Biochimica et Biophysica Acta, 689 (1982) 299–308
Elsevier Biomedical Press

BBA 71218

RESPIRATION AND SODIUM TRANSPORT IN RABBIT URINARY BLADDER

S. UNGLAUB SILVERTHORN and DOUGLAS C. EATON

Department of Physiology and Biophysics, University of Texas Medical Branch, Galveston, TX 77550 (U.S.A.)

(Received February 22nd, 1982)

Key words: Na^+ transport; Respiration; (Rabbit urinary bladder)

Respiration of rabbit urinary bladder was measured in free-floating pieces and in short-circuited pieces mounted in an Ussing chamber. Ouabain, amiloride, and potassium-free saline inhibited respiration approx. 20%; sodium-free saline depressed respiration approx. 40–50%. The coupling ratio between respiration and transport in short-circuited tissues was about two sodium ions per molecule O_2. Chloride-free saline depressed mean oxygen consumption 21% in free-floating tissue pieces; 4-acetamido-4′-isothiocyanostilbene-2,2′-disulfonic acid (SITS) and furosemide had no effect. The effect of chloride-free saline in short-circuited tissues was variable; in tissues with low transport rates, respiration was stimulated about 21% while in tissue with high transport rates respiration was reduced about 24%. Nystatin and monensin, both of which markedly increase the conductance of cell membranes with a concomitant increase in sodium entry, stimulated respiration. These data indicate that 50–60% of the total oxygen consumption is not influenced by sodium, 20–25% is linked to ($Na^+ + K^+$)-ATPase transport, while the remaining 25–30% is sodium-dependent but not ouabain-inhibitable.

Introduction

The relationship between active ion transport and oxidative metabolism has been studied recently using techniques which permit accurate assessment of both parameters simultaneously. In the majority of these experiments, amphibian tissues such as frog skin and toad bladder have been utilized. One disadvantage of studying tissues from poikilothermic animals is the effect of long-term temperature acclimation on both oxygen consumption and active ion transport. Although experimental temperatures are carefully regulated at 24–25°C, the researches often neglect to mention the holding temperatures of the animals and the environmental temperature and/or season of the year when the animals were collected. This information is important since metabolic functions in poikilothermic animals are influenced by both the ambient temperature and the past thermal history of the animal [1,2]. Animals collected from cold environments will have higher respiration rates than animals from warm habitats, even when both have been acclimated to the same temperature for a period of time [3,4]. One reason for such respiration differences may be the degree of coupling of the mitochondria. Thyroxine in vertebrates and hormonal 'cold-acclimation factors' in invertebrates uncouple mitochondrial respiration, presumably to increase cellular heat production (Ref. 5 and Carroll, A.J., personal communication). Thus differences in mitochondrial function will affect the estimation of coupling between oxidative ATP production and ion transport.

A second potential problem with the use of polikilothermic tissues is the effect of temperature on ion transport. ATPase activities are affected by

Abbreviation: SITS, 4-acetamido-4′-isothiocyanostilbene-2,2′-disulfonic acid.

temperature acclimation, presumably through changes in the saturation of the lipid component (for review, see Ref. 6). Thus, animals collected at temperatures significantly different from 25°C and not allowed adequate acclimation time in the laboratory would show different transport characteristics than animals well acclimated to 35°C. This has recently been demonstrated by Lagerspetz and Skytta [7]. The short-circuit current (I_{sc}) of frog skin from animals acclimated to lower temperatures is higher than the I_{sc} from warmer animals when both are measured at the same temperature. The sodium dependence of the I_{sc} is also affected by the previous thermal history of the animal, with a higher I_{sc} for tissues acclimated at lower temperatures at any given sodium concentration.

The use of tissue from homeothermic animals obviates the potential thermal influences associated with the use of poikilothermic tissues and facilitates replication of results by different laboratories. In the studies described below, we measured transport and respiration in rabbit urinary bladder. A preliminary report of this work has been presented [4].

Methods

Urinary bladders from male New Zealand rabbits, 11–15 kg, were dissected and stripped of the muscle layer as described by Lewis and Diamond [8]. Once the tissue was stripped of muscle, it could be used either for measurement of respiration in a Ussing chamber or, cut up, as freely floating pieces and tissue. Animals were maintained on Purina rabbit chow and tap water ('normal diet' animals), or on Purina low-sodium diet and distilled water ('low Na^+ diet' animals).

In the first series of experiments respiration of unstretched bladder pieces weighing 20–40 mg was measured using a Yellow Springs Instrument (YSI) polarigraphic oxygen probe and the standard YSI bath assembly which consists of sealed, temperature-controlled chambers with 3 ml of rabbit saline and a spin bar. The bladder pieces floated free within the chamber. Changes in oxygen content were monitered continuously on a chart recorder. Respiration is expressed as nanoliters of molecular oxygen per milligram of blotted wet tissue weight per minute. Short-circuit current measurements were made on another piece of tissue from the same bladder which had been mounted in a standard Ussing chamber. As a control, respiration measurements were also made on pieces of the muscle layer which had been stripped off the epithelium.

In the second series of experiments, rabbit bladders were mounted in a modified Ussing chamber (Fig. 1). YSI oxygen electrodes were inserted flush with the rear wall of each chamber. Bladder tissue was mounted on a pair of rings with nylon mesh supports, exposing 1 cm^2 of tissue to the bathing solutions on each side. When the rings were sealed into the chambers, saline was pumped in by a peristaltic pump. Air was eliminated through a tube in the top of the chamber. Each 6.5 ml chamber thus became a sealed system facing one surface of the bladder. Small magnetic spin bars in the bottom provided continuous stirring. Temperature of the chambers was regulated at 37 ± 0.01°C by means of water jackets around the chambers. Changes in the oxygen content of each of the chambers were monitored individually on a chart recorder. Respiration measurements were taken 30 min after the start of the experiments or 30 min after initiation of an experimental treatment to allow for stabilization of the system. Ag-AgCl electrodes on either side of the tissue were used to measure transepithelial voltage (V_t), short-circuit current (I_{sc}), and transepithelial conductance (G). In chloride-free (Cl-free) solutions Pt-Pt black electrodes were used for passing current and agar bridges used to measure membrane potential. In all cases the tissues were kept short-circuited at a potential difference of zero, using a voltage clamp similar to that described by Lewis et al. [9]. The YSI oxygen probe was modified to allow the use of the virtual ground current-recording amplifler as the probe reference potential.

All of the rabbit saline contained 1.2 mM $MgSO_4$ and 11 mM glucose. The standard saline also contained 112.2 mM NaCl, 5.8 mM KCl, 25 mM $NaHCO_3$, 1.2 mM KH_2PO_4, and 2 mM $CaCl_2$. The sodium-free (Na-free) saline substituted 25 mM choline bicarbonate for $NaHCO_3$ and either choline chloride or Tris-HCl, 112.2 mM, for NaCl. No difference in results was noted between the Tris-choline and choline solutions.

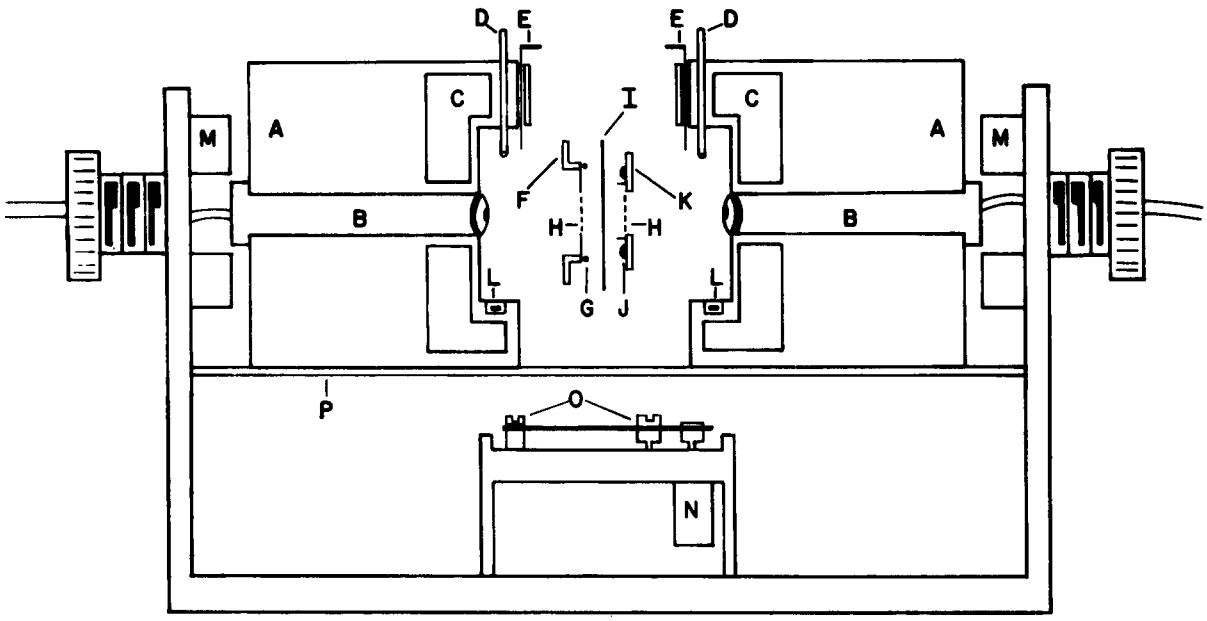

Fig. 1. Schematic cross-section of modified Ussing chamber. A = lucite chamber; B = YSI polarographic oxygen probe; C = temperature-controlled water jacket; D = tubing for filling and draining chamber, 2 per chamber; E = Ag-AgCl electrodes for membrane potential and current passing; F = plastic ring with nylon mesh (h) for supporting tissue; G-silicon sealant layer; I = piece of bladder; K = plastic ring with pins (J) around circumference and nylon mesh support (H); L = internal magnet for stirring; M = vise for holding chambers together; N = motor to drive external magnets (O); P = grooved platform into which the ridge on bottom of chambers fits to prevent lateral movement of the chambers.

The potassium-free (K-free) saline contained 118.0 mM NaCl, and 1.2 mM NaH_2PO_4 in place of KH_2PO_4. The Cl-free saline contained 56.1 mM Na_2SO_4, 25 mM $NaHCO_3$, 1.2 mM KH_2PO_4, and 2 mM calcium methanesulfonate.

Ouabain and nystatin were obtained from Sigma; amiloride was a gift from Merck, Sharpe, and Dohme. Monensin was a gift from Dr. Hammil of Eli Lilly Laboratories; SITS (4-acetamido-4′-isothiocyanostilbene-2,2′-disulfonic acid) came from ICN Pharmaceuticals, and furosemide (Lasix) came from Hoechst-Roussel. Nystatin and monensin stock solutions were prepared by dissolving the ionophores in 95% ethanol at concentrations of 5 mg/ml and 1 mg/ml. These stocks when used to produce the final concentrations of ionophores resulted in salines containing less than 0.6% ethanol which was shown to produce no effect on respiration or short-circuit current when applied alone.

Respiration rates are expressed as mean values ± standard error. The significance of the difference between means were calculated by Student's *t*-test or a paired *t*-test if appropriate. Lines were estimated by linear regression analysis; values for intercepts are ± the standard error of the regression line.

Results

Free-floating tissue pieces

The results of respiration studies on unstretched bladder pieces freely floating in saline are summarized in Table I. There was no significant difference in oxygen consumption of tissue from normal and low-sodium diet animals, so those data have been pooled. Low Na^+ diet animals usually have a higher short-circuit current than normal diet animals and consequently might be expected to have higher respiration rates. However, there is a large variability in the transport rate of normal diet animals probably due to varying levels of endogenous aldosterone production. This variability was large enough to preclude our demonstrat-

TABLE I

RESPIRATION OF TISSUE PIECES IN YSI BATH

Values are presented as mean ± S.E. nl $O_2 \cdot mg^{-1} \cdot min^{-1}$. n.s., not significant.

Treatment	Control	Post-treatment	% Change	*P*	*n*
Ouabain	22.1 ± 1.3	17.6 ± 1.4 [a]	−20	<0.001	18
Amiloride	20.1 ± 1.4	16.5 ± 1.2 [a]	−18	<0.001	13
0-K	25.8 ± 1.7	19.2 ± 1.0 [a]	−26	<0.01	7
Ouabain	22.1 ± 1.3 [b]	17.6 ± 1.4 [d]	−20	<0.001	18
0-Na	27.7 ± 1.7 [c]	14.3 ± 0.9 [e]	−48	<0.001	26
0-Na		16.0 ± 1.8		n.s.	5
0-Na + ouabain		14.4 ± 1.4			
0-Cl	22.0 ± 2.2	17.4 ± 1.7 [f]	−21	<0.001	8
0-Cl + ouabain		13.8 ± 1.3 [g]	−37	<0.001	3
0-Na	27.7 ± 1.7	14.3 ± 0.9 [h]	−48	<0.001	26
Nystatin	19.7 ± 1.6	23.4 ± 1.9	+19	<0.001	13
SITS	23.6 ± 0.3	23.6 ± 0.3	0	n.s.	3
Furosemide	17.7 ± 1.3	16.8 ± 1.0	−5	n.s.	4

[a] not significantly different from other values in the same column.
[b] and [c] significantly different, $p = 0.01$.
[d] and [e] significantly different, $p = 0.05$.
[f] and [g], [f] and [h] significantly different, $P = 0.05$
[g] and [h] not significantly different.

ing a statistically significant difference between the two populations.

Three blockers of active sodium transport (ouabain, amiloride, and K-free saline) all decreased respiration approx. 20%. A fourth treatment which blocks transport, Na-free saline, reduced respiration about 48%, with a mean post-treatment respiration value significantly lower than that for ouabain-treated tissues. This suggested a component of respiration which is sodium-dependent but not ouabain-inhibitable.

We thought that this additional component of Na^+-dependent respiration might be associated with an Na-Cl coupled system. To test this possibility, we applied a Cl-free saline. This saline produced a significant decrease in respiration of approx. 21%. When ouabain was subsequently added to tissue in Cl-free medium, respiration dropped further to a value close to that seen in tissues exposed to Na-free medium.

Although these results seem to support the possibility of an Na-Cl coupled system associated with respiration, the mechanism must be similar to the Na-Cl co-transport system of muscle [10] rather than that found in red blood cells [11] and various invertebrate preparations [12] since neither SITS or furosemide which are known blockers of Cl^- transport in the latter cases have any effect on respiration. The lack of effect of the blocking agents and the results from bladders mounted in Ussing chambers suggests some alternative mode of action for Cl-free Ringer (see Discussion).

We also examined the effect of nystatin which in previous work was found to increase short-circuit current [8]. Nystatin (30 mg/l), which appears to form pores in the cell membranes [8,9,13–16], increased respiration 19%, presumably corresponding to increased sodium entry into the cell followed by increased transport across the basolateral membrane into the serosal solution [8,9]. In several cases the initial increase in respiration was followed by a rapid decline. We speculate that in these tissues the sodium pump was unable to handle the increased intracellular sodium load leading to cell swelling and lysis. The effect of nystatin on the respiration compares closely with

its effect on short-circuit current. That is, there is a rapid increase in short-circuit current shortly after application of nystatin and then, particularly when the nystatin concentration is high, a gradual decrease of tissue resistance and short-circuit current [8].

Respiration of serosal smooth muscle

Although we attempted to remove the vast majority of the adherent muscle layer from our bladder preparations, we were concerned that there might still be some contribution of any remaining muscle. To investigate this possibility we tested the effect of the experimental treatments on samples of stripped muscle. Table II shows that Na-free saline, ouabain, and amiloride had very little, it any, effect on muscle respiration, while Cl-free saline decreased respiration by about 44%. The weight-adjusted respiration of muscle tissue is considerably lower than that of the epithelial preparation (3.4–6.4 vs. 17.7–27.7 nl $O_2 \cdot mg^{-1} \cdot min^{-1}$). Considering the small amount of muscle left on the epithelial preparation and the lower respiration rate, we have assumed that the contribution of muscle to our data is negligible.

Ussing chamber experiments

To identify the relative importance of the apical and basolateral membrane in regulating respiration, we mounted bladder pieces in a special Ussing chamber in which we could measure oxygen consumption from both the serosal and mucosal solutions. In addition to allowing manipulations of only one side of the tissue, the Ussing chambers allowed simultaneous determination of short-circuit current and oxygen consumption. To avoid the difficulty in distinguishing the transport rate of normal diet animals from those with higher transport rates on 'low Na^+ diet', all animals were maintained on their respective diets for no less than 10 days. This procedure substantially, reduced the variability in transport of the normal diet animals observed in the preceding experiments with tissue pieces, and allowed us to distinguish between the two populations.

We chose to examine the tissues under short-circuit conditions since the results would be comparable with the results from the floating tissue pieces. In both cases, there is no potential difference across the tissue: on the one hand because of the shunting properties of the electrolyte bathing media and on the other because of the voltage clamp. Thus in both cases all ion movement must be due to active transport.

In Fig. 2, we have plotted the initial short-circuit current versus oxygen consumption from all of the bladders examined in this study. If the

TABLE II

RESPIRATION OF BLADDER MUSCLE PIECES

Values are presented as mean ± S.E. ($n = 4$) nl $O_2 \cdot mg^{-1} \cdot min^{-1}$. n.s., not significant.

Treatment	Control	Experimental	% Change	P
0-Na	6.4±1.0	5.4±0.4	−16	n.s.
Ouabain	4.4±0.9	3.9±0.8	−11	n.s.
Amiloride	3.4±0.2	3.4±0.2	0	n.s.
0-Cl	4.3±1.0	2.4±0.4	−44	<0.10

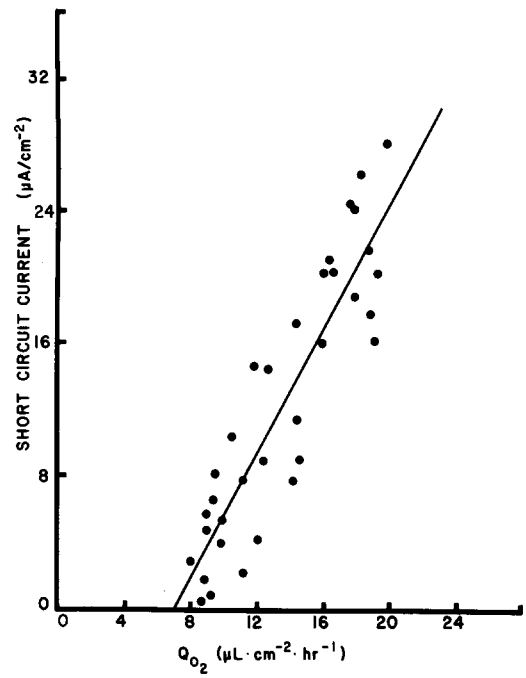

Fig. 2. The relationship between control respiration rate and short-circuit current. The dotted line is the best least-squares linear fit to the data. The intercept of the line on the horizontal axis is 7.16 $\mu l \cdot cm^{-2} \cdot h^{-1}$. The slope of the line is $6.811 \cdot 10^{-3}$ $C \cdot \mu l^{-1}$ (regression coefficient-0.898).

relationship between short-circuit current and O_2 consumption is a linear one, then the dotted line in Fig. 2 is the best least-squares fit to the data. The regression line has a slope of $6.811 \cdot 10^{-3}$ C $\cdot \mu l^{-1}$ with an intercept on the horizontal axis at 7.16 $\mu l \cdot cm^{-2} \cdot h^{-1}$ (regression coefficient = 0.898). Since the mean weight of the 3.8 cm^2 of tissue in the Ussing chamber was 55 ± 4 mg ($n = 8$), the respiration per unit area is equivalent to a respiration rate of about 8.3 $nl \cdot mg^{-1} \cdot min^{-1}$. Thus the basal rate of O_2 consumption is similar to the basal rate obtained in experiments when the tissue in the Ussing chamber was bathed on both sides in Na-free solutions and to the respiration of tissue pieces in Na-free saline.

The response to ouabain and Na-free saline of bladder pieces stretched in the Ussing chamber was similar to that which occurred in the free-floating pieces (Table III). Serosally applied ouabain decreased oxygen consumption about 25%, and 21% in normal diet and low Na^+ diet animals, respectively. Na-free saline applied to the mucosal side of the tissue decreased respiration by about 31% in normal diet animals, while application of Na-free saline in low Na^+ diet animals reduced respiration 40%. The change in Na-free saline was not as great as seen in the free-floating pieces (−48%), but this may be due to a lower initial respiration rate in the normal diet animals. The important aspect of the measurement of respiration and short-circuit current is the observation that the decrease in respiration associated with serosal application of ouabain and mucosal removal of Na^+ is correlated with short-circuit current. In Fig. 3 the change in the short-circuit current after application of ouabain to the serosa or Na-free solution to the mucosa is plotted versus the change in the respiration. The relationship appears linear with a slope of $5.638 \cdot 10^{-3}$ C $\cdot \mu l^{-1}$ if the intercept is assumed to be zero (regression coefficient = 0.813).

Na-free saline placed on the serosal surface of the bladder had a small variable inhibitory effect on respiration (Table III). The inhibitory effect was not correlated with any alteration in short-circuit current (Fig. 4).

The small response associated with the application of Na-free saline to the serosal surface of the tissue does not imply that the serosal membrane is

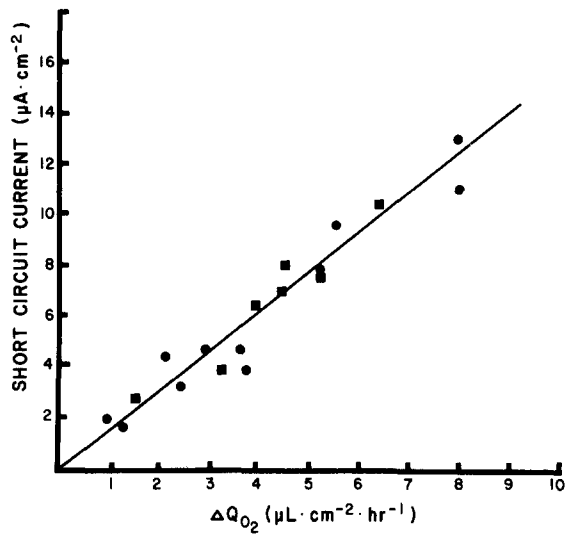

Fig. 3. The decrease in oxygen consumption caused by application of ouabain in the serosal solution (●) or by removal of Na^+ from the mucosal solution (■). For a line whose intercept is the origin, the slope is $5.638 \cdot 10^{-3}$ C $\cdot \mu l^{-1}$ (regression coefficient = 0.813).

completely impermeable to Na^+. It only suggests that under normal conditions the entry of Na^+ from the mucosal solution is much more important in generating respiratory activity. To test the possibility that there was a small component of basolateral Na^+ entry, we initially exposed several bladder pieces to Na-free solutions on both

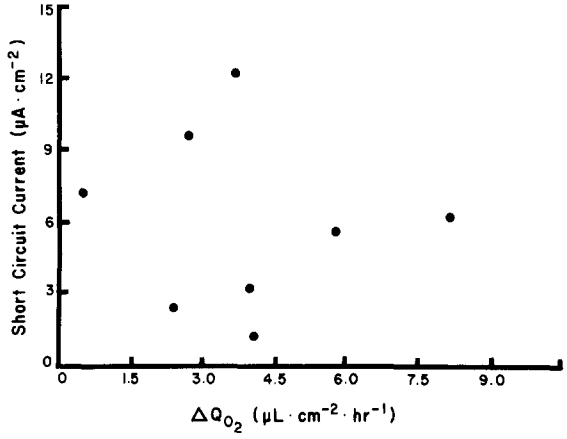

Fig. 4. The decrease in oxygen consumption associated with removal of Na^+ from the serosal solution. There is no obvious correlation between the O_2 consumption and short-circuit current.

TABLE III

TISSUE RESPIRATION IN USSING CHAMBER

Values are presented as mean ± S.E. $\mu l\ O_2 \cdot cm^{-2} \cdot h^{-1}$. n.s., not significant. Values in parentheses are calculated values for respiration based on wet tissue weight. The calculations are based on the observation that the mean weight of the 3.8 cm^2 piece of tissue in the Ussing chamber was 55 ± 4 mg (n = 8). The units are the same as those in Table I (nl $O_2 \cdot mg^{-1} \cdot min^{-1}$).

Treatment	Control	Post-treatment	% Change	*P*	*n*
Normal diet animals					
Ouabain	15.4 ± 0.8 (17.7)	11.6 ± 1.1 (13.4)	−25	<0.001	6
0-Na to both side	11.3 ± 0.9 (13.0)	8.2 ± 0.7 (9.4)	−27	<0.02	5
0-Na to serosa	14.7 ± 1.0 (16.9)	12.2 ± 1.2 (14.0)	−17	n.s.	7
0-Na to mucosa	13.2 ± 1.0 (15.2)	9.1 ± 1.1 (10.5)	−31	<0.01	7
Na to serosa after					
0-Na to both sides	8.8 ± 0.9 (10.1)	15.2 ± 2.2 (17.5)	+73	<0.01	6
0-Cl to both sides	13.9 ± 1.6 (16.0)	16.8 ± 1.9 (19.3)	+21	<0.02	6
0-Cl to serosa	11.2 ± 0.8 (12.9)	15.3 ± 0.7 (17.6)	+37	<0.02	5
0-Cl to mucosa	12.7 ± 1.3 (14.6)	15.4 ± 1.6 (17.7)	+21	<0.01	5
Monensin	13.4 ± 1.8 (15.4)	29.4 ± 3.9 (33.8)	+119	<0.05	3
Low Na^+ diet animals					
0-Na to both sides	27.3 ± 2.0 (31.4)	15.3 ± 1.2 (17.6)	−44		
0-Cl to both sides	24.2 ± 1.4 (27.9)	18.4 ± 1.7 (21.2)	−24		
Ouabain	25.6 ± 1.1 (29.5)	20.2 ± 1.4 (23.3)	−21		
0-Cl after ouabain	20.2 ± 1.4 (23.3)	15.7 ± 0.8 (18.1)	−14		

mucosal and serosal surfaces. Respiration under these conditions approximated the values seen when sodium was removed from the mucosa or from both sides of the tissue. Addition of sodium to the serosal side of the tissue after one hour in Na-free saline caused a dramatic increase in respiration (+73%) to a level equalling the highest rate observed in tissues exposed to normal sodium containing salines. The large apparent permeability of the basolateral membrane to sodium in this case is probably the result of the prolonged depletion.

The effect of Cl-free saline on stretched bladder pieces is different from the data obtained with free-floating tissue. In all three types of experiments with normal diet animals (Cl-free on mucosa only, serosa only, or on both sides), the removal of chloride increased respiration significantly. On the other hand, Cl-free saline applied to low Na^+ diet preparations caused a decrease in respiration.

Finally, monensin ($2 \cdot 10^{-5}$ g · l^{-1}) was placed on the mucosal side of several tissues. Monensin produces a large sodium-specific conductance in cell membranes [17] and to the extent that it allows Na^+ entry is similar to nystatin. Respiration in the presence of the drug increased 119%, suggesting that the bladder pieces were originally transporting sodium at less than half their maximal rate.

Discussion

State of the tissues

We initially measured the respiration of free-floating tissue pieces because it was much more difficult to measure respiration with the tissue mounted in Ussing chambers. Through these experiments we were able to relatively quickly determine many of the properties of O_2 consumption in this tissue before attempting to measure the respiration together with short-circuit current. However, using the tissue pieces left us in some doubt about the condition of the tissue. Only in the Ussing chamber experiments can we make inferences about the state of the tissue. In the Ussing chamber the tissue is in a state identical to that which has been extensively investigated previously [18]. When a tissue is transporting normally

within the range of 2–20 $\mu A \cdot cm^{-2}$, the intracellular Na^+ activity varies between 7 and 14 mM while the K^+ activity is very close to 70 mM [9,19]. Under these conditions the coupling ratio of the $(Na^+ + K^+)$-ATPase appears to be 3 Na:2 K. Unfortunately no information is available on the intracellular concentrations of ionorganic phosphate or the various adenosine phosphates. There is also no direct evidence on the degree of coupling of mitochondrial respiration to ATP production. But if basal metabolic rate were changing along with respiratory rate due to ion transport, then it seems unlikely that the relationship between oxygen consumption and short-circuit current would be linear as we have found (Fig. 2) [20].

Components of respiration

The data presented here show that in the rabbit urinary bladder approx. 30–50% of the total tissue respiration is sodium-dependent. These results are similar to data published for other epithelia. Sodium-dependent respiration in frog skin and toad bladder has been estimated at between 30 and 50% of the total oxygen consumption [21,22]. In guinea pig kidney slices, sodium-free saline decreased respiration 50% [23], while in rabbit gall bladder, a 'leaky' epithelium with coupled sodium-chloride transport, the total sodium-dependent component was 43% [24].

A portion of Na^+-dependent respiration is inhibited by ouabain and therefore linked to active sodium transport via the $(Na^+ + K^+)$-ATPase. The ouabain-inhibitable portion of the respiration is strongly stimulated by the antibiotics, nystatin and monensin. This suggests that the $(Na^+ + K^+)$-ATPase was not operating at its maximum capacity prior to the addition of the drugs. This idea is consistent with the characteristics of the ATPase described in previous work [9,19].

Besides the ouabain-inhibitable respiration, there is also a substantial sodium-dependent component which is insensitive to ouabain blockage. This finding is not new. Similar results have previously been described in tissues ranging from toad bladder [25] to guinea pig kidney [23] and rabbit gall bladder [24]. However, no evidence has been presented which describes the nature of the non-ouabain-inhibitable, sodium-dependent respiration.

It appeared initially from our studies on free-floating bladder pieces that the non-ouabain-inhibitable component might be a Na-Cl-linked system. Cl-free saline depressed respiration to an average value of 17.4 ± 1.7 nl $O_2 \cdot mg^{-1} \cdot min^{-1}$. Addition of ouabain inhibited oxygen consumption further to 13.8 ± 1.3 nl $O_2 \cdot mg^{-1} \cdot min^{-1}$, a value not significantly different from the average of 14.3 ± 0.9 nl $O_2 \cdot mg^{-1} \cdot min^{-1}$ obtained with Na-free saline. However, furosemide and SITS, two inhibitors of some forms of chloride transport, had no significant effect on respiration. These results forced us to exclude various Cl transport systems previously described in invertebrates [12]. To complicate matters, Cl-free saline applied to stretched short-circuit tissues from normal diet animals actually stimulated repiration while the same saline applied to tissues from low Na^+ diet animals decreased respiration.

The difference in the effect of Cl-free salines between tissues with high and low O_2, consumption suggests more than one mechanism for the action of Cl-free saline. At high oxygen consumption rates, the reduction of O_2 consumption associated with the application of Cl-free saline implies a Cl^- dependent utilization of ATP. Several ATP-dependent Cl^- transport processes have been described in other tissues [26,27]. In particular, there appears to be an ATP-dependent $Cl^- - HCO_3^-$ exchange system which is activated by acidification of the intracellular compartment [5,28]. At least in muscle, this system is not blocked by SITS or furosemide [10]. Such a chloride-dependent system might be expected to be active at high metabolic rates with concomitant intracellular acidification.

On the other hand, at low O_2 consumption rates, when acid extrusion might not be as important, application of Cl-free saline causes an increase in O_2 consumption. Removal of Cl^- from either serosal or mucosal solutions or both causes a marked increase in the resistance of the cellular membranes [9]. Since at least the $(Na^+ + K^+)$-ATPase transport system is electrogenic [9,29] such an increase in resistance should cause an increase in the work necessary to translocate ions. Thus, we might expect to see an increase in O_2 consumption. If this suggestion is correct, other agents which increase resistance such as Ba^{2+} [30] should also

increase O_2 consumption.

The emphasis of much of the research on respiration and epithelial transport has been toward determining the coupling ratio between ions transported and oxygen molecules consumed or CO_2 molecules produced. To calculate the coupling ratio a number of assumptions must be made which may not be valid. First, the 'basal' metabolic rate, that portion not involved in transport, must remain constant. It is known that the ATP/O_2 ratio will be affected by uncoupling agents such as thyroxine, by mitochondrial calcium uptake which channels energy into ion transport instead of ATP, and by which substrate enters the electron transport system. Succinate and substrates entering at co-enzyme Q will produce 2 ATP/O, while malate and other NAD-linked substrates will yield 3 ATP/O [31]. Because of these variables, the constancy of the basal metabolic rate may by questionable.

Additionally, it must be assumed that the various transport blockers have no secondary effects on respiration. Both Na-free saline and ouabain inhibit sodium transport as evidenced by abolition of the I_{sc} [8,32]. However, both treatments also affect the intracellular sodium pool: Na-free saline depleting it [19,33] and ouabain increasing it [19,34]. The activity of several key metabolic enzymes such as lactate dehydrogenase (EC 1.1.1.27), glutamate dehydrogenase (EC 1.4.1.2), and 3-glycerophosphate dehydrogenase (EC 1.1.1.8), is affected by changes in sodium concentration [35], so alterations in intracellular sodium levels may directly affect respiratory metabolism.

The possibility has also been raised that ouabain and amiloride have direct effects on metabolism, aside from transport and changes in the cellular sodium pool. This was suggested in light of experiments in which the drugs added to tissues in Na-free media depressed respiration even further [22,24,36,37]. There was a slight inhibition of respiration by ouabain in Na-free saline in our experiments, but the effect was not significant.

In view of the potential variables mentioned above, it is not surprising that the coupling ratios determined for various amphibian epithelia have differed between laboratories as well as between animals within a single laboratory. The values have ranged from 4 to 31 Na^+ transported per O_2 molecule consumed [21,37,38]. This is higher than the ratio of 1.9 Na^+/O_2 which we obtained for rabbit urinary bladder.

It is possible that our estimates are low due to the fact that all our experiments were run on short-circuited tissues. Lang et al. [39] have shown that at higher flux rates the Na^+/O_2 ratio will be greater for tissues where the transepithelial potential difference is not zero. An alternative possibility is that varying ratios of O_2 to sodium ion transported reflect a control mechanism for transport. There are at least two ways in which the control come about. Mitochondrial respiration could be uncoupled by altering intracellular ion gradients. Also the Na^+ to K^+ coupling ratio of the $(Na^+ + K^+)$-ATPase could be altered. As the transport system shifted from Na^+-K^+ exchange to Na^+-Na^+ exchange, net transport would decrease while ATP utilization remained nearly the same. DeWeer [40] has previously suggested this idea as a control mechanism. It may be time to examine the hypothesis more carefully.

References

1 Prosser, C.L. (1973) Comparative Animal Physiology, pp. 860–862, W.B. Saunders, Philadelphia, PA
2 Hoar, W.S. (1972) General and Comparative Physiology, pp. 335–343. Prentice-Hall, Englewood Cliffs, NJ
3 Vernberg, F.J. and Vernberg, W.B. (1970) The Animal and the Environment, pp. 103–154, Holt, Rinehart and Winston, Inc., New York, NY
4 Silverthorn, S.U. and Eaton, D.D. (1979) Physiologist 22, 115
5 Russell, J.M. and Boron, W.F. (1976) Nature 264, 73–74
6 Vernberg, F.J. and Silverthorn, S.U. (1976) in Mechanisms of Osmoregulation in Animals (Gilles, R., ed), pp. 537–562, John Wiley and Sons, New York, NY
7 Lagerspetz, K.Y.H. and Skytta, M. (1979) Acta Physiol. Scand 106, 151–158
8 Lewis, S.A., Eaton, D.C., Clausen, C. and Diamond, J.M. (1977) J. Gen. Physiol. 70, 427–440
9 Lewis, S.A., Wills, N.K. and Eaton, D.C. (1978) J. Membrane Biol. 41, 117–148
10 Aickin, C.C. and Thomas, R.C. (1977) J. Physiol. 273, 295–316
11 Cala, P.M. (1980) J. Gen. Physiol. 76, 683–708
12 Frizzell, R.A., Field, M. and Schultz, S.G. (1979) Am. J. Physiol. 236, F1–F8
13 Marty, A. and Finkelstein, A. (1975) J. Gen. Physiol. 65, 515
14 Cass, A., Finkelstein, A. and Krespi, V. (1970) J. Gen. Physiol. 56, 100–124

15 Holz, R. and Finkelstein, A. (1970) J. Gen. Physiol. 56, 125
16 Russell, J.M., Eaton, D.C. and Brodwick, M.S. (1977) J. Membrane Biol. 37, 137–156
17 Pressman, B.C. and DeGuzman, N.T. (1975) Ann. N.Y. Acad. Sci. 264, 373–386
18 Lewis, S.A. and Diamond, J.M. (1976) J. Membrane Biol. 28, 1–40
19 Eaton, D.C. (1981) J. Physiol. 316, 527–544
20 Mandel, L.J. and Balaban, R.S. (1981) Am. J. Physiol. 240, F357–F371
21 Coplon, N.S., Steele, R.E. and Maffly, R.H. (1977) J. Membrane Biol. 34, 289–312
22 Lau, Y.-T., Lang, M.A. and Essig, A. (1979) Biochim. Biophys. Acta 545, 215–222
23 Noe, G. and Crabbe, J. (1975) Arch. Int. Physiol. Biochim. 83, 343–345
24 Martin, D.W. and Diamond, J.M. (1966) J. Gen. Physiol. 50, 295–315
25 Weiner, M.W. (1979) Science 204, 187–188
26 Russell, J.M. (1976) J. Membrane Biol. 28, 335–349
27 Russell, J.M. (1979) J. Gen. Physiol. 73, 801–818
28 Schoffeniels, E. (1968) Arch. Int. Physiol. Biochim. 76, 319–343
29 Wills, N.K. and Lewis, S.A. (1980) Biophys. J. 30, 181–186
30 Kirk, K.C., Halm, D.R. and Dawson, D.C. (1980) Nature 287, 237–239
31 Lehninger, A.L. (1973) Bioenergetics, p. 75ff, W.A. Benjamin, Menlo Park, CA
32 Helman, S.I., Nagel, W. and Fisher, R.S. (1979) J. Gen. Physiol. 74, 105–127
33 Macknight, A.D.C. and Leaf, A. (1978) J. Membrane Biol. 40, 247–260
34 Macknight, A.D.C., Civan, M.M. and Leaf, A. (1975) J. Membrane Biol. 20, 387–401
35 Silverthorn, S.U. (1973) Comp. Biochem. Physiol. 45A, 417–420
36 Prosser, C.L. (1967) Molecular Mechanisms of Temperature Adaptation, p. 390, Publ. 84, Am. Assoc. Advancement of Science, Washington, DC
37 Nellans, H.N. and Finn, A.L. (1974) Am. J. Physiol. 227, 670–675
38 Vieira, F.L., Caplan, S.R. and Essig, A. (1972) J. Gen. Physiol. 59, 60–76
39 Lang, M.A., Caplan, S.R. and Essig, A. (1977) Biochim. Biophys. Acta 464, 571–582
40 DeWeer, P. (1972) J. Steroid Biochem. 3, 178–179