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Differential Effects of Aldosterone and ADH on Intracellular Electrolytes in the Toad Urinary Bladder Epithelium

Roger Rick†, Gertrud Spancken‡, and Adolf Dörge‡

†Department of Physiology and Biophysics, Nephrology Research and Training Center, University of Alabama at Birmingham, Birmingham, Alabama 35294, and ‡Department of Physiology, University of Munich, D-8000 Munich, West Germany

Summary. Quantitative electron microprobe analysis was employed to compare the effects of aldosterone and ADH on the intracellular electrolyte concentrations in the toad urinary bladder epithelium. The measurements were performed on thin freeze-dried cryosections utilizing energy dispersive x-ray microanalysis. After aldosterone, a statistically significant increase in the intracellular Na concentration was detectable in 8 out of 9 experiments. The mean Na concentration of granular cells increased from 8.9 ± 1.3 to 13.2 ± 2.2 mmol/kg wet wt. A significantly larger Na increase was observed after an equivalent stimulation of transepithelial Na transport by ADH. On average, the Na concentration in granular cells increased from 12.0 ± 2.3 to 31.4 ± 9.3 mmol/kg wet wt (5 experiments). We conclude from these results that aldosterone, in addition to its stimulatory effect on the apical Na influx, also exerts a stimulatory effect on the Na pump. Based on a significant reduction in the Cl concentration of granular cells, we discuss the possibility that the stimulation of the pump is mediated by an aldosterone-induced alkalinization.

Similar though less pronounced concentration changes were observed in basal cells, suggesting that this cell type also participates in transepithelial Na transport. Measurements in mitochondria-rich cells provided no consistent results.

Key Words: aldosterone · ADH · transepithelial Na transport · toad urinary bladder epithelium · x-ray microanalysis · intracellular electrolyte concentration

Introduction

The mineralocorticoid aldosterone stimulates active Na reabsorption and K secretion in distal segments of the mammalian kidney and other tight epithelia (for review *see* Fanestil & Park, 1981; Garty, 1986). Its natriferic effect is generally ascribed to an enhanced passive Na influx into the epithelial cell, as indicated by an increased Na transport pool (Crabbé & De Weer, 1969; Handler, Preston & Orloff, 1972), higher intracellular Na activity (Eaton, 1981), and depolarization of the cell membrane potential (Lewis, Eaton & Diamond, 1976; Nagel & Crabbé, 1980). According to electrical shot noise analysis in the toad urinary bladder, the permeability increase of the apical membrane can be explained by a recruitment of new or previously undetectable Na channels (Palmer, Lindemann & Edelman, 1982), a mechanism of action identical to that proposed for the antidiuretic hormone, ADH (Li et al., 1982).

The question of whether aldosterone has an additional effect on active Na extrusion, however, remains controversial. Investigations in the rabbit and turtle colon in which the apical membrane was permeabilized, and thereby functionally eliminated as a transport barrier, failed to show any effect on the Na pump (Frizzell & Schultz, 1978; Halm & Dawson, 1985). Also, in intracellular impalements with Na-selective microelectrodes no change in the pump kinetics was evident (Lewis & Wills, 1983). On the other hand, the dependence of the aldosterone action on metabolic substrates (Edelman, Bogoroch & Porter, 1963), the potentiation of the transport response to ADH (Sharp & Leaf, 1966), and the stimulation of enzymes of the tricarboxylic acid cycle (Kirsten et al., 1968) all point to an additional effect on the active transport step. Inasmuch as the insertion or activation of new Na channels is metabolically controlled (Garty, Edelman & Lindemann, 1983), however, these data may as well be explained by changes in the apical Na permeability.

Perhaps the best evidence for an effect of aldosterone on active Na extrusion is the demonstration in the toad urinary bladder of an enhanced biosynthesis of pump subunits (Geering et al., 1982). The increase in the number of pumps was found to be insensitive to the inhibition of the apical Na influx by amiloride, suggesting that it is independent of an increase in the intracellular Na concentration. On the other hand, the increased Na/K ATPase activity observed in isolated kidney tubules (Doucet & Katz, 1981) appears to be secondary to a stimulation of the apical Na influx (Petty, Kokko & Mar-

ver, 1981). In microelectrode studies of the toad skin epithelium a small increase in the electromotive force of the basolateral membrane was demonstrated (Nagel & Crabbé, 1980).

In the present study we investigated the effect of aldosterone on the intracellular electrolyte concentrations in the toad urinary bladder epithelium employing the technique of electron microprobe analysis. By comparing the effects of aldosterone and ADH on the intracellular Na concentration, we tried to answer the question whether the action of aldosterone is limited to a stimulation of the apical Na influx or whether the hormone has an additional effect on the Na pump. The results reveal significant differences between the mechanisms of action of the two hormones and indicate that aldosterone exerts parallel effects on both transport steps. We propose that the apparent stimulation of the pump is mediated by a change in the intracellular pH. A short account of this investigation has been given at a meeting of the Deutsche Physiologische Gesellschaft (Spahcken, Rick & Dörge, 1985).

Materials and Methods

Large female toads of the species *Bufo marinus*, obtained from the Dominican Republic through National Reagents (Bridgeport, Conn.), were used. Control animals were kept dry in plastic troughs and bathed in tap water for about 20 min on a daily basis. Salt-adapted animals were kept in troughs filled about 1 cm high with isotonic saline (0.7%). Toads were doubly pithed, the urinary bladder was cannulated, and a fluid sample taken for Na and K analysis by flame photometry. After dissection, each hemibladder was mounted over a Plexiglas® ring which was covered with a piece of nylon stocking for serosal support of the tissue. Before insertion into a custom-made Ussing-type incubation chamber, the apical surface was jet-washed with amphibian Ringer's solution to remove the adherent layer of mucus.

After initial determination of the open-circuit potential the bladders were kept short-circuited throughout the incubation. Conductance was monitored by intermittently clamping the voltage to −10 mV for 500-msec periods. Basic incubation medium was Ringer's solution. In aldosterone experiments the serosal solution was replaced with a 1:1 mixture of Ringer's and Leibovitz 15 medium (Boehringer, Mannheim, FRG) or 5 mM sodium pyruvate was added from a 100-mM stock solution. Ringer's solution contained (in mM): 110 NaCl, 2.5 $KHCO_3$, and 1 $CaCl_2$. The composition of the 50% Leibovitz 15 medium was (in mM): 106 NaCl, 3.3 KCl, 1 $CaCl_2$, 1 Na_2HPO_4, 0.1 KH_2PO_4, 0.4 $MgCl_2$, 0.3 $MgSO_4$, 2.1 Na pyruvate, 2.1 galactose, 2.1 alanine, 1.2 arginine, 0.8 asparagine, 0.4 cysteine, 0.9 glutamine, 1.1 glycine, 0.7 histidine, 0.8 isoleucine, 0.4 leucine, 0.2 lysine, 0.4 methionine, 0.6 phenylalanine, 0.8 serine, 2.2 threonine, 0.04 tryptophan, 0.7 tyrosine, and 0.7 valine.

When bubbled with air, the pH of the Ringer's solution ranged between 8.1 and 8.3, and that of the pyruvate or Leibovitz 15-containing solutions between 7.7 and 8.0. All solutions had an osmolarity of 220 mM. Aldosterone (Sigma, Munich, FRG) and ADH (arginine vasopressin, Ferring, Kiel, FRG) were added to the serosal bath from stock solutions in ethanol to give a final concentration of 500 nM and 150 mU/ml, respectively. An identical amount of solvent was given to the untreated hemibladder.

After completion of incubation, the mucosal surface of the epithelium was coated with a thin layer of a standard solution, which was prepared by dissolving 20 g% bovine albumin (Behringwerke, Marburg, FRG) in the mucosal bath. The rings were then plunged into a propane/isopentane mixture (−196°C) for shock-freezing the tissue. Approximately 5 sec elapsed between layering with the standard solution and freezing. Sections of 1 μm thickness were cut from the frozen material at −90°C in a modified cryoultramicrotome (Reichert OmU 3, Vienna, Austria) and freeze-dried at −80°C and 10^{-6} Torr in a custom-made freeze dryer based on a turbomolecular pumping unit (Balzers BAE 080T, Vaduz, Lichtenstein). Energy dispersive x-ray microanalysis of the sections was performed in a scanning electron microscope (Stereoscan S150, Cambridge, UK) to which a solid-state x-ray detecting system (LINK Systems, High Wycombe, UK) was attached. The measuring conditions were 20 kV acceleration voltage, between 0.2 and 0.5 nA beam current (determined in a Faraday cup at the level of the specimen), and 100 sec analysis time. Measurements were obtained in reduced raster mode, scanning areas of between 0.1 and 0.5 μm^2. The emitted x-rays were analyzed in the energy range from 0 to 10 keV, encompassing the α lines of the light elements Na, Mg, P, S, Cl, K, and Ca.

Quantification of cellular element concentrations and cellular dry weight fraction was achieved by a comparison of the cellular x-ray spectra with those obtained in the adherent albumin layer. Concentrations are expressed as mmol/kg wet wt or, after recalculation based on the cellular dry weight fraction, as mmol/liter cell water. Cellular dry weight fractions are given as g% (g dry matter/100 ml). All values are mean cellular concentrations derived from separate measurements in nucleus and cytoplasm or from measurement comprising both cellular compartments. Statistical significance was evaluated by Student's t-test (two-tailed). N indicates the number of bladders (experiments), n indicates the number of analyzed cells. With the exception of Figs. 3 and 5 all errors are standard deviations (SD). A detailed description of the methods has been given previously (Dörge et al., 1978; Bauer & Rick, 1978; Rick, Dörge & Thurau, 1982).

Results

Experiments were performed on toads kept under normal conditions (water-adapted) or toads adapted to saline for 4 to 6 days in order to reduce endogenous levels of aldosterone. Accordingly, the initial values of the short-circuit current (I_{sc}) and the response to aldosterone were markedly different between the two groups. As illustrated in Fig. 1, salt-adapted toads often showed low initial I_{sc} values (15.6 ± 7.3 $\mu A/cm^2$, $N = 6$) and large I_{sc} increases after addition of aldosterone. Conversely, the initial I_{sc} values of water-adapted toads were much higher (35.1 ± 14.8 $\mu A/cm^2$, $N = 8$, $2P < 0.02$), but seemingly no response to the hormone was detectable (Fig. 2). However, because of a large fall of the I_{sc} in the untreated hemibladder, the relative stimulation

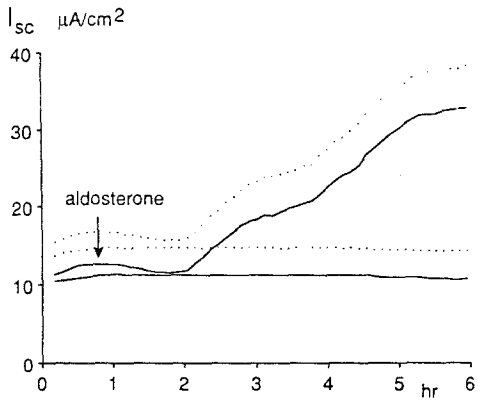

Fig. 1. Short-circuit current response to aldosterone (5×10^{-7} M, serosal bath) in a urinary bladder obtained from a salt-adapted toad (50% L-15). The distance between dots and continuous line is a measure of the transepithelial conductance (10 μA = 1 mS)

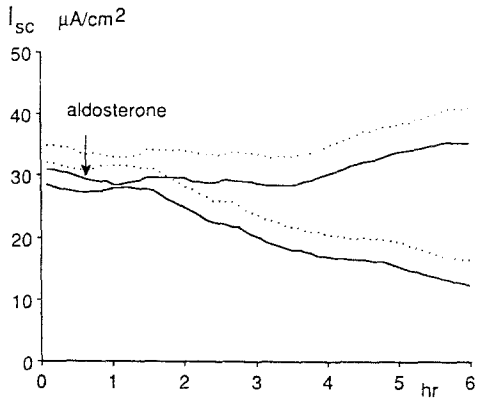

Fig. 2. Short-circuit current response to aldosterone (5×10^{-7} M, serosal bath) in a urinary bladder obtained from a water-adapted toad (50% L-15). The distance between dots and continuous line is a measure of the transepithelial conductance (10 μA = 1 mS)

Table 1. Effect of aldosterone and ADH on Na transport rate I_{sc} and transepithelial conductance g_t in urinary bladders of water- and salt-adapted toads

	I_{sc}-ctr.	I_{sc}-exp. ($\mu A/cm^2$)	I_{sc}/I_{sc}	g_t-ctr.	g_t-exp. (mS/cm^2)
Aldosterone					
Water-adapted, L-15, $N = 3$	14.0 ± 2.6	39.3 ± 16.2	2.8 ± 0.6	0.67 ± 0.44	0.83 ± 0.75
Salt-adapted, L-15, $N = 3$	9.9 ± 4.9	28.3 ± 10.4	3.1 ± 0.6	0.45 ± 0.29	0.80 ± 0.52
Salt-adapted, pyruvate, $N = 3$	12.1 ± 10.7	26.8 ± 25.4	2.1 ± 0.1	0.78 ± 0.50	1.26 ± 0.80
ADH					
Water-adapted, none, $N = 5$	16.2 ± 4.5	34.6 ± 10.2	2.0 ± 0.6	0.92 ± 0.51	1.68 ± 1.01

Means ± SD of n pairs of hemibladders. Readings were taken at the end of the incubation, immediately prior to freezing. The serosal solution was Ringer's solution with either 50% Leibovitz 15 medium (L-15), 5 mM pyruvate (pyruvate), or no metabolic substrate added (none). Relative stimulation of the short-circuit current (I_{sc}/I_{sc}) was calculated considering the spontaneous variations in the I_{sc} as monitored in the control hemibladder.

of the I_{sc} at the time of freezing was comparable to salt-adapted toads.

Addition of 50% Leibovitz 15 (L-15) medium or pyruvate did not significantly change the baseline transport rate, but had a marked effect on the transport stimulation by aldosterone. Bladders incubated without metabolic substrates often showed no I_{sc} increase and large increases in conductance. Addition of pyruvate significantly improved the response to aldosterone. However, best results were obtained when the bladders were incubated with L-15 medium. Table 1 lists the I_{sc} values and conductance values of all bladders included in this study.

The effect of aldosterone on intracellular electrolyte concentrations was investigated in nine pairs of hemibladders. The tissues were frozen between 4 and 6 hr after addition of the hormone (on average 4.9 ± 0.9 hr). In all experiments the Na concentration in granular cells of the aldosterone-treated hemibladder was increased compared to the untreated hemibladder, on average from 8.9 ± 1.3 to 13.2 ± 2.2 mmol/kg wet wt ($N = 9$, $2P < 0.001$, paired data). In only one experiment (with pyruvate Ringer's) was this change not statistically significant. Moreover, a significant drop in the Cl concentration was detectable, on average from 40.2 ± 2.5 to 35.8 ± 4.6 mmol/kg wet wt ($N = 9$, $2P < 0.01$, paired data).[1]

Similar, though somewhat smaller, concentration changes were observed in basal cells. Table 2

[1] The reported mean intracellular Cl concentration is likely an overestimation of the actual free cytoplasmic values because of the inclusion of intracytoplasmatic organelles with elevated Cl concentrations. In fact, when basing our results on nuclear measurements alone, the Cl concentration was lower by about 7 to 8 mmol/kg wet wt and the fall in Cl after aldosterone was more pronounced.

Table 2. Effect of aldosterone on intracellular electrolyte concentrations in urinary bladders of water- and salt-adapted toads

		Na	K	Cl
		(mmol/kg wet wt)		
Water-adapted toads, L-15 medium				
Granular cells	Control (n = 74)	9.0 ± 6.1	131.5 ± 21.8	39.9 ± 10.7
	Aldosterone (n = 81)	14.0 ± 9.4[a]	125.4 ± 20.3[a]	36.3 ± 12.1[a]
Basal cells	Control (n = 24)	12.7 ± 7.2	123.4 ± 19.9	39.1 ± 10.8
	Aldosterone (n = 32)	17.9 ± 7.4[a]	113.0 ± 15.6[a]	34.7 ± 8.4[a]
Salt-adapted toads, L-15 medium				
Granular cells	Control (n = 93)	8.5 ± 4.6	118.4 ± 17.6	46.9 ± 12.5
	Aldosterone (n = 121)	12.7 ± 6.5[a]	115.6 ± 14.9	40.7 ± 10.3[a]
Basal cells	Control (n = 22)	11.9 ± 6.2	120.4 ± 14.2	46.0 ± 8.3
	Aldosterone (n = 27)	13.2 ± 5.7	120.9 ± 15.1	41.4 ± 7.7[a]
Salt-adapted toads, pyruvate Ringer's				
Granular cells	Control (n = 61)	9.6 ± 3.7	123.4 ± 15.7	35.3 ± 7.7
	Aldosterone (n = 49)	13.4 ± 5.0[a]	119.6 ± 18.9	31.4 ± 8.9[a]
Basal cells	Control (n = 23)	11.1 ± 7.1	116.5 ± 19.3	33.2 ± 9.6
	Aldosterone (n = 17)	14.0 ± 6.7[a]	115.8 ± 21.4	31.4 ± 6.2

Means ± SD of n cells obtained from nine pairs of hemibladders.
[a] Significantly different from control ($2P < 0.05$). The serosal solution was Ringer's solution with either 50% Leibovitz 15 medium (L-15 medium) or 5 mM pyruvate (pyruvate Ringer's) added. Aldosterone (500 nM) was given for 4–6 hr.

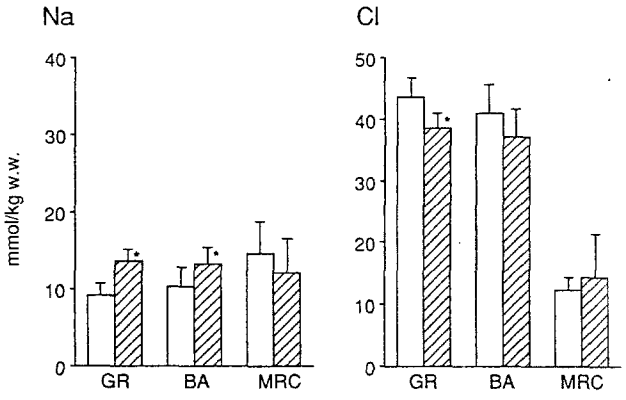

Fig. 3. Intracellular Na and Cl concentrations in granular (*GR*), basal (*BA*), and mitochondria-rich cells (*MRC*) in control (open columns) and after aldosterone (hatched columns). The experiment was in a salt-adapted toad using 50% L-15 as serosal incubation medium. Means ± 2 SEM. * Significantly different from control ($2P < 0.05$)

lists the results for both cell types according to the adaptation state of the animals and incubation medium used. The increase in the Na concentration and decrease in the Cl concentration were not significantly different between water- and salt-adapted toads, nor between bladders incubated with 50% L-15 medium or pyruvate Ringer's solution. In water-adapted toads an additional small drop in the K concentration was detectable. The dry weight fraction of granular cells varied between 19.3 and 22.1 g%, regardless of the experimental condition.

Analyses in mitochondria-rich cells and goblet cells were made difficult by the relatively small number of these cells and the large variability of their electrolyte composition. Mitochondria-rich cells typically showed very low intracellular Cl concentrations, ranging between 4.2 and 18.7 mmol/kg wet wt in individual bladders. Similar low Cl values were obtained in nuclei of goblet cells. Cytoplasmic measurements in goblet cells in the region containing mucus granules demonstrated very high Ca and S concentrations, consistent with a previous observation (Rick et al., 1978). In general, the electrolyte concentrations in goblet and mitochondria-rich cells were unaffected by aldosterone. Figure 3 shows the result of a typical experiment performed on a salt-adapted toad.

Figure 4 illustrates the effect of ADH on short-circuit current and transepithelial conductance. Compared to aldosterone the stimulation of the I_{sc} was much faster and, at least in part, transient in nature. On average, the I_{sc} increased from 17.3 ± 6.8 to a maximal value of 41.4 ± 13.8 μA/cm^2 (N = 5, $2P < 0.05$, paired data). In one case the tissue was frozen at the peak of the I_{sc} response, 12 min after addition of the hormone. In all other experiments the tissue was frozen after 30 to 40 min (on average 31 ± 8 min). The I_{sc} values at the time of freezing are listed in Table 1.

Table 3 summarizes the effects of ADH on the intracellular electrolyte concentrations of granular cells and basal cells. In granular cells a large Na

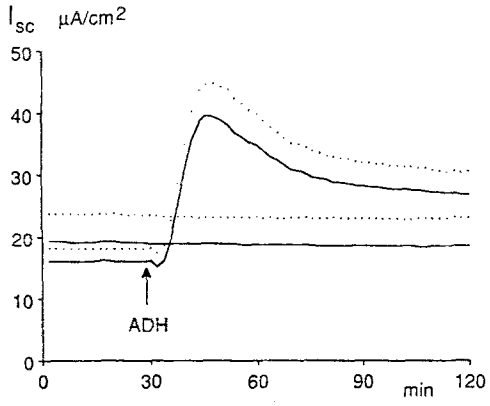

Fig. 4. Short-circuit current response to ADH (150 mU/ml, serosal bath) in a urinary bladder obtained from a water-adapted toad (normal Ringer's). The distance between dots and continuous line is a measure of the transepithelial conductance (10 μA = 1 mS)

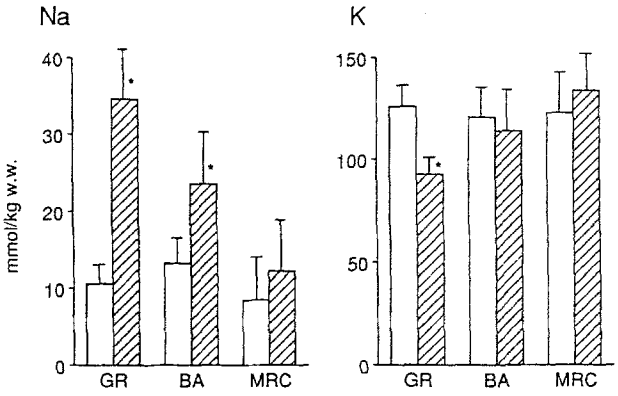

Fig. 5. Intracellular Na and K concentrations in granular (*GR*), basal (*BA*), and mitochondria-rich cells (*MRC*) in control (open columns) and after ADH (hatched columns). The experiment was in a water-adapted toad using normal Ringer's as serosal incubation medium. Means ± 2 SEM. * Significantly different from control ($2P < 0.05$)

Table 3. Effect of ADH on intracellular electrolyte concentrations in urinary bladders of water-adapted toads

		Na	K (mmol/kg wet wt)	Cl
Granular cells	Control (n = 112)	11.9 ± 4.9	124.7 ± 16.9	38.6 ± 5.9
	ADH (n = 131)	31.4 ± 12.3[a]	102.9 ± 17.4[a]	39.3 ± 6.5
Basal cells	Control (n = 41)	10.7 ± 5.6	128.2 ± 18.3	40.2 ± 5.8
	ADH (n = 44)	21.5 ± 7.8[a]	118.9 ± 20.1[a]	39.7 ± 7.0

Means ± SD of n cells obtained from five pairs of hemibladders.
[a] Significantly different from control ($2P < 0.05$). Incubation with normal Ringer's solution. ADH (150 mU/ml) was given for 31 ± 8 min.

concentration increase and an about equivalent K concentration decrease was observed. On average, Na increased from 12.0 ± 2.3 to 31.4 ± 9.3 mmol/kg wet wt ($N = 5$, $2P < 0.02$, paired data) and K decreased from 125.2 ± 5.1 to 103.0 ± 10.8 ($2P < 0.05$). Somewhat smaller changes were observed in basal cells. Other element concentrations, including that of Cl, and the dry weight fractions were not significantly changed.

In mitochondria-rich cells or goblet cells no significant concentration changes were detectable after ADH. It should be noted, however, that only in two of the bladders was a sufficiently large number of mitochondria-rich cells analyzable. Figure 5 depicts such an experiment. Compared to granular cells, the Na concentration increase in basal cells was much less pronounced, however still statistically significant, whereas in mitochondria-rich cells the Na concentration remained unchanged. The fall in the K concentration attained statistical significance only in granular cells.

Discussion

There is considerable disagreement in the literature as to the effect of aldosterone on intracellular Na. Chemical analyses of the intact toad urinary bladder failed to produce any evidence for a Na concentration increase (Handler, Preston & Orloff, 1969). Using isolated epithelial cells, Handler et al. (1972) noted a small Na increase whereas Lipton and Edelman (1971) observed no change. In the rabbit urinary bladder Wills and Lewis (1980) were unable to demonstrate any significant alteration of the intracellular Na activity while Eaton (1981) reported an almost three-fold increase.

In the present study in all but one bladder a statistically significant increase in the Na concentration was detectable after 4 to 6 hr incubation with aldosterone. On average, the Na concentration in granular cells increased from 8.9 ± 1.3 to 13.2 ± 2.2 mmol/kg wet wt ($N = 9$, $2P < 0.001$, paired data), or when expressed per liter cell water from 11.2 ±

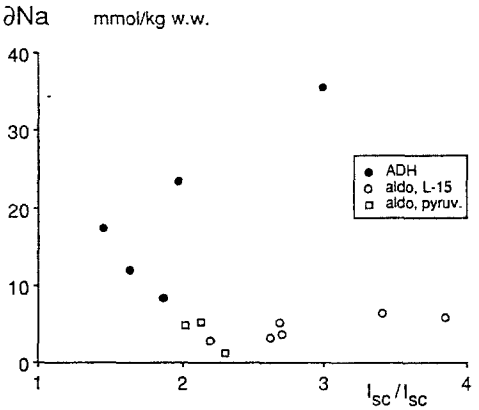

Fig. 6. Na concentration increase (∂Na) plotted as function of the relative stimulation of the short-circuit current (I_{sc}/I_{sc}). Open symbols denote experiments with aldosterone; filled symbols denote experiments with ADH. The ADH experiment demonstrating the largest Na increase was frozen at the peak of the I_{sc} response, 12 min after addition of the hormone, whereas all others were frozen after 30 to 40 min

1.6 to 16.6 ± 2.7 mmol/liter ($2P < 0.001$).[2] The observed increase in the Na concentration confirms the view that the hormone stimulates transepithelial Na transport primarily by enhancing apical Na influx.

Compared to ADH, however, the increase in the intracellular Na concentration after aldosterone was significantly less pronounced. The differential effects of the two hormones are obvious from Fig. 6, which plots the Na concentration increase in granular cells as a function of the relative I_{sc} increase. It is evident that, regardless of the degree of transport stimulation, aldosterone always produced a much smaller increase in the Na concentration. Notably, in the aldosterone experiments no correlation is apparent between intracellular Na increase and transport stimulation.

The much-attenuated Na increase suggests that aldosterone stimulates active Na extrusion, in addition to stimulating apical Na influx. This finding is in apparent conflict with data obtained in the rabbit and turtle colon, which demonstrated the absence of any Na pump stimulation (Frizzell & Schultz, 1978; Halm & Dawson, 1985). Apart from the fact that different epithelial preparations were used, the discrepancy of results may be explained by a difference in the experimental protocol applied. In both earlier investigations the polyene antibiotic amphotericin-B was used to permeabilize the apical membrane and, thereby, functionally eliminate the outer transport barrier. Since the pores formed by amphotericin-B are rather unspecific, the possibility exists that a potential intracellular messenger of the aldosterone action was eliminated as well.

A factor that immediately comes to mind is an aldosterone-induced alkalinization caused by a stimulation of the Na/H exchanger (Oberleithner et al., 1987). Indeed, the fall in the Cl concentration of granular cells may be taken as a sign of intracellular alkalinization since the increased dissociation of cellular proteins and nucleic acids would lower the requirement for small anions. In agreement with this expectation, a significant reduction of the intracellular Cl concentration was observed in rat kidney tubules during metabolic alkalosis (Beck et al., 1982). A rise in pH could very well account for the stimulation of the pump as impalements with pH-sensitive microelectrodes have shown that the Na pump rate is a function of the intracellular pH (Eaton, Hamilton & Johnson, 1984). Similarly, since the apical Na influx is known to be pH-sensitive (Mandel, 1978; Garty, Civan & Civan, 1985; Palmer, 1985), we may speculate that the effect of aldosterone on the apical membrane is, at least in part, mediated by an intracellular alkalinization.

Alternatively, the stimulation of the pump by aldosterone could be explained by an increased biosynthesis (Geering et al., 1982) and increased membrane expression of pump units. Conceivably, also the intracellular Na concentration could play a role in modulating the expression of the pump. In the frog skin epithelium we obtained some evidence for a Na-pump stimulation by ADH when the intracellular Na concentration was allowed to rise, however, not when the Na increase was prevented by blocking the apical Na influx with amiloride (Rick et al., 1984). Since the recruitment or insertion of pumps is likely to be a slow process, the differences in the intracellular Na concentrations may simply reflect the different speed of action of the two hormones.

Our results may also shed some light on the metabolic regulation of transepithelial Na transport. In experiments in which pyruvate was provided as the only metabolic substrate generally a smaller stimulation of the short-circuit current was observed than in experiments with 50% L-15 (*see* Fig. 6). In spite of this, the Na increase was similar in both groups, implying that the metabolic control of the Na influx is about as effective as the control of the pump activity. Obviously, the epithelial cell is capable of adjusting the rate of transepithelial Na

[2] This calculation presumes that all Na is dissolved in the watery phase, which need not be the case since the method measures total element concentration, regardless of binding state. Nevertheless, the close agreement with ion activity measurements (Rick et al., 1985) and the observation that most of the intracellular electrolytes are osmotically active (Rick & DiBona, 1987) would suggest only very little intracellular binding.

transport to its metabolic supplies without necessarily disturbing the intracellular Na concentration. In agreement with this view, no significant differences were observed between granular cells which were incubated with pyruvate, L-15 medium, or no metabolic substrates (*compare* controls in Tables 2 and 3).

Consistent with the view that the granular cells are the main target of the aldosterone action, this cell type experienced the largest changes in the intracellular Na and Cl concentrations. Nevertheless, statistically significant Na concentration changes were detectable in basal cells as well. This finding can be explained by a syncytial coupling between the two cell types (Rick et al., 1978). In this way, the basal cells are participating in transepithelial Na transport. Perhaps the best evidence for the existence of permeable cell-to-cell junctions between granular and basal cells is the observation that the ADH-induced Na concentration increase in basal cells was preventable by mucosal application of amiloride (Rick & DiBona, 1987). Regarding the mitochondria-rich cell, our results are less clear. Although occasionally this cell type showed a response to aldosterone, in the majority of bladders this was not the case. Further studies on the role of this cell type are needed.

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