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Research Article

ON THE MECHANISM OF IMPAIRMENT OF RENAL CONCENTRATING ABILITY IN HYPERCALCEMIA *

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A decrease in the ability of the kidneys to concentrate the urine is characteristic of many varieties of experimental and clinical hypercalcemia, hypercalciuria and nephrocalcinosis (1). Vitamin D intoxication in rats (2) and large doses of parathyroid extract in dogs (3, 4), in addition to producing hyposthenuria, have been shown to cause morphological changes which are most marked in the epithelial cells lining the collecting ducts but are also present in the distal convoluted tubules and the loops of Henle. Such lesions might impair renal concentrating capacity by interfering with the accumulation and concentration of sodium in the interstitial fluids of the medulla and papilla, or by impairing the back-diffusion of water from the lumen of the collecting duct into a hypertonic interstitium (5).

In the present studies the composition of renal cortex, medulla and papilla was compared with that of plasma and urine in hydropenic rats in which hypercalcemia had been induced by large doses of vitamin D. The results indicate that the hyposthenuria observed in such animals results at least in part from a diminished concentration of sodium in renal medulla and papilla.

MATERIALS AND METHODS

White male Sprague-Dawley rats weighing 150 to 200 g were fed a synthetic diet containing liberal amounts of sodium and potassium (6). Forty-five animals divided into 12 groups were given subcutaneously, daily for 4 days, 400,000 units of vitamin D_2 suspended in peanut oil; following this they were placed on a diet containing no sodium (6) for 5 to 10 days. Seven to 12 days after the last injection of calciferol, the animals were sacrificed and portions of renal papilla, medulla and cortex were obtained for analysis. Seventy rats divided into 18 groups served as controls, receiving a diet free of sodium for 5 days before sacrifice. In order to obtain enough tissue for accurate analysis, 3 or 4 rats were kept in a single cage and the material obtained from all animals in a group was pooled. Prior to sacrifice urine was collected for 12 hours under the influence of hydropenia and exogenous vasopressin. The techniques of collection and analysis of blood, urine and tissue have been described (6).

RESULTS

Rats given calciferol appeared lethargic and weak. Polyuria was apparent and the level of urea nitrogen in plasma was mildly elevated (Table I). At the time of sacrifice, serum calcium had risen to an average of 15.7 mg per 100 ml.

As previously reported (2), maximum urinary osmolality was greatly diminished in rats injected with vitamin D (control = 3,036 mOsm per kg; vitamin D = 1,724 mOsm per kg). This could not be ascribed to a solute diuresis, since the total solute output of the hypercalcemic animals did not differ significantly from that of controls. Despite the fact that vitamin D-intoxicated rats were kept on a sodium-free diet as long or longer than were the controls, their losses of sodium in the urine were significantly greater than in the normal group.

The sodium content of papilla and medulla of hypercalcemic rats was lower than that of normal animals (Table II). This difference was highly significant, whether expressed as milliequivalents per kilogram of tissue water or as milliequivalents per 100 g of dry solids. The concentration of urea in papilla and medulla was likewise depressed, so

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TABLE I

Effect of nephrocalcinosis on plasma and urine of rats *

	Groups/ animals	Weight	Plasma						Urine								
			Osmol-ality	Na	K	CO_2	Urea	Ca	Osmolal U/P ratio	U_{osm}	Na	K	NH_4	Urea	Ca	$U_{osm}V$	$U_{Na}V$
		g	*mOsm/kg*	*mEq/L*	*mEq/L*	*mmole/L*	*mmole/L*	*mg/ 100 ml*		*mOsm/kg*	*mEq/L*	*mEq/L*	*mEq/L*	*mmole/L*	*mg/ 100 ml*	*mOsm/ 24 hr/ 100 g*	*μEq/ 24 hr/ 100 g*
Control	18/70	250	313	150.0	4.17	24.3	6.6	10.2	9.70	3,036	15.2	395.1	226.0	1,719	8.1	3.57	17
SD		±60	±5	±2.3	±0.50	± 1.8	± 1.4	±0.3	±1.00	±184	±13.3	±108.8	±44.4	±200	± 2.2	±1.28	± 8
Vit. D	12/45	237	325	150.3	3.98	25.0	14.7	15.7	5.30	1,724	41.8	205.7	98.7	969	89.4	4.13	100
SD		±45	±5	±1.6	±0.27	± 1.2	± 3.9	±0.6	±0.74	±261	±17.2	± 38.8	±41.2	±134	±39.3	±0.80	±43
p		> 0.5	<0.01	>0.7	>0.2	> 0.2	<0.01	<0.01	<0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	>0.1	<0.01

* Values are given for mean and standard deviation.

TABLE II

Effect of nephrocalcinosis on composition of renal tissue *

	Weight	Papilla							Medulla						Cortex†				
		Water content	Na	Na	K	NH_4	Urea	Total solutes‡	Water content	Na	Na	K	NH_4	Urea	Water content	Na	K	NH_4	Urea
	mg	%	*mEq/ kg H_2O*	*mEq/ 100 g DS§*	*mEq/ kg H_2O*	*mEq/ kg H_2O*	*mmole/ kg H_2O*	*mmole/ kg H_2O*	%	*mEq/ kg H_2O*	*mEq/ 100 g DS*	*mEq/kg H_2O*	*mEq/ kg H_2O*	*mmole/ kg H_2O*	%	*mEq/ kg H_2O*	*mEq/ kg H_2O*	*mEq/ kg H_2O*	*mmole/ kg H_2O*
Control	6.6	82.6	241	114	83	101	598	1,460	83.7	141	73	75	33	302	75.6	64	103	20	33
SD	±1.3	±1.3	±32	±12	±10	±54	±142	±201	±1.1	±17	±11	±4	±7	±66	±0.8	±2	±5	±4	±6
Vit. D	7.2	84.5	178	96	84	50	374	995	85.0	108	62	77	26	212	76.5	67	103	16	40
SD	±2.7	±1.0	±36	±17	±10	±18	±57	±121	±1.3	± 8	± 5.5	±5	±6	±31	±0.8	±4	±7	±2	±9
p	NS‖	<0.01	< 0.01	< 0.01	NS	< 0.01	< 0.01	< 0.01	<0.01	< 0.01	< 0.01	NS	<0.01	< 0.01	NS	NS	NS	NS	NS

* Values are given for mean and standard deviation.
† Cortices of only 6 control and 6 vitamin D groups were analyzed.
‡ "Total solutes" = 2 (Na + K + NH_4) + urea.
§ DS = dry solids.
‖ NS = not significant ($p > 0.05$).

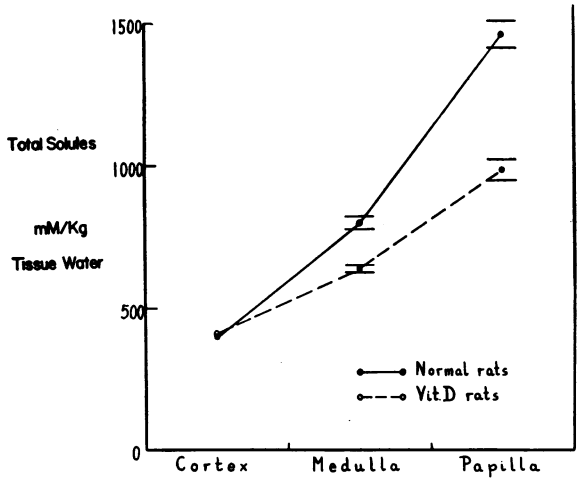

FIG. 1. THE CONCENTRATION OF "TOTAL SOLUTES," $2 \times (Na + K + NH_4)$ +UREA, IN MEDULLA AND PAPILLA WAS CONSIDERABLY DECREASED IN RATS TREATED WITH VITAMIN D. The parallel lines indicate standard error of the mean.

that the calculated concentration of osmotically active solutes [1] in water of papillary and medullary tissue was significantly diminished in rats treated with vitamin D (Figure 1). The water content of medullary and papillary tissue was slightly increased in rats given vitamin D; this may have been a reflection of their polyuria.

The decrease in urinary osmolality induced by vitamin D was considerably greater, percentagewise, than was the fall in the concentration of sodium or of "total solutes" in the water of the renal papilla. The ratio of urinary osmolality to the papillary concentration of sodium and of "total solutes" was therefore significantly decreased in hypercalcemic animals (Figure 2).

DISCUSSION

These experiments confirm findings of marked impairment of renal concentrating ability produced by hypercalcemia of several days' duration resulting from vitamin D intoxication. This change in renal function is associated with structural changes which are most prominent in the collecting ducts of the medulla but also involve the distal tubules and loops of Henle (2). The present data indicate that one reason for the decrease in maximal urinary concentration observed under these circumstances is a diminished ability of the kidneys to create and maintain a high concentration of sodium in the interstitial fluids of the medulla and papilla. Thus, the concentration of sodium in medullary and papillary tissue of hypercalcemic rats was significantly below that of normal controls, even though the concentration of sodium in the urine was greater than that of normals. The tendency of vitamin D-treated rats to lose sodium in the urine on a sodium-free diet further suggests that active reabsorption of sodium by some portion of the nephron was impaired by hypercalcemia and nephrocalcinosis.

It is of interest that the fall in maximal urinary osmolality produced by hypercalcemia was considerably greater than the decrease in sodium content or total solute concentration of the renal papilla or medulla. It is, of course, possible that changes in freezing-point of papillary tissue would not have paralleled the calculated change in concentration of tissue solutes. This might occur, for example, if the osmotic activity of cell solutes were altered owing to changes in protein-binding, or if the nature of the anions associated with sodium, potassium and ammonium were changed in the kidneys of animals treated with vitamin D. The calculated concentration of solutes in tissue water of the renal papilla in nor-

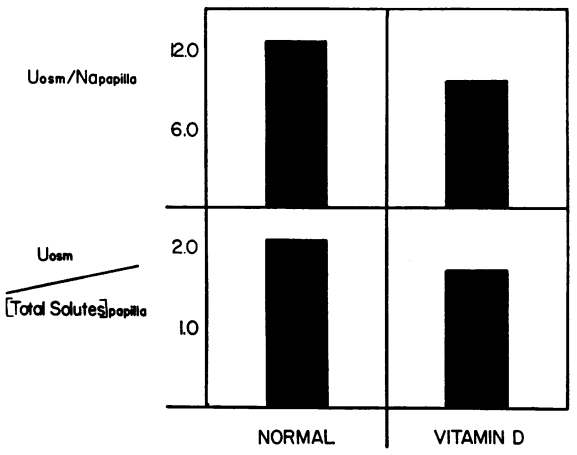

FIG. 2. THE RATIO OF MAXIMAL URINARY OSMOLALITY (U_{osm}) TO CONCENTRATION OF SODIUM AND "TOTAL SOLUTES" IN WATER OF THE RENAL PAPILLA WAS DIMINISHED IN NEPHROCALCINOTIC RATS.

[1] "Solute concentration" of renal tissue was calculated as $2 \times (Na + K + NH_4)$ + urea, where Na, K, NH_4 and urea are expressed as millimoles per kilogram of tissue water. It must be appreciated that this is only a rough approximation of the actual osmotic activity of tissue water.

mal hydropenic rats is considerably below urinary osmolality; this discrepancy is sharply diminished, however, as the terminal segment of the papilla is aproached (6). The final upturn in the concentration of sodium and total solutes at the papillary tip might be greatly reduced in nephrocalcinotic animals, without being detected in the present experiments. If that were the case, the fall in tissue sodium at the very tip of the renal papilla in rats treated with vitamin D might parallel their drop in maximal urinary osmolality, although analysis of the entire papilla would make it appear that the ratio of papillary sodium/urinary osmolality had increased.

An additional explanation for the changes illustrated in Figure 2 is that the back-diffusion of water from collecting ducts into the medullary and papillary interstitium may have been impaired in nephrocalcinotic rats, so that urinary osmolality fell more than did the concentration of sodium and other solutes in the water of the papilla. In this connection, it is of interest that the permeability to water of many cell membranes is diminished when calcium is added to their surrounding medium (7). The transfer of water out of the toad bladder in response to vasopressin is depressed when the concentration of calcium in fluid bathing the serosa is increased (8).

These results are similar to those obtained in potassium-deficient rats and dogs (6). In potassium depletion, ancillary evidence suggests that active reabsorption of sodium by the loops of Henle is unimpaired, and that the decreased concentration of sodium in the renal medulla and papilla results from interference with the reabsorption of sodium by the collecting ducts. Evidence bearing on this point in nephrocalcinotic animals might be obtained by studies of urinary dilution and by micropuncture of the distal tubule in rats intoxicated with vitamin D.

Acute decreases in glomerular filtration rate exceeding 30 per cent have been reported, by Levinsky, Davidson and Berliner (9), to reduce the sodium content of the renal medulla and papilla. Although it is not clear whether chronic depression of glomerular filtration results in similar changes, it is possible that alterations in glomerular filtration played some part in the reduction in sodium content of renal tissue noted in the present experiments.

Gill and Bartter (10) have reported that some human subjects with hypercalcemia, in whom a renal concentrating defect has been demonstrated, may reduce their urinary losses of sodium to the point of equilibrium between intake and output on a diet containing 9 mEq of sodium daily. This does not rule out the likelihood, suggested by the present studies, that sodium reabsorption in such patients is defective in some crucial segment of the nephron and that hyposthenuria is contributed to by a decreased concentration of sodium in the interstitial fluids of medulla and papilla.

SUMMARY

1. The mechanism of hyposthenuria in hypercalcemia was investigated by analyzing renal tissue from rats intoxicated with vitamin D.

2. Vitamin D intoxication produced a significant fall in the content of sodium and urea of the renal papilla and medulla. The percentile decrease in the concentration of sodium and of total solutes in water of the renal papilla was not so great as the fall in maximum urinary osmolality. Renal conservation of sodium was impaired.

3. It is concluded that the impairment of renal concentrating ability observed in hypercalcemic rats results at least in part from impaired reabsorption of sodium by the renal tubules and a diminished ability to create and maintain a high concentration of sodium in the interstitial fluids of the medulla and papilla. The possibility that the permeability of the collecting ducts to the back-diffusion of water is reduced by vitamin D intoxication is discussed.

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