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The Action of Aldosterone on Na^+ and K^+ Transport in the Rat Submaxillary Main Duct

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Summary. The main excretory duct of the submaxillary gland of normal and adrenalectomized rats was perfused with bicarbonate Ringer's solution and the following values were measured: the transepithelial electrical potential difference, the specific electrical resistance of the epithelium, and the transepithelial net fluxes for Na^+ and K^+. From the potential difference and the resistance, the short circuit current was calculated. Following adrenalectomy the short circuit current dropped to about one half, while the electrical resistance increased around twofold and the transepithelial potential difference remained constant. The reduction of short circuit current was accompanied by a 30% reduction of Na^+ reabsorption whereas K^+ secretion was only slightly diminished. Acute substitution of aldosterone to adrenalectomized animals led to a restitution of the Na^+ fluxes and showed a tendency to increase K^+ secretion. Following the administration of Actinomycin D to normal animals, Na^+ resorption declined as in adrenalectomized rats but K^+ secretion remained essentially unchanged. From these observations it is concluded that the hypothetical aldosterone-induced proteins act only on Na-resorption and that they may act by both increasing the sodium permeability of the luminal cell membrane and stimulating active Na^+ transport. The latter effect does not seem to consist of a non specific enhancement of the energy supply since it does not influence the active potassium secretion of the cell.

Key words: Submaxillary Main Duct — Electrical Resistance — Aldosterone — Na^+ Transport — K^+ Transport.

It has long been known that adrenal insufficiency increases the ratio of Na^+ over K^+ in saliva and that this effect can be reversed by aldosterone [2]. The mechanism of action of the hormone on salivary glands, however remained unclear, since both the secretory function of the salivary glands, and the action of aldosterone on ion transport in general were not fully understood. Only after micropuncture and perfusion techniques opened a new era in the investigation of salivary gland function [41, 42, 54, 55] has it become possible to study the aldosterone effect on salivary glands in more detail and to distinguish between actions on the acinar region and on the ductal region of the glands. Because of the functional similarities between salivary ducts and renal distal tubules,

whose ion transport is known to be influenced by aldosterone [26,27] we have concentrated our attention on the duct system. The main excretory duct of the rat submaxillary gland was chosen as a model tissue. It reabsorbs Na^+ actively [54] and secretes actively K^+ [54] and HCO_3^- [53], thus offering the possibility to study the hormone action on both transport mechanisms. The results indicate that active Na^+ absorption from salivary ducts is controlled by aldosterone. Active K^+ secretion however, does not seem to be directly influenced by the hormone. By comparing the transepithelial resistances from normal and adrenalectomized rats and applying a two membrane model for transepithelial Na^+ transport, further insight was obtained into the mechanism of transport stimulation, suggesting a dual action of the hormone, by 1. an increase of sodium permeability of the luminal cell membrane and 2. a stimulation of active sodium transport across the basal cell membrane.

Methods

The experiments were performed on control and adrenalectomized male Wistar rats. The average body weight was 265 and 276 g in both groups of animals respectively. Adrenalectomy was performed at least 14 days prior to the experiment. After adrenalectomy the animals were allowed to drink saline instead of water and the standard diet (Altromin) was continued. To test whether the adrenal glands had been completely removed, a waterload of 15 ml was administered through a gastric tube into the conscious animals two days prior to the experiment. Only those animals were accepted which retained more than 50% of the load in the first three hours.

During the experiments the animals were anaesthetized with Inactin®. Control rats received 100 mg/kg body weight intraperitoneally but adrenalectomized rats required only half of this amount. A tracheotomy was performed on all animals and blood pressure was controlled in the carotid artery with use of a Statham-element. It was recorded on a Schwarzer Physioscript (Model PE 4). Mean blood pressure varied between 130 and 100 mm Hg in control rats and between 130 and 85 mm Hg in adrenalectomized rats. Since blood pressure tended to decline gradually in the latter group of animals, the duration of the experiments was limited to about 2—3 h from onset of anaesthesia. Body temperature was kept constant at 37° C. The submaxillary glands were exposed through a midline neck incision. The duct was opened near gland hilum and small polyethylene catheters of 30—40 μm o.d. were inserted into it to perfuse the duct and to perform electrical measurements.

Electrical Measurements

In these experiments the duct was perfused at a rate of 2.7 μl/min with bicarbonate Ringer's solution and the transepithelial electrical potential difference was determined as well as the specific resistance of the duct wall, using cable analysis. From these data the short circuit current was calculated by applying Ohm's law as described previously [34]. This indirect way of obtaining the short circuit current had to be chosen, since direct short circuiting of the duct epithelium proved to be impossible.

The experimental set-up is depicted schematically in the inset of Fig. 1. Two thin polyethylene catheters were inserted into the duct lumen near the hilum of the

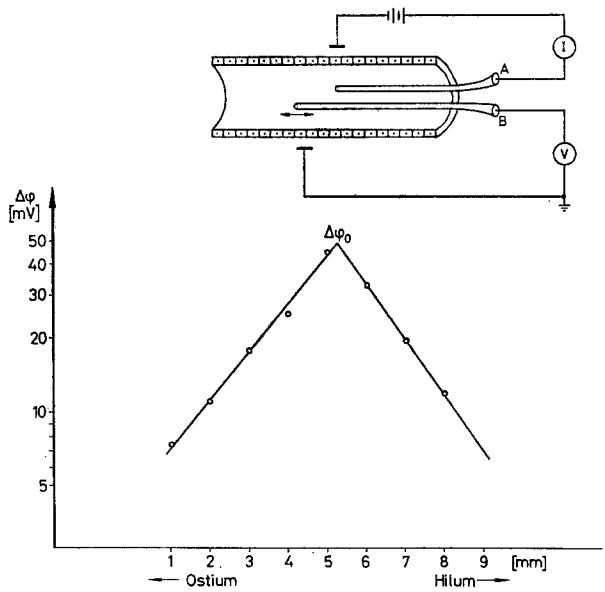

Fig. 1. Voltage attenuation along the duct lumen. Abscissa: Position of the tip of the voltage recording catheter B with respect to the arbitrary starting point during its gradual retraction through the duct lumen in steps of 1 mm. Ordinate: steady state voltage deflection in mV on a logarithmic scale. The inset gives a schematic circuit diagram. The current pulses were applied through catheter A. The voltage deflection at the point of current injection was obtained from the intersection of the regression lines. There was a small asymmetry of the slope of the regression lines in normal as well as adrenalectomized rats (see text)

gland, one for measuring the potential difference (catheter B) and one for applying current (catheter A). Both catheters were filled with bicarbonate Ringer's solution containing the following ions in mEq/l: Na^+: 140.5, K^+: 4.5, Ca^{2+}: 1.9, Cl^-: 121, HCO_3^-: 25.9. Catheter A was connected to the high input of a model 27 Knick electrometer via a saturated calomel half cell. It measured the potential difference between the lumen fluid and the interstitial fluid compartment. The skin of the rats tail was stripped and the tail was dipped into a beaker filled with bicarbonate Ringer's solution to serve as reference point. Connection to the low input of the electrometer was achieved by means of a second calomel half cell. Catheter A served for passing current to a Ag-AgCl-electrode, placed in the neck region of the animal, and for continuous perfusion of the duct with bicarbonate Ringer's solution. DC current was obtained from a battery and measured as the voltage drop across a precision resistor. Current strength was ~4 μA and the direction of current flow was such as to depolarize the epithelium.

At the beginning of the experiment, catheter B was advanced within the duct lumen almost up to the oral orifice whereas catheter A was moved only halfway downstream, to reach the middle between gland hilum and oral opening, where it remained throughout the experiment. Catheter B was then gently retracted in steps of 1 mm by means of a micromanipulator (Brinkmann, model type RP 4) to perform

the measurements. At each stop one or two current pulses of three minutes duration and three minutes interval were passed through catheter A and the response of the transepithelial voltage was measured with catheter B. All measurements were recorded on a penrecorder (Metrawatt Servogor). Bringing the catheters into their correct positions and performing a complete electrical analysis, required about 2—3 h, since extreme care had to be taken to avoid injury of the duct wall as signalized by a drop of the transepithelial potential difference.

Passing a square wave current pulse through the duct epithelium gave rise to a step-like voltage response, which was complete in approximately 0.5 sec. It was followed by a slower phase of further polarization, which reached a constant plateau value after 1 to 2 min. A similar time dependent voltage response has been observed and further analyzed in other epithelia like frog stomach [43], Necturus gallbladder [21] and toad urinary bladder (Frömter, E., unpublished observations). According to these analyses the instantaneous voltage current relation $(\partial \Delta\varphi/\partial I)_{t\to 0}$, can be interpreted as a measure of the true (zero current) membrane resistance, whereas the stationary state measurements, $(\partial \Delta\varphi/\partial I)_{t\to\infty}$ include all sorts of polarization effects, like ion concentration changes in intraepithelial compartments and changes of membrane geometry. For calculation of the short circuit current in the sense of Ussing and Zerahn [51] the steady state current voltage relation was used. Both the instantaneous and the steady state voltage deflections declined exponentially along the duct axis (Fig. 1) suggesting that cable analysis was applicable. However, in both groups of animals, control and adrenalectomized rats, the voltage attenuation was consistently steeper towards the gland hilum than toward the oral opening of the duct. A similar observation was made in isolated rabbit submaxillary main duct recently, using different measuring techniques (K. Schopow and E. Frömter, unpublished observations). It may indicate that the electrical properties of the duct wall are not constant and that the specific resistance is slightly higher near the mouth end and lower near the gland hilum. The open circuit potential, however, was constant over the entire length of the duct in both species. To ascertain constant reference conditions the length constant (λ) was always calculated from the voltage attenuation along the oral end of the duct. The effective resistance or input resistance (R_{eff}) was obtained from the voltage deflection ($\Delta\varphi_0$) at the point where the two regression lines intersected (see Fig. 1) and from the total current applied. From these data the specific resistance of the duct wall (R_m) was calculated according to the equation (see ref. [24])

$$R_m = \sqrt{8\pi\varrho R_{\text{eff}}\lambda^3} \tag{1}$$

where ϱ is the resistivity of the lumen fluid, which was taken to be 50 Ω cm. Regarding possible errors in applying the cable analysis, it should be mentioned that the occluding effect of the duct by the voltage measuring catheter was negligible. The cross sectional area of the catheter was $\sim 2\cdot 10^{-3}$ mm² compared to the cross sectional area of the duct of $\sim 5\cdot 10^{-2}$ mm². Furthermore during current injection the voltage drop in the interstitium between duct surface and reference point of the potential measurements was found to be in the order of only 3% of the transpithelial voltage drop. Hence no correction was applied.

Determination of the Net Electrolyte Fluxes

Net electrolyte fluxes were measured with the technique of Young *et al.* [54]. The ducts were perfused from hilum to mouth by means of a polyethylene catheter which was attached to a tuberculine syringe mounted on a constant perfusion pump (model Unita I, Braun). The perfusion rate was 1.1 µl/min and the composition of

the perfusate was a given above. Under these perfusion conditions, the transepithelial cation net fluxes approach maximum values and are independent of small variations of the perfusion rate [54]. The perfusate was collected under oil by means of a thin polyethylene catheter which had been introduced into the oral opening of the duct. The perfused segment of the ducts was approximately 22 mm. Sodium and potassium concentrations were determined in the collected perfusate with a flame-photometer (Eppendorf). In order to test for transepithelial water fluxes, inulin was added to the perfusion fluid and the inulin concentration of the collected perfusate was determined chemically [44]. The cation fluxes were corrected for water flux using the inulin ratios and were expressed in neq/min duct.

Statistical Analysis

In connection with the mean values standard errors (S.E.M) are given throughout the paper. The standard error of the indirectly determined values for transepithelial resistance and for the short circuit current was calculated according to Gauss' law of the propagation of errors from the standard errors of the directly measured quantities. The data from adrenalectomized rats were compared to those from control rats by means of the ungrouped student t-test.

Results

In Table 1 the results obtained from adrenalectomized rats are compared with the control data.

In good agreement with our previous observation [54], the transepithelial electrical potential difference was 71 $\pm$ 5 mV (lumen negative) in control animals. In adrenalectomized rats, a mean value of 68 $\pm$ 3 mV

Table 1. Summary of transport data of normal and adrenalectomized rats. Significance levels (P) derived from ungrouped t-test. n is the number of animals

Quantity	Control rats ($M \pm$ S.E.M.)	n	Adrenalectomized ($M \pm$ S.E.M.)	n	Probability of error
Potential difference	-71 ± 5 mV	10	-68 ± 3 mV	9	n.s.
$R_{\text{eff}\, t \to 0}$	7.0 ± 0.5 $K\Omega$	6	7.3 ± 0.4 $K\Omega$	5	n.s.
$\lambda_{t \to 0}$	0.18 ± 0.01 cm	6	0.25 ± 0.01 cm	5	$P < 0.01$
$R_{m\, t \to 0}$	218 ± 27 Ωcm^2	6	367 ± 19 Ωcm^2	5	$P < 0.01$
$R_{\text{eff}\, t \to \infty}$	10.5 ± 0.6 $K\Omega$	10	13.6 ± 1.1 $K\Omega$	7	$P < 0.01$
$\lambda_{t \to \infty}$	0.23 ± 0.01 cm	10	0.32 ± 0.01 cm	7	$P < 0.01$
$R_{m\, t \to \infty}$	400 ± 32 Ωcm^2	10	736 ± 57 Ωcm^2	7	$P < 0.001$
SCC	187 ± 19 μA/cm^2	10	93 ± 7 μA/cm^2	7	$P < 0.001$
Radius	132 ± 5 μm	10	137 ± 5 μm	7	n.s.
Inulin-ratio	1.01 ± 0.01	8	0.99 ± 0.02	6	n.s.
Net-Na-flux	39.0 ± 1.5 $\frac{\text{nanoEq}}{\text{min} \cdot \text{duct}}$	8	28.9 ± 1.7 $\frac{\text{nanoEq}}{\text{min} \cdot \text{duct}}$	6	$P < 0.001$
Net-K-flux	29.0 ± 1.7 $\frac{\text{nanoEq}}{\text{min} \cdot \text{duct}}$	8	25.3 ± 1.6 $\frac{\text{nanoEq}}{\text{min} \cdot \text{duct}}$	6	$P < 0.1$

n.s. means difference not significant.

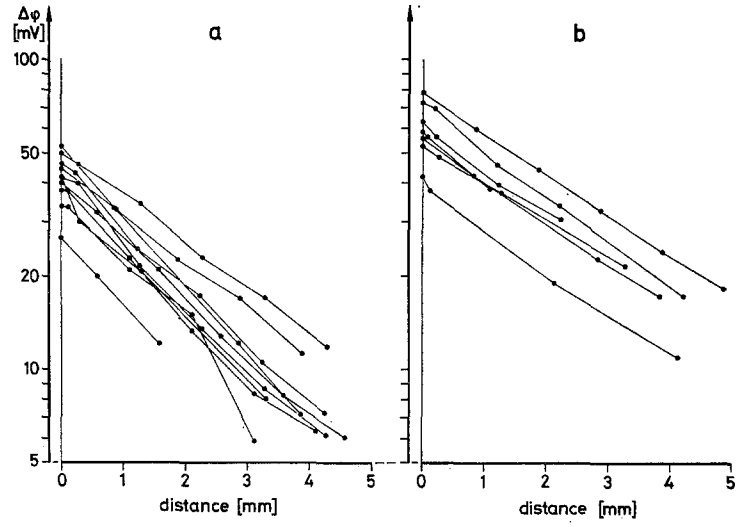

Fig. 2a and b. Voltage attenuation in salivary duct of normal rats (a) and adrenalectomized rats (b). Abscissa: distance along duct axis from the point of current injection in mm. Ordinate: current induced potential difference in mV. Current flow was 3.93 and 4.42 μA in group a and b respectively. In order to eliminate small differences in current flow between individual experiments the potential values were corrected for the deviation from the mean current

was observed. The slight difference is not significant. After adrenalectomy both the length constant (λ) and the effective resistance (R_{eff}) increased (compare Fig. 2a and b). This indicated an increase of the transepithelial resistance R_m. When determined from the instantaneous current voltage relation, R_m increased from $218 \pm 27\ \Omega\text{cm}^2$ to $367 \pm 19\ \Omega\text{cm}^2$ whereas the stationary current voltage relation yielded values of $400 \pm 32\ \Omega\text{cm}^2$ in control rats and $763 \pm 57\ \Omega\text{cm}^2$ after adrenalectomy.

The short circuit current was $187 \pm 19\ \mu\text{A/cm}^2$ in normal animals and $93\ \mu\text{A/cm}^2$ in adrenalectomized animals. In order to check the validity of the resistance measurements, the radius of the duct lumen was calculated from the mean values of λ and R_{eff} according to the equation (see reference [24])

$$r = \sqrt{\frac{\lambda \varrho}{2\pi R_{\text{eff}}}} \quad . \tag{2}$$

The radius was $132 \pm 5\ \mu\text{m}$ in normal rats and $137 \pm 5\ \mu\text{m}$ in adrenalectomized rats. These values correspond well to the actual dimension of the duct, which can be inferred from the thickness of the catheters that it accomodates.

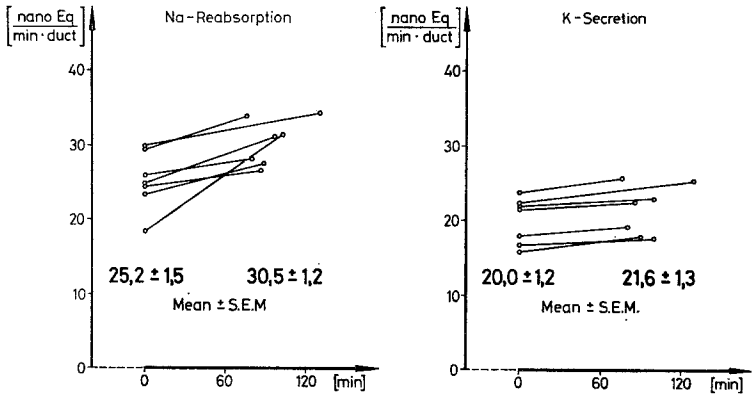

Fig. 3. The response of Na^+ resorption and K^+ secretion to acute substitution of aldosterone to adrenalectomized rats. Abscissa: time after administration of the hormone in min. Ordinate: cation net fluxes in nano Eq/min · duct

Table 1 continues with the results of flux measurements. In normal rats, net Na^+ reabsorption was 39 neq/min duct, in agreement with the data of Young *et al.* [54] and Schneyer [45]. Net K^+ secretion, however, was found to be 29 nEq/min/duct—thus being greater than the K^+ fluxes measured by the above mentioned authors. After adrenalectomy, Na^+ transport decreased by 28%, and K^+ transport decreased by 13%.

In order to test whether the difference between the results from normal and adrenalectomized rats was a specific effect of aldosterone deprivation a series of experiments was performed in which the effect of acute substitution of the hormone on Na^+ and K^+ fluxes was studied[1]. Approximately $1^1/_2$ h after substitution of aldosterone (75 µg/kg body weight i. m. and 1.25 µg/kg body weight i. v.) to adrenalectomized animals, the cation fluxes reapproached control levels. Na^+ flux increased by 21% and K^+ flux by 8% (Fig. 3).

Because of the comparatively small increase in K^+ flux, it was still questionable whether the changes in K^+ transport were directly attributable to aldosterone or were rather side effects of the adrenal insufficiency. Hence a series of experiments was performed in which Actinomycin D was administered to intact animals in a dose of 2 µg/kg body weight intravenously. This antibiotic is known to inhibit protein syn-

1 The effect of aldosterone substitution on the electrical parameters could not be investigated for technical reasons. To complete a second electrical analysis after substitution of the hormone would have required another two hours bringing the total experimental time to around five hours. As evidenced by a drop in blood pressure, adrenalectomized animals would not withstand anaesthesia in good condition over such long time periods.

thesis [6] and therefore to prevent the formation of aldosterone induced proteins within the cell. Approximately 90 min after administration of Actinomycin D, Na^+ transport was reduced to a similar extent as in adrenalectomy but K^+ transport remained essentially constant.

Discussion

1. Relation between Electrical Measurements and Flux Measurements

Since the flux measurements cover only net fluxes and since the salivary duct in contrast to frog skin does not only absorb Na^+ actively but does also secrete actively K^+ and HCO_3^-, it is not possible to compare the fluxes and the short circuit current directly. One may, however, try to compute a figure for the short circuit current from the observed fluxes under the assumption that all passive fluxes are negligibly small. Taking a surface area of the perfused duct of 0.19 mm^2 and including the HCO_3^- flux of 1.2 nEq/min · duct as determined by Young and coworkers [53] the figure would be $I_{Na} - I_K + I_{HCO_3} = 328 - 244 + 10 = 94\ \mu A/cm^2$. Although this figure is only half the short circuit current observed, the agreement is actually not as bad as it might look. The data can already be brought to a complete fit, if we allow passive fluxes to amount only to approximately 15% of the active fluxes for the above mentioned ions. Similarly the alterations of short circuit current and cation fluxes, which occurred in adrenalectomy, appear to be consistent. As will be discussed later it is likely that the change in potassium transport, which follows the same pattern as in rat kidney, involves only

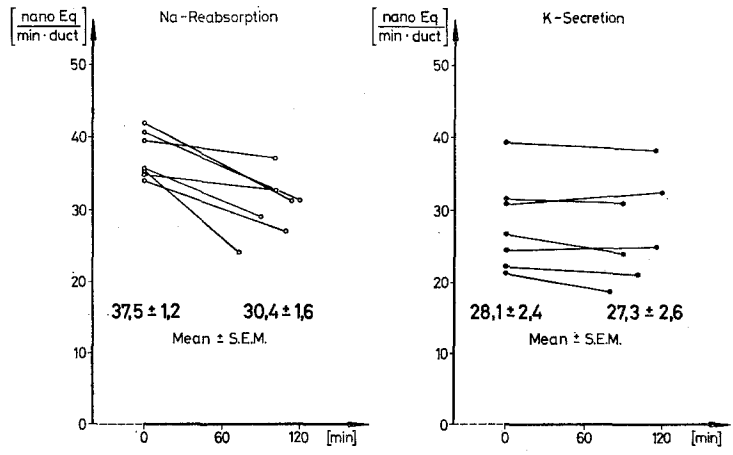

Fig. 4. The effect of administration of Actinomycin D to normal rats on Na^+ resorption and K^+ secretion. Abscissa: time after administration of Actinomycin D. Ordinate: cation net fluxes in nano Eq/min · duct

passive K^+ transport. This means that the drop of Na^+ transport, from 39.0 nEq/min · duct to 28.9 nEq/min · duct, corresponding to a change of current of 93 $\mu A/cm^2$, would then be the only major cause for the reduction of short circuit current. This figure agrees well with the observed reduction of the short circuit current which happened to be 94 $\mu A/cm^2$.

2. Salivary Glands and Aldosterone

The present experiments have shown that salivary duct sodium absorption and potassium secretion decrease after adrenalectomy. These results can explain the old observations of rising salivary Na^+/K^+ concentration ratios in adrenal insufficiency [13, 14, 36a, 38, 47] without requiring an additional action of the hormone on the formation of primary secretion in the acinar region of the glands. The latter possibility has been recently discussed [3]; the experimental evidence, however is still far from being conclusive. Quantitatively the effect of adrenalectomy was somewhat smaller in salivary duct than in other epithelia. Sodium absorption decreased by 30%. In toadbladder [9], frog skin [40], renal tubules [27, 49] and small and large intestine [12, 17] the aldosterone dependent part of sodium transport varies between 50 and 60%. Regarding potassium the difference is even more pronounced, if we compare our data for example to respective data from the kidney [52]. It is likely that this difference reflects the different modes of potassium secretion in salivary ducts and kidney tubules (see below).

3. The Mechanism of Action of Aldosterone

Studies on both amphibian and mammalian tissues have shown that the primary step in the action of the hormone involves binding of the aldosterone molecule to specific nuclear receptor sites within the epithelial cells [1, 20]. The binding triggers the production of one or more specific proteins, which according to the two-membrane model of Na^+ transport [36] are thought to stimulate Na^+ transport by 1. facilitating passive entry of Na^+ through the apical membrane into the cell, or 2. increasing the energy supply to the pump, or 3. increasing the number of pump sites operating in the basal cell membrane. A considerable body of evidence has already accumulated in support of the first [10, 16, 46] and second [16, 31, 32] hypothesis, whereas attempts to test the third hypothesis by measuring the Na^+/K^+-ATPase activity in relation to aldosterone action have led to ambiguous results [4, 15, 30, 46].

Since we have observed that the electrical resistance of salivary duct epithelium increased after adrenalectomy, our data may be taken to support the 1. hypothesis. However, before accepting this result we have to consider at least three possible objections, concerning 1. the meaning of

resistance measurements in the presence of active transport, 2. the specificity of the effect for aldosterone and 3. the site of the resistance barriers in the epithelium.

Hoshiko and Lindley [29], (see also [19]) have recently concluded that the result of the usual type of resistance measurements is a function of active transport. If this were true it could mean that the resistance increase in adrenalectomy merely reflects the simultaneous change in active Na^+-absorption. Hoshiko's conclusion follows from his definition of active transport in terms of R coefficients (measured while keeping other fluxes equal to zero), whereas the resistance measurements are usually performed while keeping other driving forces equal to zero and conform thus to L coefficients. However there is neither a justification nor an advantage of defining "true" membrane properties in terms of R coefficients as opposed to L coefficients, and it is clear that such properties like active transport and membrane resistance are independent from each other if both are considered in the same frame of reference (using either only R coefficients or only L coefficients).

The question of the specificity of the observed resistance change cannot be answered directly at present, since we have not been able to do the electrical measurements with acute hormone substitution (see footnote 1 on p. 39). However, since the flux alterations were readily reversible by aldosterone, and since Knauf and coworkers [35] have observed that the resistance of isolated rabbit salivary duct increased after Actinomycin D we may conclude that the resistance change was also caused by the lack of aldosterone in our experiments.

Regarding the third point, recent electrophysiological studies on transport-routes through epithelia [5, 21, 22, 48] have shown the presence of at least four different resistance barriers: the apical cell membrane, the basal cell membrane, the terminal bar and the lateral intercellular space, with the latter two forming the paracellular shunt. All indirect evidence suggests that the paracellular shunt path is relatively unimportant in rat submaxillary main duct (high resistance of the terminal bar) and that the electrical characteristics of this epithelium are largely determined by the serial array of the two cell membranes (see [22]). In a serial array of resistors, however, a change in a high resistance element will affect the overal resistance much more than a change in a low resistance element. Since in all epithelia studied so far the apical membrane resistance was higher than the basal membrane resistance [7, 21, 23, 37] one would expect therefore, that the large increase of the overall resistance following adrenalectomy was likely to be caused by a change in the luminal cell membrane.

As a result of this discussion, we conclude that the resistance increase following adrenalectomy reflects indeed a change of passive permeability properties of the epithelium, which is likely to be located in the luminal

cell membrane and which is at least partially caused by the lack of aldosterone. Our data thus support the first hypothesis of aldosterone-action which postulates facilitated sodium entry into the cell.

The same conclusions have been reached from resistance measurements in relation to aldosterone on toad bladder [8] and anuran skin [28] but an opposing view has also been taken by other investigators [11] who studied the effect of pyruvate on the resistance of aldosterone treated bladders rather than the effect of aldosterone itself. Our conclusions do not agree, however, with recent observations made in rat colon [18] and rat collecting duct [49,50]. In rat colon the resistance remained constant while the transepithelial potential difference increased and in collecting ducts the tracerpermeability declined slightly after administration of aldosterone to normal rats. It is interesting to note that both colon and collecting duct unlike other tissues respond already with a large increase of sodium transport, when the hormone is given to normal animals with intact adrenal glands, and that both organs act as final regulators of the sodium excretion from the body. Hence it would seem possible that these epithelia have developed special means to prevent passive sodiumloss which possibly involve simultaneous tightening of the paracellular shunt path.

4. Further Considerations of the Two-Membrane Model

Although our observations favour the first hypothesis of aldosterone-action they are not able to exclude either hypothesis 2 or 3. In fact, further consideration of the two-membrane model for active sodium-transport [36] suggests that hypothesis 1 alone cannot fully explain the mechanism of aldosterone action. In the appendix we have tried to describe in a very simplified way the relations between transport parameters of two individual membranes α and β and the transport parameters of a composite system s, containing both membranes in series. According to eq. (A.8) the relation between active transport across the overall system ($J_i^{act,s}$) and active transport across one single membrane ($J_i^{act,\beta}$) is given by

$$J_i^{act,s} = \frac{L_{ii}{}^{\alpha}}{L_{ii}{}^{\alpha} + L_{ii}{}^{\beta}} J_i^{act,\beta} \tag{3}$$

where $L_{ii}{}^{\alpha}$ and $L_{ii}{}^{\beta}$ denote the straight coefficients for passive ion flux through membranes α and β respectively. This eq. shows that the rate of active transport across the epithelium does already rise, when the luminal membrane permeability increases but the rate of active transport through the basal cell membrane remains constant. Hence the double membrane model with aldosterone induced facilitated sodium entry through the luminal cell membrane would not necessarily require a concomitant

stimulation of the sodium pump in order to yield an increase of overall active sodium transport. This increase could simply result from a reduction of passive sodium backflux across membrane β following the rise in intracellular sodium concentration. Since it has been a constant finding, however, that oxygen consumption of isolated epithelia rises in response to aldosterone and that the increase in active sodium transport depends on the availability of substrate [16,46] it would appear that aldosterone does not only influence the luminal sodium permeability but increases also the rate of active sodium transport across the basal cell membrane ($J_i^{act,\beta}$). The nature of this stimulatory effect is not known. However, from the three possibilities: 1. increased saturation of the pump with Na^+ ions, 2. increased energy supply or 3. direct stimulation of the pump, the second possibility can be excluded on the basis of our observations with the potassium fluxes, which will be discussed next.

5. Aldosterone and Potassium Transport

As compared to sodium transport, the relation between aldosterone and potassium transport has not been studied as thouroughly so far, probably because a suitable model tissue was lacking. It has long been known, that adrenalectomy reduces urinary potassium excretion, that this effect can be restored by aldosterone but cannot be elicited by Actinomycin D [52]. The failure of Actinomycin D to inhibit potassium excretion has been thought to indicate that the aldosterone induced cellular proteins do not directly act on the mechanism of potassium transport and that the alteration observed during adrenalectomy are secondary side effects [25,52]. The same conclusions seem to apply to the salivary ducts since the same pattern of transport inhibition appeared in the present experiments. This finding is of interest, however, since the mechanism of potassium transport is different in both tissues. In distal tubules which are largely responsible for the reduced K^+ excretion during adrenalectomy [25,26] potassium secretion is known to be a passive process [39], whereas in salivary duct epithelium potassium secretion is predominantly active [33,54], with only a small passive component superimposed. The salivary duct cells posses thus at least two active transport mechanisms, one for Na^+ and one for K^+. Since our experiments indicate that only one transport, the active Na^+ transport, is regulated by aldosterone, we must conclude that the action of the aldosterone induced cellular proteins must be highly specific and cannot merely consist of an unspecific increase of the energy supply within the cell.

Appendix

Since all available evidence indicates that active sodium transport is a cellular event, we may assume that sodium ions, when being reabsorbed

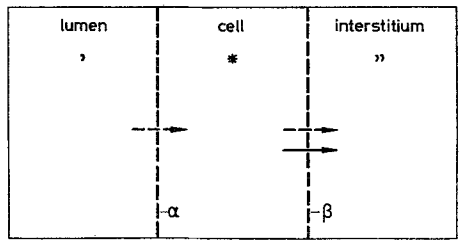

Fig. 5. Schematic diagram, representing the two-membrane model of active transport. The luminal, cellular and interstitial fluid compartment are denoted by ', * and " respectively. α and β are the luminal and contraluminal cell membranes. The dashed arrows indicate passive ion fluxes and the solid arrow indicates active ion flux

have to pass at least two membranes, the apical or mucosal and the basal or serosal cell membrane. In the following we will consider therefore the relations between single membrane transport parameters, and transport parameters of a composite membrane system, consisting of two such membranes α and γ in series. According to Ussings model [36] we assume that active transport occurs only in membrane β and that membrane α acts exclusively as a passive permeability barrier (Fig. 5). The mucosal compartment will be named ', the cellular compartment * and the basal compartment ". We will consider only the simplest conditions, will neglect ion-ion and ion water interaction and will assume the validity of linear laws. In this case the flow of ion i (J_i) through membrane (α) is given by

$$J_i^{\alpha} = L_{ii}^{\alpha} (\eta_i' - \eta_i^*) \tag{A.1}$$

and the flow through membrane β by

$$J_i^{\beta} = L_{ii}^{\beta} (\eta_i^* - \eta_i'') + J_i^{\mathrm{act},\beta} \tag{A.2}$$

where the L_{ii} are the phenomenologic cross coefficients, the η_i are the electrochemical potentials and $J_i^{\mathrm{act},\beta}$ is the active transport rate arising from a coupling of the flow of ion i to a chemical reaction within membrane β.

After rearranging and adding both equations, η^* can be eliminated and we obtain

$$\frac{J_i^{\alpha}}{L_{ii}^{\alpha}} + \frac{J_i^{\beta}}{L_{ii}^{\beta}} = \eta_i' - \eta_i'' + \frac{1}{L_{ii}^{\beta}} \cdot J_i^{\mathrm{act},\beta}. \tag{A.3}$$

Since in the stationary state

$$J_i^{\alpha} = J_i^{\beta} = J_i^{s} \tag{A.4}$$

where J_i^s denotes the flux through the overall membrane system, we obtain

$$J_i^s = \frac{L_{ii}^{\alpha} L_{ii}^{\beta}}{L_{ii}^{\alpha} + L_{ii}^{\beta}} \left(\eta_i' - \eta_i'' \right) + \frac{L_{ii}^{\alpha}}{L_{ii}^{\alpha} + L_{ii}^{\beta}} J_i^{\mathrm{act},\beta} \qquad \text{(A.5)}$$

which can also be written as

$$J_i^s = L_{ii}^s (\eta_i' - \eta_i'') + J_i^{\mathrm{act},s} \qquad \text{(A.6)}$$

with

$$L_{ii}^s = \frac{L_{ii}^{\alpha} L_{ii}^{\beta}}{L_{ii}^{\alpha} + L_{ii}^{\beta}} \qquad \text{(A.7)}$$

and

$$J_i^{\mathrm{act},s} = \frac{L_{ii}^{\alpha}}{L_{ii}^{\alpha} + J_{ii}^{\beta}} \cdot J_i^{\mathrm{act},\beta} . \qquad \text{(A.8)}$$

Equation (A.8) indicates that the overall active transport should increase when L_{ii}^{α} increases even if the active transport through membrane β remains constant. This result is independent of the presence of a third membrane element γ (not depicted in Fig. 5) arranged in parallel to membranes α and β (paracellular shunt) provided that this membrane element does not carry out active transport. If

$$J_i^{\gamma} = L_{ii}^{\gamma} (\eta_i' - \eta_i'') \qquad \text{(A.9)}$$

we obtain from equation (A.5)

$$J_i^s = \left\{ \frac{L_{ii}^{\alpha} \cdot L_{ii}^{\beta}}{L_{ii}^{\alpha} + L_{ii}^{\beta}} + L_{ii}^{\gamma} \right\} (\eta_i' - \eta_i'') + \frac{L_{ii}^{\alpha}}{L_{ii}^{\alpha} + L_{ii}^{\beta}} J_i^{\mathrm{act},\beta} . \qquad \text{(A.10)}$$

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