Sensitivity of the Renal Macula Densa to Urinary Sodium.* (30525)

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Experimental procedures that produce renovascular hypertension also increase the granularity of the juxtaglomerular cells (JGC), their renin content and the enzymatic activity of glucose-6-phosphate dehydrogenase (G6PD) and 6-phosphogluconic dehydrogenase (6PGD) of the macula densa (MD)(1-3). Sodium concentration influences these enzymes of the hexose-monophosphate shunt present in the MD. Excess dietary sodium reduces the activity of G6PD and 6PGD. A low-sodium diet enhances the enzyme activity. Both biochemically and histochemically the effect of sodium on G6PD *in vitro* is inhibitory(4).

Morphological and biochemical information suggests that the MD and JGC are functionally associated. The MD may act as a chemo- or osmoreceptor that transmits information to the JGC. We have tested this hypothesis by investigating whether the MD is sensitive to urine or blood electrolytes.

Materials and methods. Ten Wistar rats were fed a complete, well balanced diet and tap water *ad libitum*. Their weight was recorded. Thereafter the animals were placed in metabolic cages for 24 hours without food and with distilled water *ad libitum*. After this period a blood sample of 2 ml was drawn from the retroocular space and serum sodium and potassium were determined. The urine voided in 24 hours was analyzed for sodium and potassium, and its volume, pH and specific gravity recorded. Sodium and potassium determinations were carried on in a Baird-Atomic flame photometer using conventional methods.

At the end of the observation period the animals were killed by exsanguination, the blood collected for analysis and slices of the kidneys taken for routine histologic and enzymatic studies.

The MD was evaluated in hematoxylin and eosin stained sections, with the method previously described(5). The formula MD—OW was employed in which MD is the height in micra of the MD cells and OW is the height in micra of the opposite wall cells of the same tubule. The difference of MD minus OW represents the "specific height" of the MD, reflected morphologically.

G6PD was investigated with the method of Hess, Scarpelli and Pearse(6). Twenty MD in each kidney were estimated microscopically and assigned a number from 0 to 4 according to the average intensity of the diformazan precipitate.

Results. Only statistically significant information is presented. The urinary values given are the last obtained before sacrificing the animals.

The specific height of the MD was inversely correlated with the urinary sodium excreted in 24 hours, $r = -.78$; $p < .01$ (Fig. 1). The height of the MD was inversely correlated with the Na/K index of the 24-hour urine sample, $r = -.88$; $p < .008$. The use of the "specific height" of the MD improved the significance of this correlation, $r = -.95$; $p < .004$ (Fig. 2).

No correlations were found between the MD measurements and serum sodium or potassium values, urine volume or specific gravity. G6PD incubations did not show correlations with any of the variables investigated.

Discussion. Results derived from studying normal animals on a standard complete diet ought to reflect some basic mechanism by which sodium is handled. Since within normal limits variation in sodium intake would mostly be reflected in the urinary excretion, it is not surprising that blood levels did not

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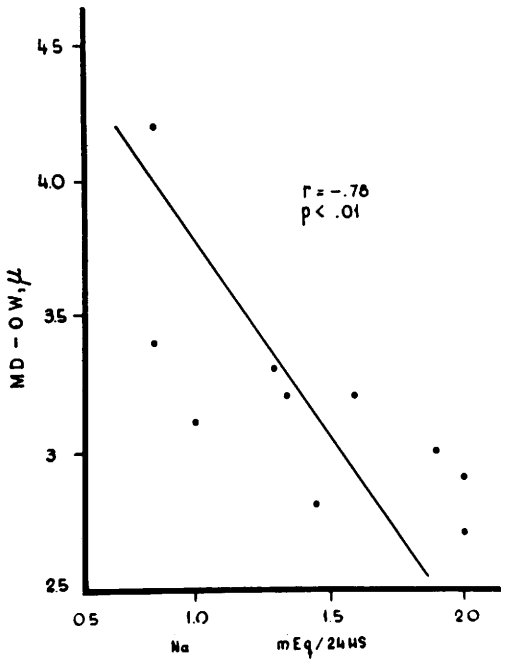

FIG. 1. Relationship between the specific height of the macula densa cells in micra and urinary excretion of sodium in mEq/24 hr is inverse and linear.

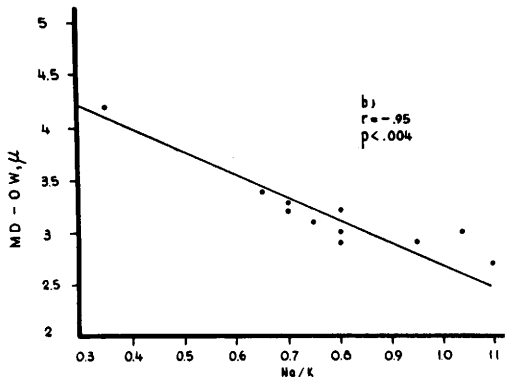

FIG. 2. A similar inverse relationship exists between specific height of the macula densa cells and ratio of urinary sodium to potassium.

correlate with any of the MD measurements. The correlations of high statistical significance found between the 24-hour urinary sodium excretion or Na/K index and the MD height can only be explained by accepting it as a highly sensitive receptor to small variations in the amount of sodium in the distal tubular fluid.

To recognize these minimal morphological changes requires an acceptable histologic method. It is evident that the MD becomes flattened as more sodium is eliminated *via* the urine. The same flattening of the MD occurs as the urinary Na/K index increases, indicating more sodium excretion. Urinary sodium itself is the most likely factor that affects the MD, since no correlation was found with blood electrolytes, urinary volume, specific gravity or potassium. The exact nature of this correlation has not been demonstrated, but since the MD cells belong to the distal convoluted tubule they evidently share its capacity to interchange tubular fluid sodium for intracellular potassium. Increased intracellular sodium and decreased intracellular potassium would lower the enzymatic activity of the hexose-monophosphate shunt. The less active cells would decrease in volume. The results obtained in this experiment thus correlate with existing information indicating an inhibitory effect of sodium on certain MD enzymes.

The high sensitivity of the MD to urinary sodium and the inhibitory effect of sodium on MD enzymes make it a convenient intrarenal chemoreceptor structure to assay the sodium that emerges with the distal tubular fluid from the critical region of renal medullary concentration. The intimately associated MD and JGC and the absence of other comparable morphologically specialized renal structures below the level of the MD would support the idea that the JGC receive signals from the MD. The association previously described between hypergranularity or increased renin content of the JGC and increased activity of the shunt enzymes in the MD strongly suggests that MD activity directly influences renin production.

Summary. Direct evidence indicates sensitivity of the renal macula densa to urinary sodium excretion. The macula densa is suggested to act as a chemoreceptor responsive to the amount of tubular sodium and transmitting signals to the juxtaglomerular cells.

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Urinary Excretion of Aldosterone in Dogs with Elevated Plasma Titers of Antirenin.* (30526)

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A substantial body of evidence supports the selective role of the renin-angiotensin system in the control of aldosterone secretion(1-5) and numerous investigations are concerned with the physiological mechanisms involved(4,6-9). The ability of antirenin, a neutralizing antibody to renin, to block *in vivo* the renin-angiotensin pressor system(2, 10), has suggested another avenue of approach to study the interactions of this mineralocorticoid control system. It would be anticipated that an inverse relationship would be demonstrable between these two parameters, *i.e.*, as antirenin titers rose, aldosterone secretion and excretion would diminish. Electrolyte clearance studies on dogs with elevated antirenin titers lent credence to this hypothesis, since these animals were found to excrete significantly larger amounts of sodium in their urine without alteration in renal hemodynamics, plasma electrolytes and potassium excretion(11,12). The present investigations were undertaken to substantiate possible correlations between the development of antirenin titers and aldosterone elaboration and to extend the observations contained in a preliminary report(13).

Methods. Six-hour urine collections were made in 7 normotensive female dogs which were trained to lie supine on padded animal boards for 8-hour periods. Food (Purina Dog Chow containing 0.5% NaCl) was removed from the animal cages on the eve of the experimental day. The following morning 20 ml/kg body weight of water (*via* stomach intubation) was given 5 minutes before the start of urine collection (time zero between 9:55-10:15 A.M.). To insure adequate hydration of the animal, additional water (10 ml/kg body weight) was given by intubation 2 hours and 4 hours after beginning urine collection. Plasma electrolytes and hematocrit were determined on venous blood samples taken 1½ hours and 5 hours after the start of the experiment. Hourly specimens of the urine obtained by bladder catheterization, which had been collected in ice-chilled containers, were refrigerated until termination of the collection. The hourly samples for each dog were pooled, the volume of the total 6-hour urine specimens measured and aliquots taken for electrolyte determination and aldosterone analysis.

Urine and blood data were collected for a 6-month period to establish control values. After this period, daily intramuscular injections of 5 Goldblatt dog units†/kg body weight of crude hog renin (1 unit/mg protein) from whole kidney were given to the animals to elicit formation of antibody(14). Plasma antirenin (AR) titers,‡ demonstrable within 3 to 4 weeks by bioassay, were maintained by daily administration of renin for a period of 20 weeks. Injections were discontinued after this 4½-month period and antibody titers were allowed to fall to zero

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† Definition: Goldblatt dog unit of Renin—That amount of renin which will produce a 30 mm Hg elevation of mean arterial blood pressure on intravenous injection into a trained unanesthetized dog.

‡ Definition: Antirenin unit (AR)—That amount of antibody capable of neutralizing completely the pressor response to 1 Goldblatt dog unit of renin.