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THE SITE OF THE ALDOSTERONE INDUCED STIMULATION OF SODIUM TRANSPORT*

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SUMMARY

The content and concentration of major ions within the scraped mucosal epithelial cells of the urinary bladder of the toad *Bufo marinus* were determined using [^{14}C]- and [^{3}H]-inulin to correct for adherent extracellular fluid. In aldosterone treated hemi-bladders the radioactive sodium content and concentration increased significantly as compared with paired control hemi-bladders when both tissues were exposed to [^{24}Na] in the mucosal bathing medium. Changes in total non-inulin space sodium were not detected but the stimulation of transepithelial sodium transport by aldosterone in this set of observations was small, averaging only some 30% above control. The results are compatible with our hypothesis that the major action of aldosterone is to facilitate the entry of sodium from the mucosal medium across the apical permeability barrier of the transporting mucosal cells.

INTRODUCTION

THE IMPORTANCE of adrenal hormones in preservation and regulation of the sodium content of the body fluids has been appreciated since the early studies of Marine and Bauman in 1927 [1]. It was not until 1953, however, that the active principle of the adrenal cortex, aldosterone, was discovered independently by three groups [2–4].

Direct physiological effects have been established in the mammal on kidneys, intestines, sweat and salivary glands, all of which reabsorb sodium salts. Although such oriented transepithelial transport of sodium may utilize the active transport mechanism believed to be present in all animal cells and which serves to maintain the characteristic low intracellular content of sodium, there is yet little evidence that aldosterone affects this universal cellular transport mechanism. A limitation of aldosterone to an effect on oriented transepithelial transport systems may indicate that aldosterone does not act on the active component of the transport system but rather on those modifications responsible for the directed transepithelial transport, i.e. the permeability properties of the plasma membrane at the lumenal or apical surface of the epithelium.

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The possibility of examining the details of the manner by which aldosterone affects sodium transport was greatly enhanced when Crabbe in 1961 demonstrated an effect of aldosterone *in vitro* on sodium transport in the toad bladder [5].

SODIUM TRANSPORT IN THE TOAD URINARY BLADDER

This tissue has been referred to extensively in this Symposium. For the present discussion the point needs only to be made that, though there are several types of cells within the mucosal epithelial layer, two major cell types predominate. Granular cells comprise some 85% of the cells and mitochondria-rich cells another 10% of this layer. There are also basal cells intercalated between the basal aspects of these cells and the fine limiting basement membrane, and scattered goblet cells are present. Careful serial section and examination with the electron microscope [6] supports the view that the functional epithelium is a single layer of cells and every cell with a surface exposed to the urine within the bladder has also a direct contact with the basement membrane. This morphological evidence is consonant with the physiological conceptualization of the transport process from which it follows that to understand the transport of sodium we must be able to explain how this ion crosses a double series permeability barrier. The two barriers are identified anatomically with the apical and the baso-lateral plasma membranes of the single layer of cells in this epithelial layer. Schematically this physiological conceptualization of the transport process for sodium can be depicted, as shown in Fig. 1. The presence of the two series permeability barriers is established by the two-step potential profile obtained when a microelectrode is

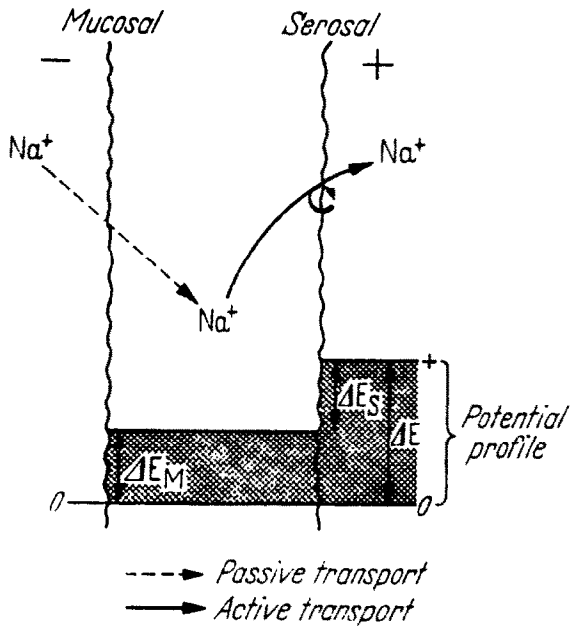

Fig. 1. Schematic representation of the transporting epithelium of the urinary bladder of the toad. The two vertical lines depict the apical and the basolateral plasma membranes of the mucosal layer of cells, the presumed two permeability barriers which the sodium ion must cross during transepithelial transport of this ion. The characteristics of these barriers and the intervening compartment are described in the text.

advanced from the mucosal medium through the apical plasma membrane and finally through the baso-lateral cell membrane into the submucosa and serosal medium. Each potential step is positive to the mucosal medium and the characteristic transepithelial potential is the sum of these two potential steps [7].

This model is supported by a considerable number of physiological studies which have sought to examine the characteristics of the mucosal and serosal permeability barriers as well as the compartment which they enclose. This compartment is often referred to as the "active sodium transport pool." Briefly the current evidence may be summarized as follows:

(A) The mucosal permeability barrier (the apical plasma membrane of the mucosal epithelial cells)

1. Sodium has been thought to move down-hill along a concentration gradient into the cell interior [8]. However, it is appreciated that the concentration of sodium in the "active transport pool" is unknown and recent studies by Biber *et al.*[9] and by Rotunno *et al.*[10] have suggested that sodium may move up-hill in penetrating the analogous outer barrier of frog skin when the concentrations of sodium are low in the outer bathing medium.

2. Even if sodium ions cross the mucosal permeability barrier passively they do not do so by simple diffusion. The entry step shows characteristics of saturation kinetics indicating that the ion interacts with one or more components of the mucosal barrier as it penetrates [8]. Other ions, i.e. Li, K and guanidinium can specifically compete with sodium ions for this entry step [11]. One diuretic, amiloride, blocks transepithelial sodium transport by preventing this first step of the transport process [12].

3. It seems likely that sodium moves across the mucosal permeability barrier bound to a transport protein, a permease, but thus far any such carrier molecule has escaped detection and, if it exists, is present at exceedingly low concentrations.

(B) The serosal permeability barrier (the baso-lateral plasma membrane of the mucosal epithelial cells).

1. Sodium is transported actively up a chemical concentratient gradient from cell interior to serosal medium and against an electrical gradient, as well [13]. Thus, this is an energy-requiring step and presumably the site where energy from metabolic processes is applied to the transport process.

2. The active transport of sodium across the serosal permeability barrier is an electrogenic process responsible for the electrical potential jump at this barrier [13]. By "electrogenic" is meant the active transport of sodium ions without obligatory coupled movement of another cation in the opposite direction or of an anion in the same direction. The electrical potential arises directly from charge separation when sodium ions are extruded from the cell.

3. The transport process at this site is thought to be via the sodium-potassium activated adenosine triphosphatase which is abundant in this tissue. The inhibition of transepithelial transport of sodium by ouabain is consistent with this assumption.

(C) The "active transport pool of sodium" (the sodium en route from mucosal to serosal media which has crossed the mucosal permeability barrier but has not yet traversed the serosal barrier).

1. Within 20 min [^{24}Na] added to the mucosal medium has achieved a constant specific activity in the sodium emerging into the serosal medium. By measuring

the radioactivity in the tissue (counts per minute) and dividing by the specific activity of [^{24}Na] in the mucosal medium (counts per minute per meq of sodium), one calculates a transport pool representing the amount of sodium having the specific activity of the mucosal medium which is somewhere within the tissue. This estimate has had little credibility as a measure of the "active transport pool of sodium" for several reasons:

a. Its location within the tissue was unknown and there was considerable suspicion that it represented sodium which had already crossed both permeability barriers[14].

b. There was no assurance that the real transport pool was not enlarged by entry of sodium from the serosal medium as well. The sodium in the noninulin or nonsucrose space of the tissue exceeded the isotopically measured active transport pool of sodium several-fold[8]. How much of this excess sodium was associated with submucosal structures, however, was unresolved.

2. Recently Macknight and associates[15, 16] have reinvestigated the active transport pool of sodium analyzing just the composition of the mucosal cells after scraping them from the submucosa and correcting for intercellular fluid with [^{14}C]-inulin and [^{3}H]-inulin*. When bladders are incubated in Ringers solution containing [^{24}Na], all sodium within the scraped mucosal cells equilibrates with [^{24}Na] in less than 30 min. After 1 h [^{24}Na] added just to the mucosal medium equilibrates with a constant amount, 23%, of the sodium in the non-inulin space of the scraped cells, while [^{24}Na] added only to the serosal medium achieves a steady state distribution of some 78% of cell sodium. This result could be compatible with a single compartment in the cell into which sodium from the serosal medium had access (or exchanged) three times as rapidly as sodium derived from the mucosal medium. However, when a sodium-free choline Ringers solution was used as the mucosal medium, ^{24}Na in the serosal medium distributed in exactly the same amount of cell sodium as it had when sodium transport was present but not all cellular sodium was uniformly labeled. With no sodium entering from the mucosal medium, the [^{24}Na] in the serosal medium was expected to equilibrate with all the intracellular sodium, if all intracellular sodium were in a single compartment. Furthermore, blocking mucosal entry of sodium with amiloride gave similar results. The inhibition of sodium transport by ouabain is associated with a large gain of cellular sodium and loss of potassium. Removing sodium from the mucosal medium markedly reduced the gain in cell sodium indicating that this sodium derives largely from the mucosal medium. The conclusion from these studies is that there are at least two cellular sodium compartments: one accessible to sodium entering the mucosal cells through their apical surface (the "active sodium transport pool") and the other entered across the baso-lateral surface by sodium from the serosal medium. Very little exchange of sodium occurs between these compartments.

EFFECT OF ALDOSTERONE ON THE "ACTIVE SODIUM TRANSPORT POOL"

As indicated some years ago[17] this physiologic model of the sodium transport process allows a simple test of the site of action of any substance which affects the rate of transepithelial sodium transport. One or the other of the two

*[^{14}C]- and [^{3}H]-inulin measures an extracellular space which is approximately ¼ smaller than [^{3}H] or [^{14}C]-sucrose. The conclusions, however, regarding compartmentation of sodium are the same using inulin or sucrose.

rate limiting permeability barriers must be affected by the substance. An agent such as aldosterone which enhances transepithelial transport of sodium may do so by accelerating the entry step across the mucosal barrier in which case the sodium content of the active transport pool must increase, or alternatively, increase the rate of exit of sodium across the serosal barrier in which case the sodium content of the active transport pool must fall. Obviously both effects might occur with no detectable change in the pool.

When this test was performed using paired half bladders from saline-soaked toads the expected stimulation of sodium transport, as shown in Fig. 2, was obtained. Table 1 shows the water, sodium, chloride and potassium content of the scraped epithelial cells. Only in the case of the [^{24}Na] measurements was there a difference between the aldosterone treated and control half bladders. This estimate, however. of the active sodium transport pool showed it to be significantly increased whether expressed as content, concentrations or percent of cell sodium which equilibrated with the [^{24}Na] from the mucosal medium. The increase in the active sodium transport pool in the aldosterone treated tissue is consistent with our other evidence[18] that the major action of this hormone in enhancing sodium transport is to facilitate the entry of sodium from the mucosal medium across the apical permeability barrier. It is appreciated that, though the changes seem real, they are quite small but so was the stimulation of sodium transport produced by aldosterone in this set of experiments. We are repeating these observations in bladders which show a more marked response to aldosterone to see whether the accompanying tissue electrolyte content will also show larger changes.

If the hypothesis we have heard earlier in this Symposium regarding the role

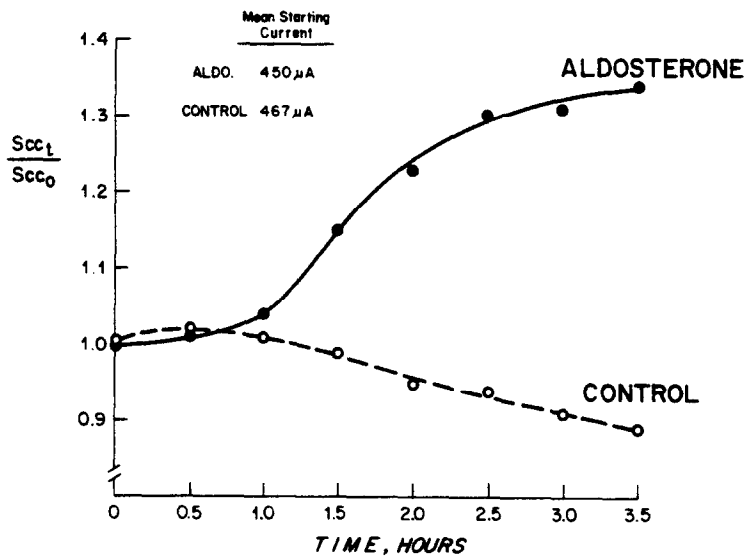

Fig. 2. The mean stimulation of sodium transport by aldosterone for the tissues, the composition of which is shown in Table 1. Results are expressed as the ratio of the short circuit current at time (t) following addition of aldosterone to that at the time (t_0) of addition of the hormone for the upper curve; the lower curve shows the comparable ratios for the paired untreated half bladder. Note that the hormonal stimulation in this set of observations was small, averaging only some 30% above the control.

Table 1. Effect of aldosterone (10^{-7}M) on the composition of mucosal cells of toad bladder

	Water (kg/kg d.w.)	Na	^{24}Na* (meq/kg cell water)	K	Cl	Na† labeled (%)
Control	3·75	44	13	133	52	29
Aldosterone	3·81	47	16	131	63	34
	0·06	3	3	−2	11	5
SEM Δ	±0·017	±4	±1	±5	±4	±0·7
P	> 0·7	> 0·3	< 0·02	> 0·7	< 0·5	< 0·001
($n = 7$)						

*^{24}Na is the concentration of sodium in the non-inulin space of the scraped mucosal cells which had a specific activity equal to that of the sodium in the mucosal bathing medium to which ^{24}Na had been added.

†"Na labeled" is the percent that the "^{24}Na" constitutes of the total sodium concentration within the non-inulin space water of the scraped mucosal cells.

of aldosterone to induce DNA-dependent RNA synthesis is correct—and it is supported by numerous observations on the effects of inhibitors but, thus far, not convincingly supported by direct observations on synthesis of new RNA or protein—then we would submit that the role of such new protein is as a "permease" enhancing the entry of sodium from urine across the apical permeability barrier into the cell.

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DISCUSSION

Alberti: Do you correlate the different pools with different cell types?

Leaf: There doesn't seem to be any cell type which corresponds in volume with the two compartments I have just described. Initially we had suspected that the basal cells might constitute the serosal compartment but they are quantitatively insufficient. The granular cells are the ones that respond to vasopressin. This seems to be the case at least with respect to water transport. The interesting

observation by Dr. Voûte, that the response to aldosterone might be located in the mitochondria-rich cells, is intriguing but we have no direct evidence to support it. Thus there is little basis at present to relate the two sodium pools to specific anatomical structures in the bladder.

Cereijido: In experiments with the frog skin we have observed that the sodium transporting compartment is only a very small fraction of the total sodium in the epithelium (*J. Physiol.* **196** (1968) 237). The sodium compartment labelled from the inside is different from the one going through the transporting pool and which is only labelled from the outside. The frog skin has intercellular spaces which are open toward the inside and closed toward the outside. We thought that part of the Na labelled from the inside might be contained there. Do you have any suggestions on the location of the Na compartment labelled from the inside in the case of the urinary bladder?

Leaf: We do have intercellular spaces closed by the basement membrane but in the collagenase and scraped cell experiments the basement membrane has gone and has already been shown to be permeable to even large molecules. We think that the inulin, mannitol or sucrose would correct for any sodium in the intercellular space. So we are quite sure that we are not dealing with sodium which is trapped between cells. Whether it is the nuclei which are the site of highest label uptake from the serosal side, I don't know. We have no single cell type which could account for the serosal versus mucosal pool.

Edmonds: Do you have any information or evidence about the kinetics of the different pools? As I showed yesterday, in the rat colon, the pool accessible from the luminal side of the gut must have an extremely fast turnover with a half time of the order of 1½ min or so. And it is likely that there exists in the gut epithelium at least one other pool with very different kinetics of sodium turnover. Do you have any information of this sort?

Leaf: We do know from earlier studies that the small mucosal pool is the active transport pool with a very rapid equilibration with radioactive sodium placed on the mucosal side and with a very fast (half time about 2 min) appearance of radioactivity on the other side, not quite as rapid as you reported for the rectum.

Crabbé: Could the quantitative differences between the data on cell ionic composition you have just presented and those of Dr. Handler result from variations in technique used? Could it be, for instance, that one method yields a larger proportion of epithelial cells as individual cells?

Leaf: I did show you the electronmicrographs of the tissue we have been using, the scraped tissue I mean. It comes off as sheets whether treated with low calcium or collagenase. So I think we are working with the same preparation. Dr. Handler has a lower content in the control tissues which might be the reason why his aldo treated group appears to be so high in sodium content. I would like to hear his comment about this and there might be something about the aldosterone working as a glucocorticoid which might help keep the tissue intact and maintain the higher normal sodium content.

Crabbé: May I ask you something else? With isolated cells you seem to confirm what you have reported 10 years ago for the intact toad bladder, regarding the changes in oxygen consumption observed during exposure to vasopressin. While the latter brings about a stimulation of sodium transporting activity which is short-lived, oxygen consumption remains high for a couple of hours at least. Obviously, aerobic metabolism is not linked only to active sodium transport in

this tissue, but the discrepancy is rather striking: Maybe you have an explanation for this?

Leaf: That is the problem that has worried us for years and I still don't have an explanation which I find satisfactory. However, the response of sodium transport to vasopressin is not always so short-lived. After 1 h the rate of transport in the vasopressin-treated bladder is often well above that of the control. Also we find that though the response may fall off rapidly, if we rinse away the vasopressin in the medium the resting rate of transport is always less than when the hormone was present. It is possible that this difference in transport rate accounts for the difference in oxygen consumption. What is clearly needed to resolve this point, however, are simultaneous and continuous measurements of both sodium transport and oxygen consumption to see how closely the two parallel each other after vasopressin treatment.

Porter: Do I understand correctly that you have measured tissue sodium contents in both fresh bladders and bladders incubated overnight?

Leaf: Yes.

Porter: Was the total sodium content in the bladder incubated overnight the same as that present in fresh tissue?

Leaf: We don't find the marked decrease in cell sodium content that Dr. Handler reports after overnight incubation of the bladders. I haven't shown the results of such long incubations of the bladder with aldosterone, since our own data is incomplete.

Edelman: As I mentioned earlier, Lipton found that aldosterone produced no significant change in intraepithelial Na^+ and K^+ concentrations. These results agree with those just shown by Dr. Leaf and are discordant with those of Dr. Handler.

Leaf: At least what has appeared in abstract indicates that your colleague failed even to detect the sizeable increase in total sodium which occurs with vasopressin. In view of this it is not surprising that he would miss the subtler changes produced by aldosterone. The changes with ouabain are so gross as to constitute no test of technique.

Edelman: Dr. Lipton's complete paper is scheduled to appear soon in the American Journal of Physiology. The electron micrographs revealed that in his preparations the basement membrane was more or less intact.

Leaf: Well, I think collagenase takes the basement membrane right off.

Edelman: This may depend on the particular preparation of collagenase used prior to scraping. I would like to ask a question concerning the interpretation of your results. How is the information processed at the apical boundary communicated to the Na^+ pump, presumably located in the basal-lateral plasma membrane? Would a change in apical Na^+ conductance produce a local change in Na^+ concentration or would it obligate an equivalent uptake of cell water and thus produce changes in cell volume?

Leaf: I am not sure you expect an answer to that! Since we are dealing with a reaction in which sodium is the substrate, the rate of the reaction is probably determined in part by the amount of sodium available as substrate. We saw this dramatically in the studies with ATPase which were shown earlier. How sensitive the reaction is to changes in sodium and whether the gain in cell volume which minimizes the change in concentration blunts the response, remain unknown. We are still very much hung up by the fact that although we can now measure the

content of sodium within the pool a little more precisely, we remain entirely ignorant about the chemical activity of this sodium and the volume of the pool in which it is contained. Obviously without this additional information we have no way of stating in what manner the pump mechanism would respond to the changes in sodium content as we see it grossly in these experiments.

Cereijido: We have recently developed a method to measure the penetration of sodium across the apical border (*J. Gen. Physiol.* **55** (1970) 716) and we have observed, in agreement with Dr. Leaf's observations, that the penetration of sodium at the apical border (J_{12}^{Na}) is increased by ADH. When the concentration of Na on the outside was 115 mM the increase of (J_{12}^{Na}) coexisted with an increase in the amount of Na labelled from the outside. Yet when the concentration of Na on the outside was low (1 mM) no increase (or even a *decrease*) of the "Na pool" was observed. In our experience the increase of a "Na transporting pool" was not a necessity for the effect of ADH. If I understood it correctly, you performed your experiments with regular Ringers on both sides (115 mM). I wonder whether in the case of the toad bladder the use of low (1–5 mM) concentration of sodium on the outside would have produced a loss of epithelial sodium.

Leaf: We haven't done experiments similar to what you describe on the scraped cells. In the whole tissue the sodium pool measured isotopically from the mucosal side always increased following vasopressin even at concentrations of sodium in the mucosal bathing medium of less than 1·0 meq/l.