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EFFECT OF ALDOSTERONE ON THE SODIUM CONTENT AND ENERGY METABOLISM OF EPITHELIAL CELLS OF THE TOAD URINARY BLADDER

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SUMMARY

Measurement of the electrolyte content of isolated epithelial cells of the toad urinary bladder indicates that vasopressin and aldosterone stimulate sodium transport by accelerating a rate limiting step for sodium entry into the cell across the mucosal cell membrane.

ALDOSTERONE stimulates active sodium transport across epithelial membranes such as the toad urinary bladder[1]. Although there is general agreement that the mechanism of action of the steroid involves induction of the synthesis of specific protein (s)[2–5], the site at which the induced protein acts to stimulate transport has not been established. The transport of sodium from the solution bathing the urinary surface of the bladder to the solution bathing the blood surface involves movement of the cation across at least two cell membranes, either of which may be the site of the rate limiting step that is affected by aldosterone.

The first step in the transport of sodium across the bladder is the entry of sodium into the cell across the cell membrane at the urinary surface, a step generally assumed to be passive. Sodium then moves from the cell across the membrane bordering the blood surface, a step generally attributed to an active pump. This study was designed to determine which of the two steps is stimulated by aldosterone. If the hormone increases transbladder flux of sodium primarily by stimulating movement across a rate limiting barrier at the urinary surface, the amount of sodium within the cell would rise. Alternatively, if the hormone accelerates a rate limiting step in the cell membrane at the blood surface of the cell, there would be a fall in the sodium content of the cell. In contrast, if an agent depresses the rate of sodium transport by inhibiting either of the two steps, the opposite changes in cell sodium would occur. Thus, by measuring the content of sodium within the transporting cells, the predominant site of action of a hormone or of an inhibitor of sodium transport can be determined.

This approach has been used in intact bladders by several investigators but interpretation of the results has been difficult. The toad bladder is not a single layer of transporting cells but in addition consists of a thick stroma of connective tissue, blood vessels, and smooth muscle bundles. There is no certain way of determining whether changes in the sodium content of the whole tissue reflect change within the transporting cells or within the supporting stroma. The problem

was avoided in this study by the use of a preparation of pure epithelial cells. Experiments were begun by perfusing the aorta with Ringer solution to remove red blood cells from the vasculature of the bladder. After incubation with collagenase in a modification of the method of Gatzy and Berndt[6], sheets of epithelial cells were scraped off the bladder and transferred to an erlenmeyer flask for incubation and experimental manipulation.

We have also used this technique to study the effect of three other agents, namely vasopressin, ouabain, and amiloride, which affect sodium transport by the toad bladder through mechanisms that do not involve protein synthesis. As will be seen the results of experiments with these agents are as predicted by the model on the basis of information from other independent studies of the site at which they affect sodium transport.

Each of the two hormones and two inhibitors used in this study produces a characteristic effect on the rate of sodium transport as a function of time. After addition of vasopressin, which acts by stimulating the production of cyclic-AMP [7], the rate of sodium transport rises to a peak in 10–15 min and then declines slowly. Aldosterone has no effect on sodium transport for at least an hour. Then the rate rises and remains elevated for over 14 h. Ouabain, a known inhibitor of the active sodium transport pump, causes a slow decline in the transport rate so that in an hour a new steady state rate is reached that is about 20% that of controls. The diuretic amiloride causes a rapid decline in the rate of sodium transport to about the same level as ouabain. In the experiments to be described, cells were prepared and incubated in Ringer solution for an hour before the addition of vasopressin, ouabain or amiloride. Cells were collected for the measurement of cell electrolyte content at the time of the maximal effect on sodium transport, i.e. 15 min after addition of vasopressin, and at least one hour after the addition of an inhibitor. In the aldosterone experiments, intact bladders were incubated with aldosterone for 14 hr, the cells were removed and then incubated with aldosterone for an additional two hours. In other experiments, the effect of agents on the metabolism of the cells was examined at this same period. Paired control and experimental tissue from the same bladder was used in all experiments. Each of the agents had an effect on oxidative metabolism similar to that seen in intact tissue. For example, in the cell preparation, aldosterone reduced flow through the hexose monophosphate shunt as estimated by the difference in the rate of oxidation of [^{14}C]-1-glucose compared to [^{14}C]-6-glucose, and increased the rate of oxidation of the latter, as in the intact bladder[8, 9].

The effect of ouabain will be considered first since there is general agreement that this agent inhibits transport by inhibiting an active pump step located at the blood surface of the cell. In nine paired experiments 10^{-4} M ouabain caused a 46 percent increase in cell sodium and a 19 percent decrease in cell potassium content (both $p < 0{\cdot}001$). The change in sodium content is the result anticipated from inhibition of sodium transport across the basal cell membrane.

10^{-5} M amiloride inhibits sodium transport to the same extent as 10^{-4} M ouabain. In contrast to the results with ouabain, the cells contained 33% less sodium than paired controls ($p < 0{\cdot}005$). This pattern would result from inhibition of a step in transport at the urinary surface of the cell.

Studies employing other experimental techniques have been interpreted as indicating that vasopressin increases net sodium transport by stimulating a rate-limiting step located at the urinary surface of the bladder[10, 11]. In seven paired cell

preparations, vasopressin caused a significant increase (63%) in the sodium content of the cells, a small decrease (11%) in the potassium content and a small increase (16%) in cell water (all $p < 0{\cdot}001$). Similar results have been reported by MacKnight *et al.*[12]. In the context of the model discussed earlier, the increase in cell sodium is interpreted as indicating that vasopressin stimulates a step in transport that is located at the urinary surface of the bladder, in agreement with the other studies[10. 11].

The changes in cells incubated with 10^{-7} M aldosterone are similar to those noted with vasopressin. There was a large increase (96%) in cell sodium ($p < 0{\cdot}001$) and a small (6%) increase in cell water ($p < 0{\cdot}01$). The increase in sodium content is interpreted as resulting from an effect of aldosterone on sodium transport at the urinary surface of the cell. The increase in water content of the cells treated with aldosterone or with vasopressin is probably secondary to the osmotic effect of the increase in the electrolyte content of the cell.

Using this epithelial cell preparation, we have examined another aspect of the effect of vasopressin and aldosterone on sodium transport, that is, the relationship between the supply of metabolic energy and the rate of sodium transport. It is well established that there is tight coupling between sodium transport and energy metabolism in this tissue[3. 13–15]. It has been suggested that hormones may stimulate sodium transport primarily by increasing the flow of metabolic energy to an energy limited step in transport[16]. Were this the mechanism of hormone action, the cell content of high energy compounds such as ATP, or the high energy reservoir, phosphocreatine, should be elevated when sodium transport is stimulated by the hormone. On the other hand, if transport is not energy limited, and the hormone stimulates sodium transport through another pathway, the increased active transport, which utilizes energy, should deplete the cell of these high energy compounds until they are replenished by glycolytic and oxidative metabolism and a new steady state level is reached. We have examined the effect of vasopressin and of aldosterone on these high energy compounds, at the time of the peak effect on sodium transport, the same time at which cell electrolytes were measured in the preceding experiments.

Vasopressin had no effect on the concentration of ATP or ADP, but the concentration of phosphocreatine was decreased in hormone treated cells in association with a reciprocal increase in the concentration of creatine. These results indicate that the effect of vasopressin on energy metabolism does not initiate the increase in sodium transport by providing more energy to an energy limited transport system, but rather that primary stimulation of sodium transport results in increased energy metabolism.

Similar results were obtained with aldosterone. There was a fall in the concentration of phosphocreatine and a rise in that of creatine in cells incubated with the hormone. The results of these experiments are interpreted similarly. They are incompatible with the suggestion that aldosterone stimulates sodium transport primarily by increasing the metabolic energy available to support transport.

In summary, the fall in the concentration of phosphocreatine in the last two series of experiments demonstrates that sodium transport by the toad bladder is not energy limited, and that neither vasopressin nor aldosterone increases the rate of sodium transport primarily by increasing the supply of metabolic energy. The effect of vasopressin and aldosterone on the cell content of sodium indicates that each hormone increases transbladder sodium transport predominantly by

accelerating the movement of sodium across a rate limiting barrier at the urinary surface of the cell.

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DISCUSSION

Snart: Can I ask you if you have followed ATP in the bladder during the self-inhibitory part of ADH action?

Handler: No, we have not.

De Weer: Dr. Handler, can you translate your ATP content figures into mmol/l?

Handler: ATP is about 5×10^{-3} M in cell water.

De Weer: Dr. Snart, maybe you have an idea about the K_m (for ATP) of the toad bladder ATPase.

Snart: No.

Jørgensen: I may help to answer this question. The apparent K_m for the hydrolysis of ATP by $(Na^+ + K^+)$-ATPase is about 0·1–0·2 mM, i.e. more than 10 times lower than the estimated concentrations of ATP in cell water. Recently, ATP binding studies have shown that the dissociation constant for the enzyme-ATP complex is very low, less than 1 μM. (J. G. Nørby and J. Jensen: *Biochim. Biophys. Acta* **233** (1971) 104).

Fanestil: A quick calculation shows that the Na concentration in cells before vasopressin was about 46 μEq/ml. Now, if one increases mucosal Na concentration from 46 to 113 μEq/ml, one does not get much of an increase in sodium transport. My question is: how can one stimulate sodium transport just by raising intracellular Na above 46 μEq/ml?

Handler: You can't measure sodium transport in the cell preparation. When we scrape the cells off the bladder they come off as a sheet, but we haven't been able to set them up and measure short circuit current. However, when we add vasopressin or aldosterone or an inhibitor of sodium transport, oxygen consumption or other manifestations of energy metabolism behave as though they were coupled to what we think is happening to sodium transport.

Fanestil: That is the question: whether or not the concentration of sodium awaiting transport from within the cell is 46 mM.

Handler: I wouldn't necessarily say that the 46 is the transported sodium but rather that the change in sodium in cells incubated with vasopressin or aldosterone is a change that is related to the stimulation of sodium transport in these cells.
Fanestil: So you would postulate compartmentation of intracellular sodium?
Handler: That of course is a possibility.
Edelman: The electrolyte measurements after 2 h of incubation of your scraped cells in vitro and the CP/C ratio remain the same under the same circumstances?
Handler: Exactly the same.
Edelman: Unfortunately, the results obtained by my colleague, Peter Lipton, differ in important respects from yours. In scraped epithelial cells either analyzed directly or after incubation *in vitro*, neither vasopressin nor aldosterone produced significant changes in intracellular Na^+ or K^+ concentrations. The corresponding effects on Na^+ transport produced about a two-fold increase in short-circuit current. In addition, I believe the results from Dr. Leaf's laboratory yielded much smaller changes in Na^+ concentration (less than 20%) at a time when short-circuit current was increased perhaps two-fold.
Leaf: These were rather small changes in current we registered, in the range of 30–40%.
Edelman: We are still faced with a discrepancy in changes in Na^+ concentration (10–20%) and changes in short-circuit current of twice this magnitude, whereas Dr. Handler records increases in Na^+ concentration of 50–100%.
Handler: Our results are not completely different from those from Boston, at least not from those they published regarding the action of vasopressin. I too am not sure why these differences exist.
Crabbé: Obviously, there is room for further discussion—and data—on this question! Maybe we'll take it up for our next meeting: What about organizing it in San Francisco, Dr. Edelman?