J. Membrane Biol. 68, 11–18 (1982)

The Journal of
Membrane Biology

The Significance of Changes in Thermodynamic Affinity Induced by Aldosterone in Sodium-Transporting Epithelia

R. Beauwens, V. Beaujean, and J. Crabbé

Department of Physiology, University of Louvain (U.C.L.), Medical School, B-1200 Brussels, Belgium

Summary. The energetics of sodium transport were examined in toad (and occasionally frog) skin, with particular emphasis on the effect of aldosterone.

Thermodynamic affinity was computed according to Essig and Caplan. Following treatment with antidiuretic hormone or drugs believed to affect only the apical membrane barrier, no change in thermodynamic affinity was observed either acutely (after one to two hours) or chronically (after 18-odd hours).

By contrast, following treatment with aldosterone overnight, thermodynamic affinity was considerably increased, whether or not incubation was conducted in the presence of sodium in the outer solution; addition of glucose at the end of incubation, whereby sodium transport was stimulated further, failed to influence affinity as measured. The stoichiometry between sodium transport and oxygen consumption was, however, unchanged by aldosterone treatment in short-circuit conditions, neither was that fraction of aerobic metabolism unrelated to sodium transport influenced.

It is concluded that the change observed with aldosterone can be directly ascribed to the hormone, as it is independent of glucose availability and of sodium transport. Aldosterone action, at least following prolonged incubation, therefore does not involve only an increase in apical conductance for sodium.

Key words amphibian epithelia · sodium transport · oxygen consumption · aldosterone · vasopressin · indanone · amiloride

Introduction

Amphibian skin is an adequate preparation for evaluation of the action exerted by aldosterone on transepithelial sodium movement: after several hours of exposure to the hormone, toad skin in particular, transports appreciably more sodium, at a stable rate; furthermore, when energy-providing substrates such as glucose are added at that stage, there is a further increase of sodium transport (Crabbé, Decoene & Ehrlich, 1971). This substrate effect, although not demonstrable in acute experiments on fresh bladder tissue, has been used as a significant argument for a hormonal influence exerted on the metabolic pathways involved in the operation of this sodium pump in target epithelia (for a review, *see* Crabbé, 1977).

Essig and coworkers concluded likewise, from changes in thermodynamic affinity observed in frog skin exposed overnight to aldosterone, that this steroid hormone influences the energetics of the sodium-transporting system (Saito, Essig & Caplan, 1973).

In the present study thermodynamic affinity was computed according to Essig and Caplan (1968) for toad skin that had been incubated overnight with aldosterone. Affinity was increased with this hormone, even after incubation overnight in the absence of glucose and/or of sodium to be transported. Glucose added in the morning did magnify the hormonal effect on sodium transport, without influencing affinity any further. On the other hand, affinity was not influenced by agents modifying acutely apical border conductance for sodium, i.e., vasopressin (stimulation), amiloride (inhibition) and its derivative dubbed MK-196 or indanone (stimulation). As the latter two drugs exert their respective influences for several hours, affinity could also be determined after overnight exposure of toad skin to them; there was no significant change in this variable either.

Therefore, the increase in thermodynamic affinity observed with aldosterone might indicate that this hormonal effect is not restricted to an increase in sodium conductance at the apical border of target epithelia.

Materials and Methods

Ventral skin of doubly pithed toads (*Bufo marinus*) kept on moist peat, or frogs (*Rana temporaria*) kept in tap water, were dissected and mounted for incubation according to Ussing and Zerahn (1951).

When prolonged treatment was required, the toad skin preparations were left in the open-circuit state overnight, after which they were short circuited for the experiment proper. Electrical potential difference (PD) was measured with a high input resistance electrometer (Keithley, model 610 C) and short-circuit

0022-2631/82/0068-0011 $01.60

current, on a DC amperemeter (Norma, model 251). The electrical potential difference readings were obtained during brief interruption of the current, for computation of ohmic conductance. The bathing solution was the usual frog Ringer's solution containing (in mM): NaCl, 115; $KHCO_3$, 2.5; $CaCl_2$, 1; pH was 7.8–8.0 at room temperature during aeration with atmospheric air; osmolality was 225 mOsm/kg H_2O. Occasionally a sodium-free solution was used on the outside, with $MgCl_2$, 57.5 mM, replacing NaCl. Concentration of Na^+ (5.75; 11.5 and 23 meq/liter) was lowered in the outside compartment in some experiments, by appropriate dilution of sodium Ringer's with sodium-free Ringer's solution. Glucose was added to the solution on the corial side only when stated, the final concentration being 10 mM.

All chemicals were reagent grade; amiloride and its analogue indanone ((6,7-dichloro-2-methyl-1-oxo-2-phenyl-5-indanyloxy) acetic acid) were gifts from Dr. Fanelli (Merck Institute, West Point, Pa.). Lysine-Vasopressin, synthetic, was a gift of Dr. Maeck (Sandoz, Brussels, Belgium); D-aldosterone, a gift of Dr. Tyberghein (Ciba-Geigy, Brussels, Belgium).

Oxygen consumption was measured by polarography in a glass chamber (membrane area: 4.15 cm^2; chamber volume on each side: 4.5 ml) equipped with inlets for electrical monitoring and for oxygen electrodes (Eschweiler, Inc., Kiel, FRG). Each side had its own reservoir and bubble air-lift device for rapid fluid circulation so that pO_2 inside both compartments of the chamber could be brought back to the initial level within 1 min. During interruption of the fluid circulation, mixing in the chamber was maintained by means of small motor-driven magnets. Oxygen consumption was summated for both compartments of the incubation chamber, so as to increase the precision of the readings.

Between experiments, chamber and connecting tubing were stored in 10% glutaraldehyde; the electrodes were kept immersed in water at room pO_2, and the Lucite holder of their Teflon membrane was covered with parafilm paper.

For oxygen consumption measurements, antibiotics (gentamicin, 10 μg/ml; penicillin G, potassium salt, 1000 U/ml) were added to all solutions that were also filtered through a millipore filter (0.22 μm). Despite those precautions, bacterial proliferation proved to be a continuous potential hazard; frequent renewal of the incubation solution on both sides of the skin seemed to be most effective in this respect. Furthermore, when pO_2 was not stable in the medium recovered at the end of an incubation, the corresponding set of data was discarded.

Incubation overnight was carried out in large section (8 cm^2) Lucite chambers with 10 ml of aerated solution on each side. The following morning, the tissue was transferred to the smaller glass chamber.

For calculation of thermodynamic affinity, the transepithelial electrical potential difference was usually set at zero first, then at −50 mV and +50 mV (with respect to the inner side) and again at zero at the end; oxygen consumption was measured each time for 10 min with 10–15 min allowed between "clampings," for new steady-state conditions to be reached (as judged by the stability of the current). When spontaneous electrical potential difference exceeded 50 mV, the preparation was "clamped" at 0, −75 or −100 mV, and at +50 mV.

The thermodynamic affinity (A), computed according to Essig and Caplan (1968), was expressed in kcal/mole O_2. Thus

$$A = J_{Na} \cdot F \cdot (\delta J_{r/\delta \Delta \Psi})^{-1}$$

in which $\Delta\Psi$ is the potential difference imposed, in mV; J_r is the corresponding rate of oxygen consumption, in moles per second; J_{Na} is the net sodium transport rate computed from short-circuit current, in equivalents per second, and F is the Faraday constant.

It should be stressed that calculation of thermodynamic affinity as performed rests on the following assumptions:

1) that affinity is stable during the period of measurement and is independent of $\Delta\Psi$. Vieira, Caplan and Essig (1972*b*) reported for frog skin, a linear relationship between J_r and imposed $\Delta\Psi$ over a range exceeding that adopted here. This point was specifically tested for toad skin in the range of −100 to +50 mV, and the data were distributed in a linear fashion, with a mean correlation coefficient of 0.92 (range: 0.82–0.99; $n=5$);

2) that electrical current as measured reflects net transcellular sodium flux and is for all practical purposes entirely supported by oxidative metabolism;

3) that the cross-coefficients in the phenomenological equations describing the system under investigation have identical values, i.e., that $L_{Na\,r} = L_{r\,Na}$ when

$$J_{Na} = L_{Na}(-F\Delta\Psi) + L_{Na\,r}A$$
$$J_r = L_{r\,Na}(-F\Delta\Psi) + L_rA$$

4) that perturbations in the transepithelial electrical potential difference are at least in part reflected across the basal lateral border of the epithelia examined, where the sodium "pump" operates.

In several instances, the aerobic cost of sodium transport was also estimated in short-circuit conditions, after subtraction of oxygen consumption when the outward-facing surface of the preparation was exposed to sodium-free Ringer's[1].

Data are expressed as means ± standard error (SE); differences were analyzed statistically by the paired t-test. All results are expressed per unit time and surface area.

Results

1. Thermodynamic Affinity in Aldosterone-Stimulated Toad Skin

In 10 instances, paired pieces of abdominal toad skin were incubated overnight in glucose-free Ringer's fluid either with or without aldosterone, 50 nM (final concentration). The following morning sodium transport by the hormone-treated preparations, was consistently increased as was affinity, whether or not sodium had been available for transport during the night (Table 1). Removal of sodium from the solution on the outside throughout the night was meant to increase the chances that the effects of aldosterone itself be evaluated, rather than possible nonspecific consequences of prolonged increased sodium-transporting activity. This manipulation merely rendered more apparent the hormonal influence on sodium transport and on thermody-

[1] One of us had concluded (Noé, Michotte & Crabbé, 1977) that residual oxygen consumption by frog skin incubated in Ringer's and exposed to large doses of amiloride (0.1 mM) on the outside, was different from that obtained after removal of sodium from the solution on the outside. As other investigators did not confirm this (Lau, Lang & Essig, 1979), the issue was reexamined and, with the improved methodology used here, we indeed failed to detect a significant difference in residual oxygen consumption by fresh frog skin examined in these two experimental conditions (Δ ±SE: 6.1 pmol $O_2/cm^2 \cdot sec \pm 4.0$; $n=6$).

Table 1. Effects of aldosterone on the sodium-transporting activity of toad skin

	Short-circuit current ($\mu A/cm^2$)		Electrical potential difference (mV)		Thermodynamic affinity (kcal/mole O_2)	
	Untreated	Aldosterone[a]	Untreated	Aldosterone[a]	Untreated	Aldosterone[a]
1) *Ringer's solution on the outside throughout*						
	6	19	12	24	5	72
	6	30	23	48	13	40
	8	12	30	28	17	28
	24	53	55	54	40	47
	30	51	38	33	22	36
2) *Sodium-free solution on the outside overnight*[b]						
	11	39	62	63	17	49
	2	16	6	11	6	43
	5	12	11	9	14	21
	32	97	50	42	23	84
	25	83	43	70	55	72
$\bar{x}$	14.9	41.2	33.0	38.2	21.2	49.2
$\Delta \pm$SE:	26.3 ± 6.4 ($P<0.01$)		5.2 ± 3.9 ($P>0.2$)		28.0 ± 6.8 ($P<0.01$)	

[a] The hormone was added the preceding evening to Ringer's solution on the inside, at the final concentration of 50 nM.
[b] Experiments were carried out during the second and third hours which followed replacement of this solution with standard Ringer's in the morning.

Table 2. Effects of glucose on the sodium-transporting activity of toad skin exposed overnight to aldosterone

	Short-circuit current ($\mu A/cm^2$)		Electrical potential difference (mV)		Thermodynamic affinity (kcal/mole O_2)	
	Aldosterone	+Glucose[a]	Aldosterone	+Glucose[a]	Aldosterone	+Glucose[a]
	16	31	28	55	30	22
	30	36	48	58	40	31
	21	30	40	57	37	61
	29	39	45	48	38	29
	20	28	27	44	30	36
	25	38	35	40	27	31
$\bar{x}$	23.5	33.2	37.2	50.3	33.7	35.0
$\Delta \pm$SE:	9.7 ± 1.4 ($P<0.01$)		13.1 ± 3.7 ($P<0.01$)		1.3 ± 5.3 ($P>0.8$)	

[a] The preparations were examined before and again after addition of glucose to Ringer's solution on the inside (final concentration: 10 mM); 1 hr at least had to be allowed for sodium transport activity to stabilize in the presence of glucose.

namic affinity. Therefore, all data were pooled for statistical analysis.

Addition of glucose to the incubation medium after exposure of toad skin to aldosterone overnight led to a further, rapid increase in sodium-transporting activity, with a new steady state reached by the second hour (Crabbé et al., 1971). Thermodynamic affinity did not change in these conditions (Table 2).

Tissue conductance, markedly increased by treatment with aldosterone ($\Delta \pm$SE: $0.68\,mS/cm^2 \pm 0.14$) was not influenced further by exposure to glucose ($\Delta \pm$SE: $0.05\,mS/cm^2 \pm 0.05$).

2. *Thermodynamic Affinity in Vasopressin-Treated Frog Skin*

Sodium transport can be stimulated acutely with vasopressin, as a result of increased sodium conductance at the apical border of skin epithelium (Nagel, 1978). Furthermore, in the case of fresh frog

Table 3. Effects of vasopressin on the sodium-transporting activity of frog skin

	Short-circuit current ($\mu A/cm^2$)		Electrical potential difference (mV)		Thermodynamic affinity (kcal/mole O_2)	
	Untreated	Vasopressin[a]	Untreated	Vasopressin[a]	Untreated	Vasopressin[a]
	20	47	17	39	33	39
	16	34	15	34	29	28
	12	23	21	37	17	19
	53	71	54	51	66	58
	12	39	8	36	17	33
	23	43	26	36	29	43
	23	61	41	45	21	49
	55	68	49	50	43	37
$\bar{x}$	26.8	48.3	28.8	41.0	31.8	38.0
$\Delta \pm$SE:	21.5 ± 3.1 ($P<0.01$)		12.2 ± 3.9 ($P<0.01$)		6.2 ± 4.3 ($P>0.1$)	

[a] Values were obtained during the second hour which followed addition of lysine-vasopressin to Ringer's solution (final concentration: 100 mU/ml) on the inside of fresh preparations that had been examined first in the untreated state.

Table 4. Effects of indanone on the sodium-transporting activity of toad skin

	Short-circuit current ($\mu A/cm^2$)		Electrical potential difference (mV)		Thermodynamic affinity (kcal/mole O_2)	
	Untreated	Treated	Untreated	Treated	Untreated	Treated
			Acute treatment[a]			
	13	20	23	26	38	58
	31	45	17	45	31	72
	17	57	36	50	26	47
	46	60	35	49	48	68
	69	92	40	70	47	39
	11	20	9	16	42	32
	62	71	48	73	32	46
$\bar{x}$	35.6	52.1	29.7	47.0	37.7	51.7
$\Delta \pm$SE:	16.5 ± 4.4 ($P<0.01$)		17.3 ± 4.0 ($P<0.01$)		14.0 ± 6.7 ($P>0.05$)	
			Chronic treatment[b]			
	19	26	34	37	22	21
	33	68	62	92	41	33
	36	38	58	75	36	34
	41	51	45	64	35	45
	19	29	17	31	27	26
	27	77	30	77	30	50
$\bar{x}$	29.2	48.2	41.0	62.7	31.8	34.8
$\Delta \pm$SE:	19.0 ± 7.8 ($P<0.05$)		21.7 ± 6.2 ($P<0.02$)		3.0 ± 4.2 ($P>0.5$)	

[a] Preparations were examined before and during the second and third hours which followed addition of the drug, 1 mM in Ringer's on the outside.
[b] Data were obtained for matched skin pieces incubated overnight; one piece of each pair was exposed throughout to indanone added on the outside (final concentration: 1 mM).

skin, the stimulation lasts for several hours (Fuhrman & Ussing, 1951), making it possible to deal with stable preparations both before and after hormonal treatment.

As appears from Table 3, thermodynamic affinity was not significantly modified by this peptide hormone.

Transepithelial conductance was little influenced by this hormone in the present case ($\Delta \pm$SE: $0.17 \text{ mS/cm}^2 \pm 0.11$).

Table 5. Effects of amiloride on the sodium-transporting activity of toad skin

	Short-circuit current ($\mu A/cm^2$)		Electrical potential difference (mV)		Thermodynamic affinity (kcal/mole O_2)	
	Untreated	Treated	Untreated	Treated	Untreated	Treated
			Acute treatment[a]			
	39	10	22	17	37	57
	35	21	34	29	36	30
	71	29	38	27	55	65
	13	3	11	2	14	14
	29	12	36	24	26	14
	63	29	45	36	44	48
	54	22	45	24	57	75
$\bar{x}$	43.4	18.0	33.0	22.7	38.4	43.3
$\Delta \pm$SE:	25.4 ± 4.5 ($P<0.01$)		10.3 ± 2.1 ($P<0.01$)		4.9 ± 4.5 ($P>0.2$)	
			Chronic treatment[b]			
	57	25	50	58	29	20
	34	16	32	16	53	15
	67	32	71	60	32	32
	34	26	37	43	67	53
	27	14	23	21	25	29
$\bar{x}$	43.8	22.6	42.6	38.1	41.2	29.8
$\Delta \pm$SE:	21.2 ± 5.3 ($P<0.02$)		4.5 ± 6.2 ($P>0.4$)		11.4 ± 7.3 ($P>0.1$)	

[a] Preparations were examined before and during the second and third hours which followed addition of the drug, 0.5 μM, in Ringer's on the outside.

[b] Data were obtained for matched skin pieces incubated overnight; one piece of each pair was exposed throughout to amiloride on the outside (final concentration: 0.5 μM).

3. Thermodynamic Affinity in Toad Skin Treated with Indanone or Amiloride

For proper interpretation of the difference with respect to affinity when vasopressin and aldosterone are compared, two factors at least have to be considered: animal species and duration of incubation — since prolonged incubation is required for full expression of aldosterone action on amphibian skin (Crabbé et al., 1971).

Therefore, experiments were conducted with toad skin exposed to indanone, a drug chemically related to amiloride (Fanelli, Bohn, Scriabine & Beyer, 1977). Unlike the latter, indanone added to the outside stimulates transepithelial sodium transport, through an increase in apical border conductance (Fisher, Fanelli & Helman, 1978).

Furthermore, this effect was observed to last for several hours, making it possible to compare preparations stimulated acutely or chronically with the same agent.

The pertinent data are given in Table 4: neither short-term nor prolonged exposure of the outer surface of toad skin to indanone led to significant changes in thermodynamic affinity.

Transepithelial conductance was modified by this treatment neither acutely ($\Delta \pm$SE: 0.11 $mS/cm^2 \pm 0.58$) nor after several hours ($\Delta \pm$SE: 0.01 $mS/cm^2 \pm 0.07$).

Analogous experiments were conducted with amiloride that inhibits sodium transport instead, in a dose-dependent and fully reversible fashion (Bentley, 1968; Ehrlich & Crabbé, 1968). The action of this drug has been ascribed to a decrease in apical border conductance for sodium (Helman & Fisher, 1977; Sudou & Hoshi, 1977). Again, neither in acute nor in chronic conditions did thermodynamic affinity change as a consequence of treatment with small amounts of amiloride (Table 5).

Tissue conductance decreased for both sets of preparations treated with amiloride ($\Delta \pm$SE: 0.44 $mS/cm^2 \pm 0.12$ and 0.50 $mS/cm^2 \pm 0.11$, respectively).

4. Aerobic Cost of Sodium Transport by Toad Skin

One could expect changes in thermodynamic affinity at the energy-requiring step (s) accounting for active sodium transport by amphibian skin, to be reflected in shifts in the stoichiometry at the "pump" site (Al-Awqati, 1977). This was thus examined in short-

Table 6. Aerobic cost of sodium transport by toad skin treated with aldosterone.

	Baseline oxygen consumption[a] (pmol/cm² sec)		Stoichiometric ratio[a] (eq/mole)	
	Untreated	Aldosterone[b]	Untreated	Aldosterone[b]
	42	46	8.3	12.1
	38	29	17.9	20.7
	44	44	12.5	13.5
	61	36	12.3	18.1
	63	44	14.2	13.7
	32	36	13.1	13.0
	32	33	13.1	11.1
	34	40	9.7	12.6
	108	88	12.7	14.9
$\bar{x}$	50	44	12.6	14.4
$\Delta \pm$SE:	6 ± 4 ($P > 0.1$)		1.8 ± 0.8 ($P > 0.05$)	

[a] After incubation overnight, oxygen consumption was measured first in the short-circuit state for preparations maintained in Ringer's solution and after partial or total replacement of Ringer's on the outside with sodium-free solution. The stoichiometric ratio $\delta J_{Na}/\delta J_r$ was calculated by regression analysis in individual experiments.

[b] The steroid hormone had been added at the outset to Ringer's solution on the inside at the final concentration of 50 nM.

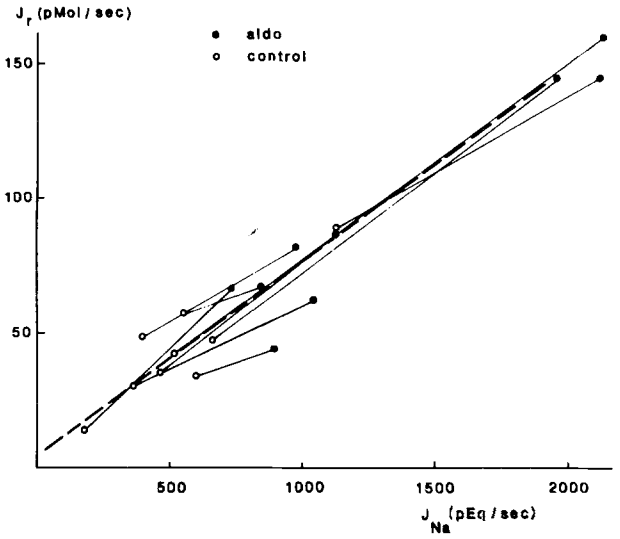

Fig. 1. Aerobic cost of sodium transport by toad skin treated with aldosterone. Matched pieces of the abdominal skin of *Bufo marinus* were incubated overnight in Ringer's fluid *vs.* Ringer's containing aldosterone, 50 nM on the inside. The following morning, sodium transport (J_{Na}) was evaluated by means of short-circuit current; and aerobic metabolism (J_r), measured polarographically, was taken as the *difference* between the value obtained in the short-circuit state and after withdrawal of sodium from the solution on the outside. The solid lines connect preparations of the same animal treated or not with aldosterone, with mean slope of 18.9 ± 2.5 eq $\cdot Na^+$ per mole of O_2 consumed. The broken line describes the relationship between J_{Na} and J_r for the nine untreated skins ($r = 0.90$); the relationship for the aldosterone-treated skin ($r = 0.95$) is not statistically different. Sodium transport averaged 33 peq/cm² · sec for untreated preparations, and 80 peq/cm² · sec in the presence of aldosterone

circuit conditions for nine pairs of aldosterone-treated and matched untreated toad skin.

Oxygen consumption was measured after overnight pre-incubation, before and after partial or total removal of sodium from the outside compartment.

The results are presented in Table 6: neither the residual oxygen consumption in the absence of sodium in the outside compartment, nor the relationship between sodium transport and related oxygen consumption were significantly altered by hormonal treatment.

In Fig. 1, suprabasal oxygen consumption is related to sodium transport rate in reference conditions (sodium Ringer's in both compartments, no substrate) for all pieces of skin, treated or not with aldosterone: One obviously deals with a homogeneous population. From regression analysis of the nine untreated preparations (of as many animals), a stoichiometric ratio of 14.0 ± 2.5 moles Na^+ per mole O_2 consumed is arrived at, while for the nine aldosterone-treated preparations, the ratio is 14.4 ± 1.6.

Discussion

Thus, thermodynamic affinity was clearly increased after treatment of toad skin with aldosterone. These results confirm in essence the report of Saito et al. (1973); they make it possible in addition to ascribe the change in affinity to the hormone itself rather than to the availability of glucose as energy-providing substrate and/or of sodium on the outside during the overnight preincubation period.

With respect to affinity, aldosterone seems to differ from vasopressin and from indanone, both of which bring about a stimulation of transepithelial sodium transport without influencing affinity; the latter was unaffected when transport was partially inhibited by small doses of amiloride. As indicated, these three substances exert their effects on sodium transport through changes in conductance for sodium at the apical border of amphibian skin epithelium.

Admittedly, it is reasoned that toad and frog skins are qualitatively similar in terms of response to vasopressin; as the effect of this hormone is sustained only in the case of frog preparations, the latter were selected.

Since indanone and amiloride remain active for several hours as stimulus *vs.* inhibitor of sodium transport, respectively, these drugs allowed an eval-

uation of late, possibly indirect changes of affinity related to prolonged incubation.

In fact, affinity was not significantly affected when toad skin was exposed overnight to indanone or amiloride. Whether affinity increases transiently during prolonged exposure to amiloride (Saito et al., 1973) cannot be ruled out.

On the other hand, the increase in thermodynamic affinity after aldosterone treatment stands in apparent contrast with the lack of change in aerobic cost of sodium transport in short-circuit conditions (Fig. 1 and Table 6).

Essig (1978) suggested that thermodynamic affinity is equal to the phosphorylation potential of the adenine nucleotides in the cytoplasm of transporting cells; this potential is defined as

$$\Delta G_{ATP} = -\Delta G_o + RT \ln \frac{(ATP)}{(ADP)(P_i)}$$

Slater, Rosing & Mol, 1973) in which (ATP), (ADP) and (P_i) stand for the cytoplasmic activity of adenosine triphosphate, adenosine diphosphate, and inorganic phosphate, respectively; ΔG_o is the standard potential of hydrolysis of ATP.

On this basis, one possible explanation for the increase in thermodynamic affinity following prolonged incubation with aldosterone, could lie in an increase in the number of functionally active mitochondria. Although there is actually a morphological counterpart to such a hypothesis (Voûte, Dirix, Nielsen & Ussing, 1969), measurements of ATP and ADP concentrations in toad bladder tissue failed to disclose changes after stimulation with aldosterone (Sharp & Leaf, 1966; Handler, Preston & Orloff, 1972).

Nevertheless, assuming a P/O ratio of 3, a value for ΔG_{ATP} of 6.5 kcal/mole ATP can be calculated for fresh untreated toad skin preparations for which thermodynamic affinity averaged 38 kcal/mole O_2 (*cf.* Tables 4 and 5). This value is not totally out of range when compared with that obtained from direct measurements of (ATP), (ADP) and (P_i), i.e.: 11.3 kcal/mole ATP in isolated hepatocytes (Wilson et al., 1974) and 11.0 kcal/mole ATP in turtle bladder under freeze clamped conditions (Dixon & Al Awqati, 1981); the value ascribed to ΔG_o by these authors may not, however, reflect the conditions prevailing in the cytoplasm.

Assuming an overall electromotive force of 120 mV for the sodium "pump" in toad skin (Nagel & Crabbé, 1980), and on the basis of ΔG_{ATP} derived from thermodynamic affinity (Dixon & Al Awqati, 1980), a ratio of 2.3 Na^+ transported per ATP consumed is computed for the data presented. From simultaneous measurements of sodium transport and oxygen consumption (Fig. 1), assuming a P/O ratio of 3, the number of Na^+ ions translocated per ATP molecule hydrolyzed ranges from 2.1 to 3.1.

The relationship between sodium transport and oxygen consumption for different toad skin preparations was quite uniform in the present study (Fig. 1); this stands in contrast to reports for frog skin (Vieira, Caplan & Essig, 1972*a*) and toad bladder (Al Awqati, Beauwens & Leaf, 1975). It is possible that there is a link between stability of the preparations in terms of short-circuit current and the relationship illustrated in Fig. 1, since in the studies quoted, a spontaneous decline of sodium transport was a constant observation.

It must be emphasized at this stage that theoretical objections have recently been raised regarding thermodynamic affinity computed according to Essig and Caplan (Canessa, Labarca, DiBona & Leaf, 1978). In our view, an important, unsolved question is the extent to which imposed transepithelial potential differences influence the electrical potential across the sodium "pump" proper.

Bearing this reservation in mind, with respect to thermodynamic affinity is still remains that aldosterone stands in contrast with vasopressin, amiloride, and indanone. Since the latter three substances that do not modify affinity significantly are thought to influence apical conductance for sodium only, the increase in affinity upon prolonged exposure to aldosterone possibly reflects a change in the cellular metabolism triggered by the hormone, aside from its effect on apical sodium conductance. In fact, recent electrophysiological measurements have led to the same conclusion (Nagel & Crabbé, 1980).

We thank Drs. Qais Al Awqati, Alvin Essig, and Wolfram Nagel for comments on various aspects of these studies.

This work was supported in part by the Fonds National de la Recherche Scientifique, Brussels, Belgium.

A preliminary account of this work has been published (Beaujean, Beauwens & Crabbé, 1979).

References

Al-Awqati, Q. 1977. Effect of aldosterone on the coupling between H^+ transport and glucose oxidation. *J. Clin. Invest.* **60**:1240–1247

Al-Awqati, Q., Beauwens, R., Leaf, A. 1975. Coupling of sodium transport to respiration in the toad bladder. *J. Membrane Biol.* **22**:91–105

Beaujean, V., Beauwens, R., Crabbé, J. 1979. Hormonal influences on the driving force for sodium transport in amphibian epithelia. *J. Physiol. (London)* **295**:48–49*P*

Bentley, P.J. 1968. Amiloride: A potent inhibitor of sodium transport across the toad bladder. *J. Physiol. (London)* **195**:317–330

Canessa, M., Labarca, P., DiBona, D.R., Leaf, A. 1978. Energetics of sodium transport in toad urinary bladder. *Proc. Natl. Acad. Sci. USA* **75**:4501–4595

Crabbé, J. 1977. Mechanism of action of aldosterone. *In:* Receptors and Mechanism of Action of Steroid Hormones. J. Pasqualini, editor. Part II, Chap. 10. Marcel Dekker, New York: Modern Pharmacology-Toxicology series. Vol. 8, pp. 513–568

Crabbé, J., Decoene, A., Ehrlich, E.N. 1971. Some characteristics of the response of the ventral skin of the toad *Bufo marinus*, to aldosterone *in vitro*. *Arch. Int. Physiol. Biochim.* **79**:805–808

Dixon, T.E., Al-Awqati, Q. 1980. H^+/ATP stoichiometry of proton pump of urinary toad bladder. *J. Biol. Chem.* **255**:3237–3239

Dixon, T.E., Al-Awqati, Q. 1981. Ion transport as the pacemaker of cellular metabolism in the turtle urinary bladder. *Kidney Int.* **19**:238

Ehrlich, E.N., Crabbé, J. 1968. The mechanism of action of amipramizine. *Pfluegers Arch.* **302**:79–96

Essig, A. 1978. Evaluation of kinetic and energetic parameters of active sodium transport. *J. Membrane Biol.* **Special Issue**:15–27

Essig, A., Caplan, S.R. 1968. Energetics of active transport processes. *Biophys. J.* **8**:1434–1457

Fanelli, G.M., Jr., Bohn, D.L., Scriabine, A., Beyer, K.H., Jr. 1977. Saluretic and uricosurie effects of (6,7-dichloro-2-methyl-1-oxo-2-phenyl-5-indanyloxy) acetic acid (MK-196) in the chimpanzee. *J. Pharmacol. Exp. Ther.* **200**:402–412

Fisher, R.S., Fanelli, G.M., Jr., Helman, S.I. 1978. Effect of (6,7-dichloro-2-methyl-1-oxo-2-phenyl-5-indanyloxy) acetic acid (MK-196) on Na transport of isolated frog skin. *Kidney Int.* **14**:758(*A*)

Fuhrman, F.A., Ussing, H.H. 1951. A characteristic response of the isolated frog skin potential to neurohypophysial principles and its relation to the transport of sodium and water. *J. Cell. Comp. Physiol.* **38**:109–130

Handler, J.S., Preston, A.S., Orloff, J. 1972. Effects of ADH, aldosterone, ouabain and amiloride on toad bladder epithelial cells. *Am. J. Physiol.* **222**:1071–1074

Helman, S.I., Fisher, R.S. 1977. Microelectrode studies of the active Na transport pathway of frog skin. *J. Gen. Physiol.* **69**:571–604

Lau, Y.T., Lang, M.A., Essig, A. 1979. Evaluation of the rate of basal oxygen consumption in the isolated frog skin and toad bladder. *Biochim. Biophys. Acta* **545**:215–222

Nagel, W. 1978. Effects of antidiuretic hormone upon electrical potential and resistance of apical and basolateral membranes of frog skin. *J. Membrane Biol.* **42**:99–122

Nagel, W., Crabbé, J. 1980. Mechanism of action of aldosterone on active sodium transport across toad skin. *Pfluegers Arch.* **385**:181–187

Noe, G., Michotte, A., Crabbé, J. 1977. Oxygen consumption by frog skin and its isolated epithelial layers as a function of their sodium-transporting activity. *Biochim. Biophys. Acta* **461**:231–238

Saito, T., Essig, A., Caplan, S.R. 1973. The effect of aldosterone on the energetics of sodium transport in the frog skin. *Biochim. Biophys. Acta* **318**:371–382

Sharp, G.W.G., Leaf, A. 1966. Mechanism of action of aldosterone. *Physiol. Rev.* **46**:593–633

Slater, E.C., Rosing, J., Mol, A. 1973. The phosphorylation potential generated by respiring mitochondria. *Biochim. Biophys. Acta* **292**:334–353

Sudou, K., Hoshi, T. 1977. Mode of action of amiloride in toad urinary bladder. An electrophysiological study of the drug action on sodium permeability of the mucosal border. *J. Membrane Biol.* **32**:115–132

Ussing, H.H., Zerahn, K. 1951. Active transport of sodium as a source of electric current in the short-circuited isolated frog skin. *Acta Physiol. Scand.* **23**:110–127

Vieira, F.L., Caplan, S.R., Essig, A. 1972*a*. Energetics of sodium transport in frog skin. I. Oxygen consumption in the short-circuited state. *J. Gen. Physiol.* **59**:60–76

Vieira, F.L., Caplan, S.R., Essig, A. 1972*b*. Energetics of sodium transport in frog skin. II. The effects of electrical potential on oxygen consumption. *J. Gen. Physiol.* **59**:77–91

Voûte, C.L., Dirix, R., Nielsen, R., Ussing, H.H. 1969. The effect of aldosterone on the isolated frog skin epithelium (*R. temporaria*), a morphological study. *Exptl. Cell. Res.* **57**:448–449

Wilson, D.F., Stubbs, M., Veech, R.L., Erecinska, M., Krebs, H.A. 1974. Equilibrium reactions between the oxidation-reduction reactions and the adenosine triphosphate synthesis in suspensions of isolated liver cells. *Biochem. J.* **140**:57–64

Received 7 July 1981; revised 9 October 1981 and 22 December 1981