MORPHOLOGY OF THE RENAL MEDULLA IN RATS ON A SUSTAINED SALT-RICH DIET

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After unilateral nephrectomy rats received 1.5% salt solution instead of drinking water for 8 months, after which an increase in width of the renal medulla and a relative increase in size of the renal papilla, with hypertrophy of the collecting tubules and an increased number of lipid granules in the interstitial cells were observed. These changes are regarded as the morphological manifestation of hyperfunction of the medulla as a whole and of the renal papilla in particular, as a consequence of the reduced proximal tubular reabsorption of sodium and water in these animals, so that a larger volume of fluid enters the medulla.

Most studies of the morphology of the kidney during an excessive intake of sodium chloride are concerned with the structures of its cortex [1]. The important role of the medulla in diuresis and results showing that the medulla secretes lipid substances possessing antihypertensive and sodium-excretory properties [5, 6, 8, 13] are the reasons for interest in the study of its morphological changes during chronic salt overloading. The present investigation was undertaken for this purpose.

EXPERIMENTAL METHOD

Male Wistar rats (12), weighing initially 125-130 g, underwent unilateral nephrectomy and were then divided into two equal groups: group 1 received dried food in pellet form with 1.5% sodium chloride solution for drinking ad lib; group 2 (control) received tap water instead of salt solution. Once a month, and 24 h before sacrifice, the arterial pressure of the animals was measured by the method described previously [2]. The animals were decapitated 34 weeks from the beginning of the experiment. The kidney was removed, the pole was cut away, and the remaining segment was divided exactly through the apex of the papilla. One half of the segment was fixed in formalin and embedded in paraffin wax, and sections from it were stained with hematoxylin-eosin and by Hale's reaction with dialyzed iron in conjunction with the PAS reaction. A piece of the renal medulla in the middle third of the renal papilla was excised from the other half, fixed in buffered 1% OsO_4 solution, and embedded in Epon. Sections, 1 μ in thickness, were cut on the LKB ultratome and stained with a mixture of 1% solutions of methylene blue and azure II (in equal amounts). The number of osmiophilic granules in the interstitial cells was counted in the stained sections by the method of Tobian et al. [14]. An ocular grid consisting of 9 squares with side 2.7 mm long was used to count the granules. A 12.5 ocular and HI 100 immersion objective (Zeiss) were used and granules were counted in 56 fields of vision (500 squares for each animal), choosing areas with the largest numbers of granules. Depending on the number of granules, each square of the grid was labeled by an index during counting: 0) no granules present; I) from 1 to 3 granules per square; II) from 4 to 12 granules; III) from 13 to 24, IV) from 25 to 34; and V) more than 35. The mean sum of the indices for 100 squares was taken as the index of granule content of the interstitial cells.

The area of the renal papilla, the area of the medulla, and the total area of the section were measured with a planimeter on photographs from histological sections magnified 8 times, and the ratio of the

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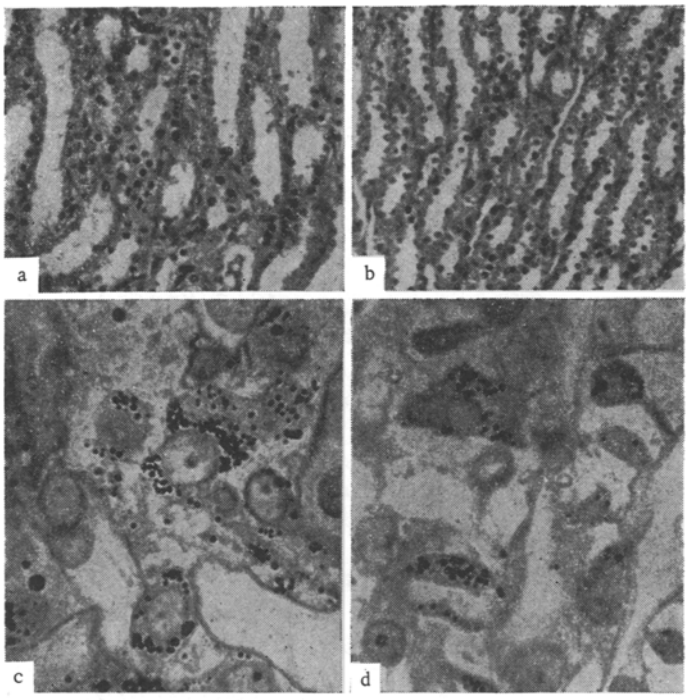

Fig. 1. Renal medulla of a rat kept on a salt-rich diet (on the left; control on the right): a, b) outer medulla (hematoxylin-eosin, 300 ×); c, d) osmiophilic granules in interstitial cells of renal papilla (methylene blue and azure II, 1200 ×).

area of medulla to the total area of section and the ratio of the area of papilla to the area of medulla were calculated. In this way the size of the medulla relative to the kidney and the size of the papilla relative to the medulla could be estimated. Using a screw-adjusted MOV × 15 ocular micrometer the mean diameter of the glomeruli was measured (the mean of the measurements of the largest and smallest diameters in the glomerulus), a hundred glomeruli being measured in each case. The height of the epithelium in the collecting tubules of the renal papilla was also measured (100 cells in 5 tubules of the same zone at the apex of the papilla).

EXPERIMENTAL RESULTS

Throughout the experiment the rats of both groups remained normotensive. The weight of the kidney in the rats receiving an excess of salt was much greater than in the controls. The renal medulla of these animals was widened and the renal papilla more massive. The ratio between the area of the medulla and the total area of cross section of the kidney was 0.24 in these rats compared with 0.19 in the controls. The ratio between the area of the renal papilla and the total area of the medulla rose to 0.42 (control 0.35). The collecting tubules in the renal papilla of this group of rats were enlarged, their lumen dilated, and their epithelium larger and higher; the lumen of the thin segment of the loop of Henle was also slightly widened, but the epithelium there was unchanged. The capillaries were poorly filled with blood, and the interstices of the papilla in sections examined under low power appeared slightly widened (Fig. 1a, b).

Branching interstitial cells lying in parallel rows between the thin segments of the loop of Henle, the capillaries, and collecting tubules were clearly visible in sections cut from pieces embedded in Epon. The cytoplasm of these cells contained large osmiophilic granules, stained brownish-purple in color. The number of granules in the interstitial cells of rats receiving an excess of salt was greater than in the control, and the granules themselves were somewhat larger (Fig. 1c, d). The index of granule content in the two groups was 194 ± 12 and 163 ± 5, respectively (Table 1).

The ascending portions of the loop of Henle and also the straight part of the proximal tubules in the outer medulla were moderately dilated (Fig. 1a, b), but the epithelium was substantially unchanged. The

TABLE 1. Morphometric Characteristics of the Medulla, Weight of the Kidney, Diameter of the Cortical Glomeruli, and Index of Granule Content of Intestitial Cells of the Papilla in Rats after Salt Overloading (M ± m)

Group of rats	Weight of kidney (in g)	Diameter glomeruli (in μ)	Area of medulla: Total area of section	Area of papilla: Area of medulla	Height of epithelium of collecting tubule (in μ)	Index of granule content of cells
Experimental	2,5±0,15	108±0,5	0,24±0,005	0,42±0,01	6,7±0,3	194±12
Control	1,9±0,07	102±0,8	0,19±0,01	0,35±0,01	4,3±0,2	163±5
P	<0,01	<0,01	<0,01	<0,01	<0,01	<0,05

proximal tubules of the cortex also were slightly dilated and their epithelium was flattened. The diameter of the cortical glomeruli was increased, the basement membranes of the capillaries slightly thickened, and the PAS reaction was brighter.

An excess intake of sodium chloride produces certain functional changes in the kidney, leading eventually to high excretion of sodium required to compensate the sodium metabolism and to protect the animal against sodium overloading.

The increased sodium excretion in acute salt overloading is not directly connected with an increase in the level of glomerular filtration [9, 15], although the increased size of the glomeruli observed in the present experiments and also previously [1] during chronic salt loading in rats suggests that increased glomerular filtration evidently had some part to play in the compensatory sodium excretion under chronic experimental conditions.

One of the principal components of the "escape" response from sodium overloading is a decrease in the tubular reabsorption of sodium [9]. This is also reflected in decreased activity of oxidation-reduction enzymes in the epithelium of the proximal tubules in rats on a high sodium intake [1].

If reabsorption in the proximal tubules is reduced (and, possibly, if glomerular filtration is increased) the volume of fluid entering the distal part of the nephron and the collecting ducts is increased. Under these conditions the concentrating mechanism (the countercurrent system) of the renal medulla and the increased reabsorption of water in the collecting ducts of the renal papilla acquire compensatory importance. These factors as a whole determine the hyperplasia and widening of the renal medulla. The increase in mass of the renal papilla, in the size of the collecting ducts, and in the thickness of their epithelium indicates a state of hyperfunction of this structure and is evidence of increased reabsorption of water in rats on a high salt intake.

The need for increased reabsorption of water is obvious: with the excretion of large quantities of sodium (because of reduced reabsorption in the proximal tubules) the animal runs the risk of dehydration.

Quantitative changes in the granule content of the interstitial cells must probably be considered from the standpoint of their participation in the working hypertrophy of the renal papilla. Most investigators regard the granule content of the interstitial cells as a manifestation of their secretory activity, and they link it with the production of antihypertensive lipid agents [3, 10, 11, 14], including prostaglandins [12], in the medulla. At the same time, the topography of the cells which form a communication between the system of tubules and the blood capillaries of the renal medulla suggests that these cells play a transporting role in the countercurrent concentration system [11].

According to Tobian et al. [14], a decrease in the number of granules in the interstitial cells correlates with a decrease in the sodium and urea concentrations in the renal papilla. With this in mind, the increased granule content of the interstitial cells of rats kept on a high-salt diet can evidently be regarded as indirect evidence of an increase in the concentration gradient for sodium and urea in the renal medulla, thus accounting for the high osmolarity of its interstices. This last factor is crucial to the intensive reabsorption of water by the renal papilla.

LITERATURE CITED

1. A. M. Vikhert, Yu. V. Postnov, and N. M. Pozdnyunina, Kardiologiya, No. 11, 107 (1967).
2. Yu. V. Postnov, K. A. Fofanova, and G. A. Fedina, Kardiologiya, No. 11, 15 (1970).

3. R.E. Bulger and B. F. Trump, Am. J. Anat., 118, 658 (1966).
4. J. H. Dirks, W. J. Girksena, and R. W. Berliner, Fed. Proc., 24, 520 (1965).
5. J. Herzog, H. Johnston, and D. P. Lauler, Clin. Res., 14, 491 (1966).
6. R. B. Hickler, D. P. Lauler, C. A. Saravis, et al., Canad. Med. Ass. J., 90, 280 (1964).
7. H. Lapp and A. Nolte, Frankfurtz. Z. Path., 71, 617 (1962).
8. J. B. Lee, B. G. Covino, B. H. Takman, et al., Clin. Res., 13, 310 (1965).
9. N. G. Levinsky and R. C. Lalone, J. Clin. Invest., 42, 1261 (1963).
10. R. C. Muehrcke, A. K. Mandal, M. Epstein, et al., J. Lab. Clin. Med., 73, 299 (1969).
11. R. C. Muehrcke, A. K. Mandal, and F. Volini, Circulat. Res., 26, Suppl. 1, 1 (1970).
12. H. M. Nissen, Z. Zellforsch., 83, 76 (1967).
13. S. Sens, R. R. Smeby, and F. M. Bumpus, Biochemistry, 6, 1572 (1967).
14. L. Tobian, M. Ishii, and M. Duke, J. Lab. Clin. Med., 73, 309 (1969).
15. H. E. Wardenerde, J. H. Mills, W. F. Clapham, et al., Clin. Sci., 21, 249 (1961).