a 10- μ l injection of 10% lissamine green. Animals with transit times >15 s, distal retention of dye, or a mean arterial blood pressure <100 mm Hg were discarded.

The rats were then given an infusion of 10% inulin in Ringer solution at a rate of 20 μ l/min. During the inulin equilibration period, two or three 10- μ l injections of lissamine green were given to localize the surface nephron segments to be sampled. Depending on the protocol used, micropuncture studies were then carried out with samples obtained from three or more of the following structures: late proximal tubule, early distal tubule (EDT),¹ late distal tubule (LDT), loop of Henle (LH), ascending vasa recta (VR), and the base or tip of the papillary collecting duct. VR samples

were obtained by the method of Johnston et al. (11), and papillary base and tip punctures were performed as described (8). During the micropuncture, timed urine collections were obtained from the right kidney for clearance determinations, and blood samples were taken immediately before and after each collection. Three series of experiments were performed.

Series I: clonidine-induced water diuresis studies. In preliminary studies we were unable to consistently establish a water diuresis in an anesthetized, surgically prepared micropuncture animal without significant dilutional hyponatremia and, therefore, significant volume expansion. This initial series of studies, therefore, used the centrally acting α -adrenergic agonist, clonidine, which has been shown to effectively inhibit the release of antidiuretic hormone and produce a striking increase in urine flow when acutely administered to laboratory animals (12, 13). Four groups of animals were studied in this series of experiments. In each group micropuncture samples were obtained from the late proximal tubule of superficial nephrons, the LH, adjacent VR, and the tip of the exposed papilla. All samples were obtained within 15 min of exposure of the papilla. The following groups were studied:

(a) Hydropenia ($n = 6$). Animals were prepared as described above and received an intravenous infusion of 10% inulin in Ringer solution at a rate of 20 μ l/min.

(b) Clonidine-induced water diuresis alone ($n = 8$). Clonidine, 1 mg/100 g, was administered intravenously in 2.5% dextrose and water at 10 ml/kg per h. When a stable water diuresis was established as determined by two consecutive urine osmolalities <300 mosmol/kg H₂O (within 15–45 min) after the beginning of the infusion, the left ureter was removed, clearance collections were begun, and micropuncture samples were obtained.

(c) 2% body weight Ringer alone ($n = 6$). After preparation of the animals and equilibration of the inulin infusion, 2% body weight Ringer solution was infused over 10 min. The infusion rate was then decreased to a rate equal to urine flow, and the left papilla was exposed, and clearance and micropuncture specimens were collected.

(d) Clonidine-induced water diuresis plus 2% Ringer loading ($r = 8$). A water diuresis was established as in group B rats, and a 2% Ringer infusion was then given as in group C animals. The Ringer infusion rate was then decreased to the average rate given to group C animals (20 μ l/min), and collections were begun.

Series II: papillary exposure studies ($n = 7$). In this group of studies, instead of the administration of clonidine, medullary tonicity was decreased by exposure of the papilla for 1 h, a maneuver previously shown to result in impaired concentrating ability (14). Thus, medullary washout was limited to the left kidney, and the right kidney provided control clearance data. Animals were prepared as described above, including removal of the left ureter. After 1 h, clearance collections from the right kidney were begun, and micropuncture samples were obtained from the VR, the LH of juxtamedullary nephrons, and the papillary base and tip. After initial hydropenic clearance and micropuncture collections were obtained, animals were volume expanded with 2% body weight Ringer solution as described above. Micropuncture was then performed on the LDT of superficial nephrons as well as on those sites sampled during the hydropenic period.

Series III: distal delivery studies. To localize the effect of medullary washout on tubular sodium reabsorption, additional micropuncture studies were performed according to the following protocols:

¹ Abbreviations used in this paper: EDT, early distal tubule; FE_{Na}, fractional sodium excretion; GFR, glomerular filtration rate; LDT, late distal tubule; LH, loop of Henle; PGE, prostaglandin E; VR, ascending vasa recta.

TABLE I
Clearance Results in Series I Experiments

Group	Uosm ^t	C _{in}	U _{Na} V	FE _{Na}	[Na] _p	B/P
	mosmol/Kg H ₂ O	ml/min	μeq/min	%	meq/liter	
A	1360±205	0.83±0.03	0.14±0.05	0.11±0.04	145±2	133±5
B	186±23*	0.63±0.04*	0.21±0.02	0.20±0.02	143±1	125±5
C	1508±110*	1.08±0.04*	0.92±0.19*	0.56±0.09*	145±1	134±4
D	145±8*	0.80±0.04*	3.66±0.47*	3.16±0.38*	144±1	125±5

Uosm^t, urine osmolality; C_{in}, inulin clearance; U_{Na}V, absolute urinary sodium excretion; FE_{Na}, fractional sodium excretion; [Na]_p, plasma sodium concentration, B/P, arterial blood pressure. All values are expressed as mean±SEM.

* $P < 0.05$ or less vs. immediately preceding group.

(a) 2% Ringer loading alone ($n = 11$). Animals were prepared in a manner identical to those in group I c. In five rats samples were obtained from the EDT of superficial nephrons and the papillary tip. In the remaining six animals, micropuncture samples from the LDT of superficial nephrons, the papillary base, and the papillary tip were obtained.

(b) Clonidine-induced water diuresis plus 2% Ringer loading ($n = 12$). A protocol identical to that used in group I d was followed in these studies. In five animals EDT and papillary tip samples were obtained, and in seven rats the LDT, the papillary base, and the papillary tip were punctured. All samples were obtained within 15 min of removal of the left ureter.

Analytical methods. Plasma and urine inulin concentrations were determined by the anthrone method (15), and the concentration of inulin in tubular fluid samples was measured by the method of Vurek and Pegram (16). Sodium concentrations in tubular fluid and VR were determined with a helium-glow photometer (American Instrument Co., Travenol Laboratories, Inc., Silver Springs, Md.) and that in urine and plasma with a flame photometer (Instrumentation Laboratory, Inc., Lexington, Mass.). Urine osmolality was measured on a vapor pressure osmometer, and tubular fluid and VR osmolality were determined on a nanoliter osmometer (Clifton Technical Physics, Hartford, NY).

Calculations. (a) Single nephron glomerular filtration rate = $TF/P_{in} \times V_f$, where TF/P_{in} is the tubular fluid to plasma inulin concentration ratio and V_f is the tubular fluid flow rate in nanoliters per minute (b) The fractional delivery of sodium to any nephron site, $FD_{Na} = (TF/P)_{Na/in} \times 100$, where $(TF/P)_{Na/in}$ is the TF/P_{Na} ratio divided by the TF/P_{in} ratio. The data were analyzed by paired or unpaired Student's t test.

RESULTS

Series I: clonidine-induced water diuresis studies.

The results of the right kidney clearance studies are summarized in Table I. Clonidine alone resulted in a marked decrease in urine osmolality compared with hydropenic animals, values averaging 1,360 and 186 mosmol/kg H₂O, respectively. Although total kidney glomerular filtration rate (GFR) was reduced in the clonidine-infused rats, no change in either absolute or fractional sodium excretion could be detected, the latter being 0.11% of the filtered load in hydropenia and

0.20% in clonidine-induced water diuresis. 2% body weight Ringer loading alone (group I c) failed to reduce urine osmolality, the mean urine osmolality of 1,508 mosmol/kg H₂O being similar to that of hydropenic rats. A small increase in both absolute and fractional sodium excretion was noted in these animals. When the same Ringer load was administered to animals undergoing a water diuresis (group I d), a much larger increase in urinary sodium excretion was seen. In these rats, excreting urine with an average osmolality of 145 mosmol/kg H₂O, absolute urinary sodium excretion and fractional sodium excretion (FE_{Na}) averaged 3.7 μeq/min and 3.2%, respectively ($P < 0.005$ for both vs. 2% Ringer loading alone). The serum sodium concentration was similar in all four groups of rats.

The micropuncture findings are shown in Table II. As can be seen when compared with hydropenic animals (group I a), clonidine alone (group I b) failed to alter sodium delivery from the proximal tubule of either superficial or juxtamedullary nephrons. Similarly, no difference in FE_{Na} at the papillary tip was noted between these groups. VR osmolality, an index of medullary tonicity, was markedly reduced in animals undergoing a clonidine-induced water diuresis, however. Volume expansion with 2% body weight Ringer loading (group I c) significantly increased the fractional delivery of sodium from the proximal tubule of both superficial and juxtamedullary nephrons, values averaging 60 and 48% of the filtered load, respectively ($P < 0.05$ for both vs. hydropenia). Despite this increment in distal delivery, no increment in FE_{Na} was detected at the papillary tip. VR osmolality was lower in group I C (2% Ringer loading) than in hydropenic rats ($P < 0.05$) but higher than that seen in water diuretic rats ($P < 0.001$). More importantly, however, a sodium gradient between the LH and VR persisted despite this moderate fall in VR osmolality. When animals undergoing a water diuresis and having a marked reduction in medullary tonicity (group I d)

TABLE II
Micropuncture Results in Series I Experiments

Group	LPT			LH			PB			PT			VR _{osm} <i>mosmol/Kg</i> <i>H₂O</i>	(LH/VR) _{Na}	
	(TF/P) _{Na}	FD _{Na}	\dot{V}	(TF/P) _{Na}	FD _{Na}	\dot{V}	(TF/P) _{Na}	FD _{Na}	\dot{V}	(TF/P) _{Na}	FD _{Na}	\dot{V}			
		%	nl/min		%	nl/min		%	nl/min		%	nl/min			%
A	2.38±0.09	41.6±1.5	28±1	6.33±0.10	2.13±0.15	33.8±2.2	57±8	—	—	—	114±18	0.37±0.12	0.31±0.13	1004±126	—
B	2.60±0.08	38.2±1.1	22±3	3.70±0.29*	1.24±0.05*	35.0±2.7	49±7	30±5	0.11±0.02	0.45±0.15	38±7*	0.02±0.02*	0.06±0.02	390±19*	—
C	1.64±0.08*	60.8±2.6*	30±1	3.39±0.14	1.55±0.15*	48.3±5.4*	55±4	80±17*	0.56±0.07*	0.86±0.25	110±16*	0.25±0.06*	0.25±0.11	750±60*	1.25±0.09
D	1.77±0.09	56.8±2.6	29±2	2.69±0.07*	1.25±0.05*	46.9±2.4	50±5	8.3±1.0*	0.30±0.06*	3.9±8*	10.7±1.0*	0.21±0.06	2.2±0.6*	342±12*	1.04±0.02*

LPT*, late proximal tubule; LH, loop of Henle; PB, papillary base; PT, papillary tip; VR_{osm}, ascending vasa recta osmolality; (LH/VR)_{Na}, LH to VR sodium concentration ratio; (TF/P)_{Na}, tubular fluid to plasma inulin ratio; (TF/P)_{Na}, tubular fluid to plasma sodium concentration ratio; FD_{Na}, fractional delivery of sodium; \dot{V} , single nephron GFR.

* $P < 0.05$ or less vs. immediately preceding group.

experienced the same 2% body weight Ringer load, FE_{Na} at the papillary tip increased dramatically to 2.2% of the filtered load. This increase in sodium excretion occurred despite the fact that distal delivery of sodium was comparable to that seen in animals receiving 2% body weight Ringer solution alone (group I c). Finally, it is important to stress that the sodium gradient between the LH and adjacent VR was virtually abolished in rats undergoing 2% Ringer loading plus water diuresis (LH/VR $_{Na}$ = 1.04).

Series II: papillary exposure studies. Although the micropuncture studies just described suggested that clonidine alone did not alter renal tubular sodium transport, it is possible that some undetectable pharmacologic or physiologic effect of this agent accounted for the greater sodium excretion in animals undergoing 2% Ringer loading plus clonidine-induced water diuresis (group I d). The second series of studies was therefore performed without this drug, and medullary tonicity was reduced by exposure of the papilla for 1 h before clearance and micropuncture samples were obtained. This maneuver markedly reduced VR osmolality in the left kidney, values averaging 536 ± 29 and 457 ± 16 mosmol/kg H₂O during hydropenia and 2% Ringer loading, respectively. As shown in Fig. 1, urine osmolality differed significantly between kidneys, urine osmolality from the left, "exposed," kidney averaging 564 mosmol/kg H₂O compared with 1,026 mosmol/kg H₂O from the contralateral control kidney. FE_{Na} did not differ between the two kidneys during hydropenia, however, values being 0.30 and 0.12% of the filtered load from the left and right kidneys, respectively. In response to 2% body weight Ringer loading, the urine osmolality remained elevated in the control kidney, 1,166 mosmol/kg H₂O compared with 485 mosmol/kg H₂O in the exposed kidney. Although sodium excretion increased significantly in both kidneys, the increment in FE_{Na} was much greater in the

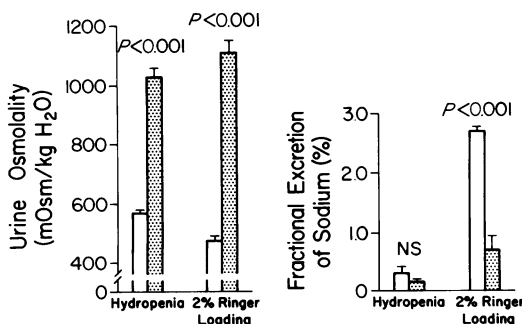


FIGURE 1 Effect of papillary exposure (left kidney) on urine osmolality and fractional excretion of sodium during hydropenia and after volume expansion with 2% body weight Ringer loading. □, left kidney; ▨, right kidney.

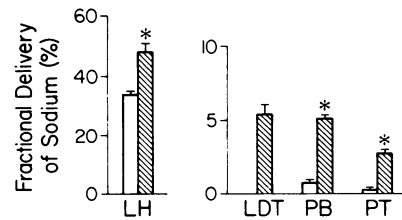


FIGURE 2 Effect of 2% body weight Ringer loading on fractional delivery of sodium to the bend of Henle's loop of juxtamedullary nephrons (LH), the late distal tubule of superficial nephrons (LDT), and the base (PB) and tip (PT) of the papillary collecting duct. Asterisk indicates $P < 0.05$ or less vs. hydropenia. □, hydropenia; ▨, 2% Ringer loading.

exposed left kidney, averaging 2.7% of the filtered load vs. 0.7% in the right unexposed kidney.

The results of the left kidney micropuncture studies are summarized in Fig. 2. As can be seen, 2% body weight Ringer loading significantly increased sodium delivery from the proximal tubule of juxtamedullary nephrons from 34.1 to 48.1% of the filtered load. The latter value is virtually identical to that seen in animals receiving the same Ringer load in the first series of experiments (Table II). Fractional delivery of sodium to the LDT of superficial nephrons was not determined during hydropenia, but, as can be seen in Fig. 2, delivery to this site after 2% Ringer loading (5.3%) was similar to that found at the papillary base (5.1%). During both hydropenia and Ringer loading, nearly half of the filtered sodium delivered to the papillary base was reabsorbed along the remainder of the papillary collecting duct.

Series III: distal delivery studies. This final series of studies was performed in an attempt to identify the nephron population and site responsive to changes in medullary tonicity. The results obtained in animals undergoing micropuncture of the EDT of superficial nephrons are summarized in Fig. 3. Fractional sodium delivery to the EDT of superficial nephrons was comparable in animals receiving a 2% body weight Ringer

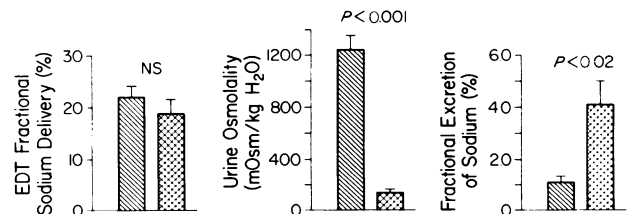


FIGURE 3 Effect of 2% body weight Ringer loading with and without water diuresis on the fractional delivery of sodium to the early distal tubule of superficial nephrons (EDT) and on urine osmolality and fractional sodium excretion. ▨, 2% Ringer loading; ▩, 2% Ringer loading plus water diuresis.

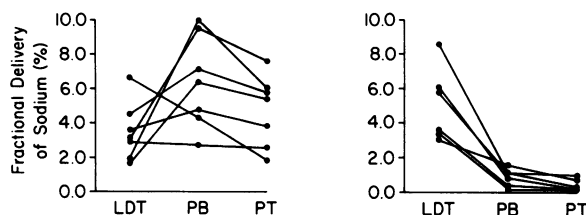


FIGURE 4 Effect of 2% body weight Ringer loading with (left panel) and without (right panel) water diuresis on sodium delivery to the late distal tubule of superficial nephrons (LDT), papillary base (PB), and papillary tip (PT).

load alone and those receiving the same infusion superimposed on a water diuresis, average deliveries being 22.3 and 18.9% of the filtered sodium in these respective groups. As in the initial series of experiments, the urine osmolality was markedly reduced in the clonidine-infused rats (152 mosmol/kg H₂O) compared with the 2% Ringer-loaded rats (1,227 mosmol/kg H₂O). Further, FE_{Na} averaged 4.1% of the filtered load in the former group compared with 1.1% in the latter ($P < 0.01$).

Comparable clearance data were obtained in series III animals undergoing micropuncture of the LDT, papillary base, and papillary tip. Urine osmolality was again low in the rats receiving clonidine plus 2% Ringer loading, values averaging 142 mosmol/kg H₂O compared with 1,470 mosmol/kg H₂O in animals receiving a 2% Ringer load alone ($P < 0.001$). Fractional sodium excretion was higher in the former group, 4.2% of the filtered load, vs. 1.0% in the latter ($P < 0.001$). The results of the micropuncture studies are shown in Fig. 4. Fractional delivery of sodium to the LDT was comparable in the two groups, 3.5% in rats undergoing a water diuresis plus 2% Ringer loading and 5.1% in those receiving the same Ringer infusion alone. Fractional delivery of sodium to the papillary base was significantly greater in the former group, however, average values being 6.3 and 0.87%, respectively ($P < 0.001$). Further, as is shown on the left panel of Fig. 4, net sodium addition between the LDT and papillary base was observed in five of the seven water diuretic animals. In contrast, all rats receiving 2% Ringer loading alone demonstrated net reabsorption of sodium between these sites (Fig. 4, right panel). Finally, net reabsorption of sodium was observed along the papillary collecting duct of both groups, the fractional delivery of sodium to the papillary tip being 4.7% in clonidine plus Ringer loading and 0.41% in Ringer loading alone ($P < 0.001$).

DISCUSSION

Previous studies in this laboratory have demonstrated that volume expansion with 10% body weight Ringer

loading preferentially inhibits sodium reabsorption in juxtamedullary nephrons (8, 10, 17). We postulated that this phenomenon was at least in part due to washout of the normally hypertonic medullary interstitium, which resulted in a loss of the gradient favoring passive sodium chloride reabsorption in the thin ascending limb of the LH. If this postulate is correct, one would anticipate that any maneuver that results in medullary washout, including water diuresis, would similarly decrease sodium reabsorption by this segment and thereby increase urinary sodium excretion. The present studies, as well as those of others (18, 19), clearly show, however, that water diuresis per se is not associated with an increase in sodium excretion. In this setting, avid proximal reabsorption persists at hydroponic levels, and, as a result, delivery of sodium to nephron sites beyond the ascending limb may be limited and well within their capacity to effectively remove sodium from the tubular fluid. Thus, the present studies indicate that when distal sodium delivery is adequately increased, relative medullary hypotonicity allows a significant portion of that sodium to reach the final urine.

Several considerations regarding the first series of experiments deserve comment. In these studies clonidine was used to establish a water diuresis and washout of the medullary interstitium. This pharmacologic agent has been shown by Roman et al. (13) to inhibit the central release of antidiuretic hormone when acutely administered to the rat. These investigators also reported that, when given in the same dose used in the present studies, clonidine caused a small but significant increase in urinary sodium excretion. Further, on the basis of free-water clearance data, they suggested that the inhibition of sodium reabsorption occurred at some site proximal to the diluting segment. We were unable to detect an effect of clonidine on either urinary sodium excretion or proximal reabsorption. The reason for this discrepancy is unclear but could relate to the fact that Roman and colleagues (13) analyzed paired data, whereas we performed group comparisons. Thus, the small changes in sodium excretion noted by those investigators may have been obscured by the scatter inherent in group comparisons. Nonetheless, using the same analytical approach, a significant and easily demonstrable increase in sodium excretion was found in animals undergoing a simultaneous water diuresis and 2% Ringer load. Further, it should be pointed out that a natriuretic response to clonidine has not been universally observed (20, 21).

As was noted above, clonidine alone resulted in a significant fall in total kidney GFR, and single nephron GFR tended to be decreased, though the fall did not achieve statistical significance (Tables I and II). Although this effect on GFR may explain the absence

of a detectable difference in sodium excretion between hydropenic animals (group I A) and rats receiving clonidine alone (group I B), it cannot explain the augmented natriuretic response to 2% Ringer loading seen in animals undergoing a clonidine-induced water diuresis (group I D). Despite a GFR similar to that seen in hydropenic rats and less than that of animals receiving 2% Ringer loading alone, both absolute and fractional sodium excretion were increased.

Finally, with regard to the first series of experiments, Hebert et al. (22) reported that antidiuretic hormone increases sodium reabsorption in the isolated mouse medullary thick ascending limb. If a similar effect is operative in the rat, it is conceivable that the clonidine-induced fall in circulating levels of this hormone would result in a decrease in sodium reabsorption by this segment.

In view of these considerations, we cannot totally exclude some direct or indirect pharmacologic or physiologic effect of clonidine that is independent of the medullary washout. Therefore, the second series of studies was performed, in which medullary tonicity was decreased unilaterally by exposure of the extrarenal papilla. Thereby, all extrarenal factors that might have influenced distal sodium transport (e.g., antidiuretic hormone) in the clonidine series were excluded. The results clearly demonstrate that the kidney with the reduced medullary tonicity has an exaggerated natriuretic response to an increment in distal delivery.

The nephron site responsible for this natriuresis cannot be identified with certainty. Delivery of sodium from the proximal tubule of both superficial and juxtamedullary nephrons was similar in nondiuretic and water diuretic rats receiving a 2% Ringer infusion. That fractional sodium delivery to the early and late portion of superficial distal tubule (series III experiments) did not differ under these conditions excludes an effect of medullary tonicity on the LH and distal tubule of this nephron population. Fractional reabsorption by the papillary collecting duct was between 25 and 50% of the filtered load delivered to that segment. In view of similar findings in other natriuretic conditions (8–10, 17, 23), it seems unlikely that medullary tonicity influenced reabsorption by this segment. In previous studies of segmental sodium transport during more massive volume expansion, we (10, 17) and others (8, 9, 23) found net addition of sodium between the LDT of superficial nephrons and the papillary base, indicative of preferential inhibition of sodium reabsorption in juxtamedullary nephrons. Using pharmacologic manipulation of transport, we suggested that this inhibition was localized to the thin ascending limb of the LH (10). In the present studies net addition between these sites was found in five of

seven water diuretic animals with enhanced distal delivery (series III, Fig. 4). Although this addition did not achieve statistical significance, the observation of net reabsorption by the papillary collecting duct and the likelihood of reabsorption by the cortical collecting tubule indicate that delivery to the LDT of juxtamedullary nephrons must have exceeded that to the LDT of superficial nephrons.

In view of these considerations, it is likely that a reduction in medullary tonicity preferentially diminished sodium reabsorption beyond the bend of Henle's loop of juxtamedullary nephrons. Without the ability to experimentally determine sodium reabsorption along the segments comprising this distal nephron, we cannot localize this effect with absolute certainty. On the other hand, the observation that the gradient for passive sodium reabsorption across the thin ascending limb is virtually abolished in water diuretic rats receiving a 2% Ringer load (Table II) suggests that this site may be involved by providing a mechanism to explain the diminished reabsorption in this setting. Further, at least partial participation of this mechanism is consistent with our earlier findings in more massively expanded animals (10).

Finally, it should be pointed out that an alternative mechanism could involve renal prostaglandin E (PGE) production. Pharmacologic studies by Olsen (24) indicate that clonidine increases urinary excretion of PGE, and indirect observations in our laboratory (14) suggest that exposure of the renal papilla may enhance renal PGE production. Unfortunately, both of these maneuvers are associated with an increase in urine flow rate, which has a direct relationship to PGE excretion (25, 26). It is not clear, however, whether the increased PGE excretion reflects an increase in synthesis of the hormone. Stokes (27) has reported that PGE inhibits sodium reabsorption in the medullary thick ascending limb of the LH. Fine and Trizna (28), however, were unable to demonstrate such an effect, and clearance studies have produced conflicting data regarding the influence of prostaglandins on renal sodium excretion (29). The observation in the present studies that sodium delivery to the EDT of superficial nephrons is similar in water diuretic and nondiuretic animals receiving a 2% Ringer load suggests, however, that medullary thick ascending limb transport was not involved in the difference in sodium excretion between these groups.

In summary, the data obtained in these studies indicate that medullary tonicity is capable of influencing distal sodium reabsorption. This inhibitory effect of relative hypotonicity probably occurs primarily in the thin ascending limb of the LH of juxtamedullary nephrons and is detected in the final urine only when delivery out of the proximal tubule is increased. These

findings may explain the importance of increased papillary plasma flow in partially mediating the natriuretic response to renal vasodilatation reported by Fadem et al. (30) and Lameire et al. (31). It is also possible that failure to reduce medullary tonicity could participate in the blunted natriuretic response to saline loading in a variety of edematous states.

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REFERENCES

- Brenner, B. M., and R. W. Berliner. 1968. Relationship between extracellular volume and fluid reabsorption by the rat nephron. *Am. J. Physiol.* **217**: 6-12.
- Howards, S., B. Davis, F. Knox, F. Wright, and R. Berliner. 1968. Depression of fractional sodium reabsorption by the proximal tubule of the dog without sodium diuresis. *J. Clin. Invest.* **47**: 1561-1572.
- Knox, F., E. Schneider, L. Willis, J. Strandhoy, and C. Ott. 1973. Effect of volume expansion on sodium excretion in the presence and absence of increased delivery from superficial proximal tubules. *J. Clin. Invest.* **52**: 1642-1646.
- Knight, T. F., and E. J. Weinman. 1977. Renal sodium reabsorption following induction of and recovery from volume expansion. *Am. J. Physiol.* **233**: F416-F420.
- Ladd, M. 1951. Effect of prehydration upon renal excretion of sodium in man. *J. Appl. Physiol.* **3**: 603-609.
- Earley, L. E., and R. M. Friedler. 1964. Observations on the mechanism of decreased tubular reabsorption of sodium and water during saline loading. *J. Clin. Invest.* **43**: 1928-1937.
- Humphreys, M. H., R. M. Friedler, and L. E. Earley. 1970. Natriuresis produced by vasopressin or hemorrhage during water diuresis in the dog. *Am. J. Physiol.* **219**: 658-664.
- Stein, J. H., R. W. Osgood, and R. T. Kunau. 1976. Direct measurement of papillary collecting duct sodium transport in the rat. *J. Clin. Invest.* **58**: 767-773.
- Higashihara, E., T. D. DuBose, and J. P. Kokko. 1978. Direct examination of chloride transport across papillary collecting duct of the rat. *Am. J. Physiol.* **235**: F219-F226.
- Osgood, R. W., H. J. Reineck, and J. H. Stein. 1978. Further studies on segmental sodium transport in the rat kidney during expansion of the extracellular fluid volume. *J. Clin. Invest.* **64**: 311-320.
- Johnston, P. A., C. A. Battilana, F. Lacy, and R. Jamison. 1977. Evidence for a concentration gradient favoring outward movement of sodium from the thin loop of Henle. *J. Clin. Invest.* **59**: 234-240.
- Humphreys, M. H., and I. A. Reid. 1975. Suppression of antidiuretic hormone secretion by clonidine in the anesthetized dog. *Kidney Int.* **7**: 405-412.
- Roman, R. J., A. W. Cowley, and C. Lechene. 1979. Water diuretic and natriuretic effect of clonidine in the rat. *J. Pharmacol. Exp. Ther.* **211**: 385-393.
- Chuang, E. L., H. J. Reineck, R. W. Osgood, R. T. Kunau, and J. H. Stein. 1978. Studies on the mechanism of reduced urine osmolality after exposure of the renal papilla. *J. Clin. Invest.* **61**: 633-639.
- Fuhr, J., J. Kaczmarczyk, and C. D. Kruttsch. 1955. Eine einfache colorimetrische Methode zur Inulinbestimmung für Nieren-Clearance-Untersuchungen bei Stoffwechselgesunden und Diabetikern. *Klin. Wochenschr.* **33**: 729-730.
- Vurek, G. G., and S. E. Pegram. 1966. Fluorometric method for the determination of nanogram quantities of inulin. *Anal. Biochem.* **16**: 409-419.
- Reineck, H. J., R. Parma, J. L. Barnes, and R. W. Osgood. 1980. Nephron heterogeneity in renal excretion of sodium and potassium in the rat. *Am. J. Physiol.* **239**: F187-F193.
- Jamison, R. L., and F. B. Lacy. 1972. Evidence for urinary dilution by the collecting tubule. *Am. J. Physiol.* **223**: 898-902.
- Jamison, R. L., J. Buerkert, and F. Lacy. 1973. A micropuncture study of Henle's thin loop in Brattleboro rats. *Am. J. Physiol.* **224**: 180-185.
- Chrysant, S. G., and A. R. Cavender. 1975. Direct renal hemodynamic effects of clonidine. *Arch. Int. Pharmacodyn. Ther.* **218**: 202-222.
- Reid, I. A., D. M. McDonald, B. Pachino, and W. F. Ganong. 1975. Studies concerning the mechanism of suppression of renin secretion by clonidine. *J. Pharmacol. Exp. Ther.* **192**: 713-721.
- Hebert, S. C., R. M. Culpepper, B. S. Misanko, and T. E. Andreoli. 1980. ADH induces anti-saliuresis in medullary but not cortical thick ascending limbs. *Clin. Res.* **28**: 533A.
- Haas, J. A., T. J. Berndt, S. P. Youngberg, and F. G. Knox. 1979. Collecting duct sodium reabsorption in deoxycorticosterone-treated rats. *J. Clin. Invest.* **63**: 211-214.
- Olsen, U. S. 1976. Clonidine-induced increase of renal prostaglandin activity and water diuresis in normal dogs. *Eur. J. Pharmacol.* **36**: 95-101.
- Wright, L., and M. D. Lifschitz. 1979. High urine flow rate increases urinary prostaglandin E excretion in conscious dogs. *Kidney Int.* **16**: 845.
- Kirschenbaum, M. A., and E. R. Serros. 1980. Effect of alterations in urine flow rate on prostaglandin E excretion in conscious dogs. *Am. J. Physiol.* **238**: F107-F111.
- Stokes, J. B. 1979. Effect of prostaglandin E₂ on chloride transport across the rabbit thick ascending limb of Henle. *J. Clin. Invest.* **64**: 495-502.
- Fine, L. G., and W. Trizna. 1977. Influence of prostaglandins on sodium transport of isolated medullary nephron segments. *Am. J. Physiol.* **232**: F383-F390.
- Lifschitz, M. D., and J. H. Stein. 1981. Renal vasoactive hormones. In *The Kidney*. B. M. Brenner and F. C. Rector, editors. W. B. Saunders Co., Philadelphia, PA. 2nd ed. 1: 650-720.
- Fadem, S., R. Patak, M. Lifschitz, and J. Stein. 1977. Studies on the mechanism of increased sodium excretion during drug-induced vasodilatation. *Kidney Int.* **12**: 557.
- Lameire, N., R. Vanholder, S. Ringoer, and I. Leusen. 1980. Role of medullary hemodynamics in the natriuresis of drug-induced renal vasodilatation in the rat. *Circ. Res.* **47**: 839-844.