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COMPOSITION OF THE RENAL MEDULLA DURING WATER DIURESIS *

By HOWARD LEVITIN,† ALVIN GOODMAN,‡ GILLES PIGEON § and FRANKLIN H. EPSTEIN ||

(From the Department of Internal Medicine, Yale University School of Medicine, New Haven, Conn.)

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During antidiuresis, sodium is concentrated in the papilla and medulla of mammalian kidneys as a result of active sodium reabsorption from medullary tubules and countercurrent flow through the loops of Henle and the medullary capillaries. It is clear from chemical (1) and cryoscopic (2, 3) analysis of sections of kidney, and from direct micropuncture (4–6), that under these circumstances the osmolality of medullary interstitial fluid is approximately that of collecting-duct urine and that sodium concentration rises progressively along a gradient from cortex to medulla.

The precise role of antidiuretic hormone (ADH) in establishing and maintaining this gradient is not entirely clear, partly because comparable studies in the absence of vasopressin (i.e., during water diuresis) have been more difficult to accomplish. Ullrich, Jarausch and Drenckhahn demonstrated that the osmolality and sodium concentration of papillary water approached that of peripheral plasma in dogs during water diuresis (1, 7). That medullary interstitial fluid is actually hypertonic to plasma in the absence of ADH was suggested by the demonstration (8) that compression of the renal artery of one kidney during water diuresis led to production of hypertonic urine by that kidney. Recently, Bray reported that the melting point of the contents of small medullary tubules in kidney sections from rats undergoing water diuresis was lower than that of cortex (3). Finally, direct micropuncture of loops of Henle and vasa recta in hamsters with diabetes insipidus yielded fluid with an osmolality higher than that of vena cava plasma but lower than that observed in normal animals excreting a concentrated urine (6).

The present experiments were designed to clarify the action of antidiuretic hormone on the sodium content of the renal medulla. The chemical composition of papilla, medulla, and cortex of dogs was measured during hydropenia, during water diuresis, and at varying intervals after the infusion of vasopressin. The results confirm previous evidence that medullary fluid is hypertonic during water diuresis, although not as concentrated as during hydropenia. They further indicate that, in addition to the fall in sodium concentration expected as a result of an increase in water content, water diuresis is associated with a decline in the absolute amount of sodium per unit of dry solids in the renal medulla. The latter is restored by infusions of vasopressin or by dehydration. The data suggest that, in addition to increasing the permeability of the collecting ducts to the back-diffusion of water, ADH enhances the sequestration of sodium in the interstitial fluids of medulla and papilla.

METHODS

Unanesthetized female mongrel dogs weighing 8 to 13 kg were trained to lie quietly with a urethral catheter in place. Their standard dog ration was supplemented with half a pound of horsemeat daily. All of the animals had been previously tested and were not used if their urinary osmolality after 24 hours of dehydration did not exceed 1,400 mOsm per kg of water.

Four groups of dogs were studied. The first group (6 dogs) was studied during a water diuresis which had been induced 2 hours earlier by the intragastric administration of water amounting to 5 per cent of their body weight.

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† Established Investigator of the American Heart Association.

‡ Postgraduate Fellow of the U. S. Public Health Service.

§ Traveling Fellow of the R. Samuel McLaughlin Foundation, 1960–1961; Research Fellow of the Connecticut Heart Association, 1961–1962.

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TABLE I

Composition of urine, papilla, medulla, and cortex of nonfrozen dog kidneys during water diuresis and at varying stages of antidiuresis

	Urine	Papilla			Medulla			Cortex		
	mmoles/kg H_2O	*mmoles/kg H_2O*	*mmoles/ 100 g DS*	*g H_2O/100 g wet tissue*	*mmoles/kg H_2O*	*mmoles/ 100 g DS*	*g H_2O/100 g wet tissue*	*mmoles/ kg H_2O*	*mmoles/ 100 g DS*	*g H_2O/100 g wet tissue*
					Water diuresis					
Water content				87.8 ± 1.4			87.9 ± 0.77			80.5 ± 0.85
Osmolality	70.6 ± 16.6*									
Na	2.8 ± 2.5	118 ± 23.1	86.4 ± 12.6		134 ± 17.7	97.5 ± 7.5		68.1 ± 5.7	28.2 ± 1.8	
K	5.8 ± 3.7	48.6 ± 4.4	35.5 ± 3.8		45.8 ± 5.0	33.2 ± 3.6		77.2 ± 4.0	31.9 ± 1.9	
NH_4	5.3 ± 2.5	6.8 ± 2.8	4.9 ± 2.0		3.9 ± 0.51	2.9 ± 0.36		716 ± 0.71	3.1 ± 0.32	
Urea	42.1 ± 16.4	33.4 ± 9.1	24.7 ± 8.4		31.1 ± 9.9	22.9 ± 8.3		10.0 ± 2.5	4.1 ± 1.1	
Sum of osmoles†	69.9 ± 16.5	381 ± 50.7			401 ± 32.5			315 ± 13.9		
					30-min post-vasopressin infusion					
Water content				*85.0 ± 1.7‡*			*86.8 ± 0.99*			80.0 ±0.85
Osmolality	*773 ± 198*									
Na	*28.1 ± 23.3*	*177 ± 30.9*	*100 ± 12.6*		*154 ± 10.5*	102 ± 8.4		74.0 ± 9.6	29.7 ± 4.7	
K	*170 ± 76.5*	47.5 ± 4.1	*27.1 ± 4.2*		*40.1 ± 3.4*	*26.6 ± 3.4*		72.4 ± 9.0	*29.0 ± 2.2*	
NH_4	31.5 ± 32.3	*14.1 ± 4.5*	*7.8 ± 1.7*		*9.4 ± 1.6*	*6.3 ± 1.3*		8.1 ± 5.1	3.3 ± 0.19	
Urea	*306 ± 70.5*	*301 ± 145*	*164 ± 61.5*		*199 ± 63.9*	*131 ± 42.8*		13.4 ± 4.0	5.3 ± 0.60	
Sum of osmoles	*766 ± 226*	*780 ± 208*			*607 ± 159*			322 ± 15		
					2-hr post-vasopressin infusion					
Water content				*84.8 ± 1.2*			*86.5 ± 0.78*			80.0 ± 0.62
Osmolality	*1,012 ± 232*									
Na	*148 ± 34.4*	*206 ± 27.5*	*115 ± 13.7*		*168 ± 11.0*	*108 ± 12.4*		*80.5 ± 5.4*	*32.1 ± 2.5*	
K	*120 ± 75.6*	45.6 ± 6.1	*25.5 ± 4.0*		*40.5 ± 4.0*	*25.4 ± 2.6*		*69.1 ± 2.7*	*27.6 ± 1.7*	
NH_4	*23.4 ± 19.4*	*13.6 ± 4.5*	*7.8 ± 2.2*		*10.7 ± 2.3*	*7.1 ± 1.7*		*8.4 ± 0.6*	3.3 ± 0.24	
Urea	*484 ± 225*	*391 ± 102*	*216 ± 47.0*		*267 ± 70.6*	*176 ± 49.1*		*13.4 ± 3.9*	5.3 ± 1.5	
Sum of osmoles	*1,069 ± 267*	*881 ± 130*			*706 ± 83.5*			*329 ± 9.4*		
					24 hrs of dehydration					
Water content				*81.1 ± 1.9*			*84.0 ± 1.5*			*78.9 ± 0.65*
Osmolality	*1,725 ± 361*									
Na	*153 ± 60.0*	*320 ± 48.4*	*139 ± 15.4*		*258 ± 18.7*	*136 ± 14.1*		*80.8 ± 8.9*	30.2 ± 3.6	
K	*162 ± 70*	52.7 ± 5.5	*23.0 ± 4.2*		44.5 ± 14.2	*23.6 ± 4.0*		80.5 ± 7.9	30.1 ± 3.2	
NH_4	*76.2 ± 59.5*	*21.3 ± 2.3*	*9.3 ± 1.5*		*14.2 ± 3.2*	*7.5 ± 1.7*		7.5 ± 0.30	*2.8 ± 0.26*	
Urea	*991 ± 228*	*855 ± 160*	*368 + 66.7*		*532 ± 127*	*280 ± 51.5*		*21.0 ± 4.6*	*7.9 ± 1.8*	
Sum of osmoles	*1,771 ± 328*	*1,645 ± 255*			*1,165 ± 127*			*358 ± 27.5*		

* Mean ± standard deviation.
† Sum of osmoles = 2(Na + K + NH_4) + urea.
‡ Italics indicate that $p < 0.05$ when compared with same value during water diuresis.

Group 2 (5 dogs) and group 3 (5 dogs) received a similar water load, followed 2 hours later, at the height of the ensuing water diuresis, by the intravenous infusion of vasopressin at the rate of 50 mU per kg per hour. Group 2 received the vasopressin infusion for 30 minutes, and group 3 received the vasopressin infusion for 2 hours. Group 4 (6 dogs) was studied during antidiuresis which resulted from 24 hours of food and water deprivation. In all dogs, timed urine collections were obtained prior to the conclusion of the experiment, at which time an intravenous injection of pentobarbital was given, and both kidneys were removed in 1 to 2 minutes.

The pelvis of the kidney was opened and the longitudinal papillary ridge was exposed. A thin strip of this ridge (papilla), 2 mm in thickness, was cut. A deeper longitudinal strip (medulla), 4 mm thick, was then dissected free. The capsule was stripped and pieces of cortex obtained. Duplicate samples of papilla, medulla, and cortex were examined. One sample was weighed by tare to constant weight for water content. The other sample was ground in electrolyte-free, fine mesh carborundum and diluted with 7 ml of water (papilla and medulla) or 10 ml of water (cortex) and analyzed for sodium, potassium, ammonia, and urea, employing techniques previously published (9). The average weight of the samples was as follows: papilla, 138 mg; medulla, 390 mg; cortex, 291 mg. Urine samples were analyzed for the same solutes, as well as for osmolality, with a Fiske osmometer.

In order to eliminate possible artifacts arising from losses of blood and interstitial fluid from the severed pedicle of an unclamped kidney (10), two further groups of 5 dogs each were studied—one group during water diuresis and the other during hydropenia. In these experiments the renal pedicle was occluded with two large clamps. The renal artery, vein, and ureter were cut, and the kidney with attached pedicle clamp was immediately frozen in acetone and dry ice. Cross sectional cuts near the middle of the frozen kidney were made with a power-driven scroll saw. Duplicate samples of papilla, medulla, and cortex, weighing an average of 169, 218, and 783 mg, respectively, were obtained. One sample was weighed by tare and dried to constant weight for water content. The other sample was placed in a weighed 10-ml volumetric flask to which 4 ml of concentrated nitric acid was added. Digestion was accomplished for 24 hours on a steam table, after which the clear nitric acid digest was brought to volume and analyzed for sodium and potassium. Urea and ammonia were not determined in this series of experiments.

In order to estimate the rate of turnover of medullary sodium during water diuresis and during hydropenia, 0.04 to 0.09 μc of $Na^{22}Cl$ per kg of body weight per minute was infused intravenously into trained, unanesthetized dogs, using a syringe-type, constant infusion pump. At 3 to 4 minutes after the beginning of the infusion, samples of papilla, medulla, and cortex were analyzed for sodium as described above, after their radioactivity had been measured in a γ-ray, well-type scintillation counter. In some experiments blood was obtained by cardiac puncture at the time the kidneys were clamped; in these cases the specific activity of plasma sodium corresponded closely to that of the sodium of renal cortex, suggesting that in analyzing the data the specific activity of medullary or papillary sodium might conveniently be referred to that of cortex. Four dogs were studied during water diuresis and 5 after 24 to 48 hours of dehydration.

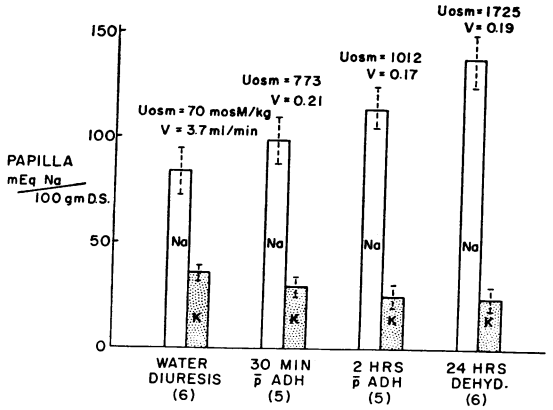

FIG. 1. PAPILLARY CONTENT OF SODIUM AND POTASSIUM PER 100 G DRY SOLIDS DURING WATER DIURESIS AND AT VARYING STAGES OF ANTIDIURESIS.

RESULTS

Composition of papilla, medulla, and cortex of unclamped kidneys (Table I, Figure 1). The concentration of solutes in tissue water [calculated as the sum of the concentrations of urea + $2(Na + K + NH_4)$] increased progressively from cortex to papilla in dehydrated dogs. This gradient was markedly reduced during water diuresis, a result of decreases in the concentration of sodium and urea. Nevertheless, at the height of water diuresis, while the kidneys were elaborating a urine of concentration below 100 mOsm per kg, papilla and medulla were distinctly hypertonic to plasma (average 381 and 401 mOsm per kg, respectively, while mean plasma osmolality was 285 mOsm per kg). It is of interest that under these circumstances the concentration of sodium and of total solutes was consistently slightly higher in water of the inner medulla than at the papillary tip, in contrast to the pattern seen in hydropenia.

The quantity of sodium per unit of dry solids (DS) declined significantly in papilla and medulla during water diuresis. When water diuresis was interrupted by infusing vasopressin, sodium content of papilla per 100 g of dry solids rose progressively

TABLE II

Composition of urine, papilla, medulla, and cortex of frozen dog kidneys during water diuresis and after 24 hours of dehydration

	Urine	Papilla		Medulla		Cortex	
	mmoles/kg H_2O	*mmoles/ kg H_2O*	*mmoles/ 100 g DS*	*g H_2O/100 g wet tissue*	*mmoles/ kg H_2O*	*mmoles/ 100 g DS*	*g H_2O/100 g wet tissue*	*mmoles/ kg H_2O*	*mmoles/ 100 g DS*	*g H_2O/100 g wet tissue*
					Water diuresis					
Water content				89.2 ± 0.72			89.9 ± 0.75			83.3 ± 0.85
Osmolality	61.2 ± 20*									
Na	3.8 ± 5.1	118 ± 6.8	98.0 ± 2.0		157 ± 13.4	139 ± 9.1		88.8 ± 12.7	44.3 ± 6.4	
K	5.2 ± 2.3	34.2 ± 4.5	28.5 ± 4.5		35.9 ± 1.7	31.8 ± 3.3		55.0 ± 8.0	27.4 ± 4.1	
					24 hrs of dehydration					
Water content				*84.0 ± 0.77*			88.6 ± 1.4			*79.6 ± 0.94*
Osmolality	*1,790 ± 187†*									
Na	*58.7 ± 53.9*	*276 ± 58*	*144 ± 24*		*212 ± 31.7*	*165 ± 4.4*		*92.8 ± 10.3*	*36.3 ± 4.2*	
K	*140 ± 27.9*	*38.6 ± 5.3*	*20.3 ± 2.5*		*33.3 ± 6.4*	*25.9 ± 23.7*		*71.9 ± 8.0*	*28.1 ± 2.6*	

* Mean ± standard deviation.
† Italics indicate that $p < 0.05$ when compared with same value during water diuresis.

(Figure 1). Papillary sodium was higher in dogs killed after 2 hours of vasopressin infusion than after 30 minutes, and was higher still after 24 hours of dehydration. The rate of urine flow was similar in all three groups of antidiuretic animals. Cortical sodium was little affected by the transition from diuresis to antidiuresis. Tissue potassium tended to decrease slightly when water diuresis was replaced by antidiuresis.

It seems unlikely that changes in the concentration of sodium and potassium in the urine contributed importantly to the observed changes in tissue content of these ions. Bray estimated the volume of urine in segments of papilla from antidiuretic kidneys of dogs at not more than 5 per cent of the water of tissue (11). Despite a change in potassium concentration in the urine from 6 mEq per L during water diuresis to 160 mEq per L during antidiuresis, there was no increase in medullary potassium.

Tissue composition of kidneys clamped and frozen (*Table II*). Kidneys clamped and frozen before analysis contained more water and sodium than those processed by the usual technique, while potassium was unaffected. The increase in water content was most pronounced in the cortex of both diuretic and antidiuretic kidneys. These observations are consistent with those of Swann and coworkers (10, 12) and Hanssen (13), who called attention to the prominence of the interstitial space in the cortex of kidneys clamped and frozen before sectioning.

When compared with kidneys from dehydrated dogs, water diuresis was again clearly associated with a decrease in the quantity of sodium per 100 g of dry solids in the renal papilla and medulla. Perhaps as a result of increased distention with extracellular fluid, sodium content of cortex, referred to dry solids, was actually higher during water diuresis than in hydropenia.

Histological sections of frozen kidneys were fixed by a freeze-substitution technique. Portions of medulla were examined with particular attention to the extent of the "interstitial" area between tubules. No significant difference was apparent in this respect between diuretic and antidiuretic kidneys.

Rate of equilibration of Na^{22} in cortex and medulla (*Table III*). When Na^{22} was infused, the rate of enrichment of the sodium of papilla and

TABLE III

Relative specific activity of sodium in various portions of the dog kidney after injection of Na^{22}

	Specific activity			Sodium content			
	Papilla/cortex	Medulla/cortex	U_{Osm}	Papilla	Medulla	Cortex	Duration of Na^{22} infusion
			mOsm/kg		*mEq/100 g DS*		*min*
Dehydrated 5 dogs	0.348*	0.455	1686	136.6	140.6	31.3	3.43
	±0.145	±0.147	±530	±17.4	±22.3	±6.9	±0.36
Water diuresis 4 dogs	0.539	0.679	67	106.4	116.8	36.2	3.64
	±0.177	±0.104	±21	±29.5	±9.4	±6.6	±0.45
p	<0.05	<0.01	<0.01	<0.05	< 0.02	NS†	NS

* Mean ± standard deviation.
† NS = not significant.

medulla, as compared with cortex, was significantly more rapid in dogs undergoing water diuresis than in dogs excreting a maximally concentrated urine.

DISCUSSION

These experiments add to the accumulating evidence that, in the absence of ADH, medullary interstitial fluid is hypertonic, although not as strongly so as when a maximally concentrated urine is being excreted. Calculated osmolality of tissue water in medulla and papilla was about 400 mOsm per kg in water-loaded dogs. These values are similar to those obtained by Gottschalk in fluid aspirated from the loop of Henle and the vasa recta of the papilla in hamsters with diabetes insipidus (6). It would seem that the countercurrent mechanism for concentrating sodium in the medulla continues to operate, although at reduced efficiency, during water diuresis.

It was suggested by Kiil and Aukland (14) that more water is actually reabsorbed from the medullary collecting ducts during water diuresis than during hydropenia, since in the former state, large volumes of dilute urine, unable to equilibrate with isotonic interstitial fluid in the distal convoluted tubules, are delivered to the medulla. Under these circumstances one might expect the concentration of sodium in medullary fluid to be diluted. The present experiments show clearly that the absolute amount of sodium in medulla and papilla, as well as its concentration in tissue water, is depressed by water diuresis and increased by ADH.

Several possible mechanisms might account for this action of posterior pituitary hormone. It is conceivable that active reabsorption of sodium chloride from the ascending loop of Henle is accelerated by ADH, resulting in an *increased deposition* of sodium salts in medullary interstitial fluid. Active transport of sodium by frog skin and toad bladder is enhanced by vasopressin (15, 16). Although infusions of vasopressin have not been shown to decrease sodium excretion, the total quantity of sodium necessary to raise the content of sodium of the medulla from that observed during water diuresis to that present during hydropenia is very small (40 μEq per minute for only 10 minutes, in a dog with kidneys weighing 50 g each). Such a transient diminution in urinary excretion of sodium would be difficult to detect.

A second possibility is that ADH *decreases the rate of removal* of sodium from the medulla by diminishing medullary blood flow. Circulation time through the medulla has in fact been shown to be shortened by water diuresis and prolonged by vasopressin (17). The observation that urinary oxygen tension is elevated by water diuresis (18) is also compatible with the hypothesis that blood flow through the countercurrent arrangement of capillary loops in the medulla is depressed by hydropenia and increased in the absence of ADH. Such an increase in blood flow through the medulla during water diuresis might result either from the removal of a vasoconstrictive action on the vasa recta induced by vasopressin itself, or from increased diffusion of water back through the collecting ducts, as a consequence of the delivery of a larger volume of dilute urine to the medulla.

Studies of the rate of turnover of sodium in me-

dulla and cortex were undertaken in an attempt to clarify these questions. When radioactive sodium was infused, the rate of enrichment of medullary sodium was accelerated during water diuresis and retarded during antidiuresis. These data are consistent with the hypothesis that ADH promotes the sequestration of sodium in the medullary interstitial fluid by slowing its removal via capillary blood. Because net transfer of sodium out of the renal tubule is such a small fraction of the unidirectional flux (19), these relatively crude measurements do not, of course, rule out the possibility that ADH increases active outward transfer of sodium by cells lining the loop of Henle.

It seems likely that the mechanism of action of vasopressin in producing a concentrated urine consists of at least two parts. By increasing the permeability of distal tubule and collecting duct, reabsorption of water from tubular urine is enhanced. In addition, ADH increases the concentration and the total quantity of sodium in the interstitium of the renal medulla. This is accomplished, at least in part, by retarding its removal via the capillary circulation.

SUMMARY

1. The composition of renal papilla, medulla, and cortex was studied in dogs during water diuresis, during hydropenia, and after interruption of water diuresis by vasopressin.

2. During water diuresis the total concentration of solutes in water of papilla and medulla was distinctly higher than the osmolality of plasma.

3. Water diuresis was associated with a reduction in the quantity of sodium per unit of dry solids in medulla and papilla, as well as in the concentration of sodium in the water of these tissues. Sodium content of the renal medulla is increased by infusions of vasopressin or by hydropenia.

4. The rate of turnover of sodium in medulla and papilla, as compared with that of cortex, is accelerated by water diuresis.

5. It is concluded that antidiuretic hormone enhances the sequestration of sodium in the renal medulla. This is accomplished, at least in part, by retarding its removal via the capillary circulation.

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