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Renal Concentrating Mechanism: Possible Role for Sodium-Potassium Activated Adenosine Triphosphatase

Abstract. *Sodium-potassium activated adenosine triphosphatase activity was found to be almost twice as high in renal medulla as in cortex. Infusion of digoxin, a specific inhibitor of the enzyme, into one renal artery of the dog resulted in unilateral natriuresis, impaired concentrating capacity, and reduction of the enzyme activity in both cortex and medulla. It is suggested that the sodium-potassium adenosine triphosphatase plays an important role in urine concentration mechanisms.*

It is well established that the sodium-potassium activated adenosine triphosphatase (Na^+,K^+-adenosine triphosphatase) is intimately related to the transport of sodium across most biological membranes (*1, 2*). The kidney tubular epithelium is no exception. When cardiac glycosides, specific inhibitors of this enzyme, are injected into the renal circulation of the dog, inhibition of net sodium reabsorption occurs (*3*). The sodium diuresis that results from intrarenal administration of these preparations has been attributed to inhibition of sodium reabsorption in portions of the nephron localized in the cortex (proximal and distal convolutions) (*3*). We have recently demonstrated (*4*), however, that in the dog digoxin inhibits sodium transport in the ascending limb of Henle's loop, since it diminished both free water clearance (C_{H_2O}) and free water reabsorption ($T^c_{H_2O}$). Since the loop of Henle is anatomically located in the medulla, our results suggested that if the Na^+,K^+-adenosine triphosphatase is a receptor for cardiac glycosides, the enzyme may be of primary importance in the mechanism of sodium transport in the medulla. Therefore, this enzyme system may contribute significantly to renal concentrating and diluting functions.

Mongrel dogs of either sex were utilized for all the experiments. Under pentobarbital anesthesia, each ureter was cannulated through a suprapubic incision. Through a flank incision, a curved needle was introduced into the left renal artery, into which normal saline or saline containing digoxin in a concentration of 0.01 mg/ml was infused at 1 ml/min. In four control dogs the saline infused into the renal artery did not contain digoxin. A comparison between the results in these control animals and the experimental animals demonstrated that the infusion of digoxin into one kidney did not significantly affect the contralateral organ either physiologically or biochemically; therefore, the latter is adequate as an internal control. All animals received 5 units of Pitressin in oil intramuscularly 1 hour prior to the experiment in addition to aqueous Pitressin (60 milliunits per kilogram of body weight per hour) intravenously, throughout the duration of the experiment. The clearance of iothalamate iodine-125, present in the sustaining infusion, was utilized as an index of glomerular filtration rate (*5*). All animals were undergoing a modest hypertonic (10 percent) mannitol diuresis; infusion rate was 1.0 ml/min. Control urine samples were obtained from each kidney, after which the infusion of digoxin was begun. An increase in urine flow in the experimental kidney was maximum between 60 and 105 minutes after the infusion of digoxin. In control animals, a waiting period of at least 90 minutes was observed before terminating the experiment. When the maximum digoxin effect was obtained, the kidneys were removed and separated into cortex and medulla without any attempt to separate the latter into an inner and outer portion. The tissue was processed for Na^+,K^+-adenosine triphosphatase activity as previously described (*2, 6*). The assay was carried out by a spectrophotometric procedure (*7*).

In the control dogs, the Na^+,K^+-adenosine triphosphatase specific activity in medullary tissue was approximately twice [72 ± 5 (S.E.M.) μmole of P_i per milligram of protein per hour] that found in the cortex [41 ± 4 (S.E.M.) μmole of P_i per milligram of protein per hour]. Similar values were observed for the internal control renal tissues (uninfused kidney) (Table 1). Furthermore, in all instances the administration of digoxin decreased the enzyme activity in both cortex (proximal and distal convolution) and medulla (loops of Henle). The inhibition appeared to be associated with a sharp rise in the fraction of filtered sodium excreted [C_{Na}/100 ml glomerular filtration rate (GFR)] and an increase in osmolar clearance (C_{osm}/100 ml GFR), three to four times that of control (Table 1). Under these circumstances, free water reabsorption rose only slightly. This failure of $T^c_{H_2O}$ to rise

Table 1. Effect of digoxin on the kidney: functional and biochemical correlates. C_{Na} is clearance of sodium; C_{osm}, osmolar clearance; and $T^c_{H_2O}$, free water reabsorption. All renal function terms were corrected for 100 ml GFR and expressed as percentages. Each figure represents the mean values ± S.E.M. of five experiments. The enzyme activity is expressed in micromoles of inorganic phosphate per milligram of protein per hour.

Test	Renal function			Na^+,K^+-adenosine triphosphatase activity			
	Control		Digoxin	Cortex		Medulla	
	Right	Left	Left	Control	Digoxin	Control	Digoxin
C_{Na}	1.8 ± 0.1	2.0 ± 0.2	9.3 ± 0.7*				
C_{osm}	4.2 ± 0.4	4.3 ± 0.5	14.3 ± 2.0*				
$T^c_{H_2O}$	2.6 ± 0.2	2.5 ± 0.3	3.5 ± 1.0	35 ± 5	20 ± 5*	91 ± 27	44 ± 27*

* The mean of the differences is statistically significant at $.001 < P < .01$.

in the face of mounting osmolar clearance indicates that $T^c_{H_2O}$ was inhibited from 40 to 70 percent of its expected value at that level of C_{osm}.

The demonstration of in vivo inhibition of both cortical and medullary adenosine triphosphatase at a time when net sodium reabsorption by the kidney was also depressed suggests that in both these regions the enzyme may participate in active sodium transport. The depression in $T^c_{H_2O}$ [in view of our demonstration that digoxin inhibits renal diluting capacity (*4*)] further indicates that the Na^+,K^+-adenosine triphosphatase plays a principal role in renal concentration and dilution. Since the bulk of sodium transport is believed to occur in the cortex (*8*), the finding of a significantly and reproducibly higher Na^+,K^+-adenosine triphosphatase activity in the medulla requires explanation. There are two possibilities: (i) the rate of sodium transport in this region is greater than in the cortex, and, therefore, there is a requirement for a more active transport enzyme and (ii) the medullary site may represent an area of the kidney which transports Na^+ against a concentration gradient significantly higher than any other area in the organ. Although it is impossible at present to distinguish between the alternatives, current data would support the latter as the most likely explanation. These data represent additional evidence that Na^+,K^+-adenosine triphosphatase is an important pharmacological receptor for cardiac glycosides (*7*).

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Separation of Type 2 Toxins of Vibrio cholerae

Abstract. *Choleragenic toxin was separated from vascular permeability factor by ion-exchange chromatography of supernatants of dialyzed peptone cultures of* Vibrio cholerae. *The choleragenic toxin eluted from columns of QAE-Sephadex with low-ionicity systems is free of permeability factor activity. Further elution of these columns with 0.5*M *NaCl removes both the permeability factor and residual choleragenic toxin. When this latter material is chromatographed on columns of carboxymethyl-Sephadex, the permeability factor toxin is eluted by 0.02*M *phosphate buffer and is free of choleragenic activity. Therefore, choleragenic and permeability factor activities of the type 2 cholera toxins are different and can be separated by these procedures.*

Cholera in man is considered an intoxication because the vibrios appear to be confined to the intestinal lumen and do not invade the tissues. It is now recognized that, in addition to the endotoxin, *Vibrio cholerae* produce exotoxins which can induce in animals an acute diarrheal disease resembling that of human beings. These choleragenic toxins are found in culture filtrates as well as in the stool of patients with acute cholera. According to the classification and interrelations of the several cholera toxins, reviewed by Burrows (*1*), those most likely to take part in the pathogenesis of cholera are designated type 2. These toxins may be demonstrated by their capacity to induce fluid accumulation in the small bowel of the infant rabbit (*2*) or in the ligated ileal loop of the adult (*3*), and by increased vascular permeability when injected intradermally in rabbits (*4*).

It is important to distinguish those toxins which truly play a role in pathogenesis and to devise effective methods for assessing their presence and immunogenic potency. Although the fluid-inducing toxins seem to be directly related to the disease process, the status of the vascular permeability factor (PF) is somewhat uncertain in its relation to choleragenic toxin and to the pathogenesis of cholera. Only a few investigators studied both choleragenic activity and PF in the same toxin preparation. Evans and Richardson (*5*) described the production, in liquid cultures, of toxin choleragenic for the infant rabbit and vascular permeability factor. In a semisynthetic medium which gave high yields of PF, their cultures also elaborated choleragenic toxin. They further purified these toxins by precipitation with dextran sulfate (*6*) and again observed high PF activity associated with choleragenic activity, although exact quantitation of the latter was not attempted.

By the use of chromatography on ion-exchange polymers, we have shown that the choleragenic and PF activities can be separated from one another and, therefore, do not appear to be two manifestations of the same toxin.

Choleragenic toxin was titrated in the ligated ileal loop of young adult rabbits by a modification of the methods developed in Burrows' laboratory (*7*). The toxin unit we have used is that amount which results in fluid accumulation of 1.0 ml per centimeter of intestinal loop. Although this system gives results approximating those of Burrows' group, it is somewhat less precise.

The vascular permeability factor was titrated by the methods of Craig (*4*). The test material was injected in 0.1 ml amounts intradermally in the rabbit, 18 hours later the rabbit was given an intravenous injection of Niagara sky-blue dye. The toxin unit is the bluing dose and is defined as that amount of toxin which yields a blue area, at the injection site, with a mean diameter of 0.7 cm.

Crude toxin was produced in dialyzed peptone medium with *Vibrio cholerae* 569B according to the procedure of Coleman *et al.* (*7*). The cultures were grown for 6 hours on a rotary shaker at 37°C, the cells were removed by centrifugation, and the supernatant was sterilized by membrane filtration. This peptone supernatant was concentrated by flash evaporation and exhaustively dialyzed to remove medium constituents. Final concentration was 50- to 100-fold over the original culture filtrate.