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Rapid Report

Osmotic swelling and membrane conductances in A6 cells

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Hyposmotic basolateral perturbations (−30 mosmol/kg) in cultured renal layers (A6) increased basolateral membrane conductance more than 2-fold within 10 min; the increase was partly due to upregulation of K^+ conductance, but other conductive pathways were also activated. The raise in apical membrane amiloride-sensitive Na^+ conductance was less pronounced; it appears to be due to secondary effects.

Very often epithelial cells are exposed to important changes in medium osmolalities. Transepithelial Na^+ transport is usually increased after incubation with hyposmotic media, whereas opposite effects are exerted by hyperosmotic bathing solutions [1]. Recently, it has been reported that exposure of A6 epithelia to basolateral hyposmotic media quickly (5 min) results in cell swelling [2] and increased transcellular Na^+ reabsorption [3]. Data from noise analysis revealed that the increase in Na^+ transport was associated with activation of apical Na^+ channels [3]. Effects at the basolateral membrane were not measured in these studies. In the present study, we examined the response of basolateral membrane transport properties on hyposmotic swelling using microelectrode impalement of the epithelial cells and equivalent circuit analysis. These techniques can reliably be applied to A6 cell preparations [4].

Conditions for growth and subculturing of A6 cells (American Type Culture Collection, Bethesda, USA), originally derived from the renal distal tubular segment of *Xenopus laevis* were recently described [4]. Electrical analysis of the confluent layers was done 15–20 days after plating on microporous filter membranes (Millicell-HA, Millipore PIHA 030050). The monolayers were transferred with the supporting filter membrane to a modified Ussing-type chamber, short-circuited and impaled with microelectrodes as reported recently [4]. At the beginning of the experiments, both sides were separately and continuously superfused with a solution containing (in mM): NaCl, 70; KCl, 2.5; $CaCl_2$, 1; $MgCl_2$, 1; KH_2PO_4, 1; $NaHCO_3$, 18; Hepes, 5; sucrose, 30; pH was maintained at 7.4 with 5% CO_2. Osmolality of this solution was 200 mosmol/kg. Hyposmotic challenge was achieved by omission of sucrose from the basolateral standard perfusion solution. Short-circuit current and intracellular potential of the short-circuited monolayer are indicated by I_{sc} and V_{sc}. Apical and basolateral membrane conductances, g_a and g_b, were calculated from standard equivalent circuit analysis using the relationships

$$g_a = (G_t - G_t')/(fR_a' \cdot fR_a) \quad (1)$$

$$g_b = (G_t - G_t')/(fR_a' - fR_a) \quad (2)$$

where G_t and fR_a reflect transepithelial conductance and fractional apical resistance, respectively. Values with ′ indicate measurements in the presence of 10^{-5} M amiloride at the apical side. The apparent transference number for K^+, t_K, of the basolateral membrane was obtained after blocking the apical membrane with 10^{-5} M amiloride, comparing the change in intracellular voltage (ΔV_{sc}) to a short-term increase in basolateral K^+ concentration from 3.5 (c_1) to 20 mM (c_2) with the theoretical value predicted by the Nernst equation:

$$t_K = \Delta V_s'c/(RT/ZF \ln c_2/c_1) = \Delta V_s'c/43.9\ mV \quad (3)$$

Basolateral K^+ conductance was derived from t_K and g_b using the relationship

$$g_K = t_K \cdot g_b \quad (4)$$

Basolateral membrane rectification properties were estimated from the relationship between I_{sc} and V_{sc} at

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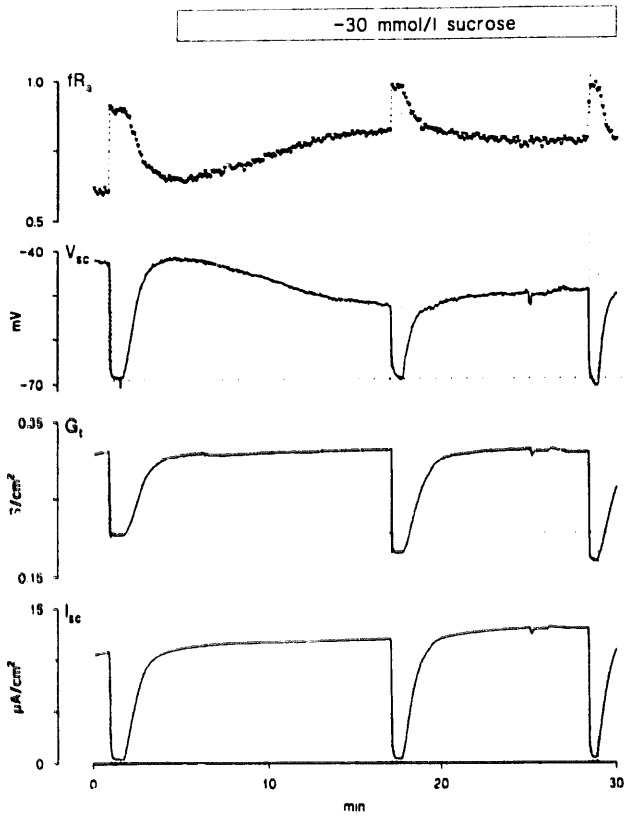


Fig. 1. Transepithelial and cellular parameters of an A6 epithelium during control and hyposmotic media at the basolateral side. I_{sc}, G_t, V_{sc} and fR_a indicate short-circuit current, transepithelial conductance, membrane potential at short-circuit and apical fractional resistance. Vertical bars indicate short-term application of 10^{-5} M amiloride at the apical side.

different concentrations of apical amiloride [5]. Mean values are reported ±S.E. Significance of differences was calculated using paired Student's *t*-test, considering $P < 0.01$ as significant.

Fig. 1 shows a response of an A6 layer on reduction of osmolality in the basolateral perfusion solution. Initially, the preparation was equilibrated with the standard solution (200 mosmol/kg; control) at both sides. Under this condition, the I_{sc} was 10.7 $\mu A/cm^2$; it was near-completely blocked by 10^{-5} M amiloride. Amiloride-inhibitable conductance, $(G_t - G_t')$, was 104 $\mu S/cm^2$. The characteristic fast increase in fR_a to 0.91, associated with hyperpolarization of the cell from −42 to −69 mV, indicates a reliable impalement [4]. From equivalent circuit analysis using the amiloride-sensitive conductance and the change in fR_a we obtain apical and basolateral membrane conductances of 160 $\mu S/cm^2$ and 400 $\mu S/cm^2$, respectively. Change to a lower osmolality (170 mosmol/kg) of the basolateral medium led promptly to notable increase in fR_a and hyperpolarization of V_{sc}. Steady values of V_{sc} and fR_a, approached within 10 min, were considerably different from the control levels. G_t and I_{sc}, on the other hand, were only slightly affected compared with the values before hyposmotic challenge. Application of amiloride after this time shows that V_{sc}' (i.e., the magnitude of V_{sc} during amiloride, which reflects the effective electromotive force at the basolateral membrane) remained unchanged compared with the control value, whereas fR_a' (i.e., fR_a in the presence of amiloride) increased to 0.98. In the depicted experiment, amiloride-insensitive shunt conductance decreased slightly by 12%, but this response was not always observed. Using as above the values of amiloride-sensitive conductance, which increased despite constancy of G_t, and fR_a for equivalent circuit analysis, it can be calculated that g_b doubled to 806 $\mu S/cm^2$, whereas g_a remained essentially unchanged until the test 10 min after hyposmotic perturbation. The gain in g_b is the reason for the much smaller amiloride-induced change in V_{sc} in hyposmotic than in control medium, since the change in V_{sc} reflects the Ohmic $R \cdot I$-drop across the basolateral membrane and I_{sc} remained almost at the control level. During the following 10 min of perfusion with hyposmotic solution, fR_a and V_{sc} decreased slightly from the maximal values. These changes together with increases in I_{sc} and amiloride-sensitive G_t are consistent with activation of apical membrane conductance. Indeed, at the end of this period, g_a had increased to 176 $\mu S/cm^2$, whereas g_b was slightly reduced to 703 $\mu S/cm^2$. The EMF of the basolateral membrane, reflected by V_{sc}', remained constant during the entire experimental period.

Table I summarizes the observations for transepithelial and transmembrane parameters from eight similar experiments. It is evident that the major change on perfusion with the hyposmotic basolateral solution occurred during the first 10 min (early period) and mainly consisted of a remarkable and highly significant increase in g_b. Basolateral membrane conductance was, in relative terms, 2.4-fold higher in the hyposmotic than in the control medium. The gain is reflected by the rise of fR_a. It can be deduced from the temporal

TABLE I

Effect of reduced osmolality on electrical membrane parameters in A6

The periods 'early' and 'late' indicate 10 respectively 20 min after hyposmotic challenge by omission of 30 mmol/l sucrose from the basolateral perfusion solution. Values are means ± S.E. of eight A6 layers. Values with ' indicate measurements in the presence of 10^{-5} M amiloride at the apical side.

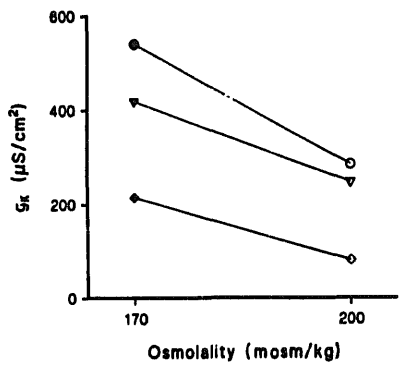
	Control (200 mosmol/kg)	Early (170 mosmol/kg)	Late (170 mosmol/kg)
I_{sc} ($\mu A/cm^2$)	8.7 ± 1.2	10.9 ± 1.1	12.5 ± 1.2
G_t ($\mu S/cm^2$)	329 ± 25	363 ± 24	401 ± 24
fR_a	0.77 ± 0.03	0.87 ± 0.02	0.82 ± 0.02
V_{sc} (mV)	−40 ± 4	−46 ± 4	−42 ± 4
V_{sc}' (mV)	−58 ± 5	−59 ± 5	−59 ± 6
g_a ($\mu S/cm^2$)	127 ± 18	156 ± 16	190 ± 19
g_b ($\mu S/cm^2$)	594 ± 111	1437 ± 259	1253 ± 25

evolution of changes in fR_a and V_{sc} that the major fraction of increase in g_b was complete within 8–10 min after hyposmotic challenge. Similar time course (11 min) has been reported for the gain in cellular volume after reduction of basal osmolality [2]. During the following 10 min (late period), g_b decreased slightly from the maximal level, but remained considerably elevated above the control. Increase in g_b at unchanged EMF of the basolateral membrane (reflected by constancy of V_{sc}') explains the hyperpolarization of V_{sc}, which occurred during the early and late observation period despite increase in I_{sc}. In view of the stable effective emf of the basolateral membrane, a fraction of the gain in g_b must be due to activation of K^+ channels, since the negative value of V_s'c can only be explained by K^+.

Fig. 2 illustrates the influence of a 10 min lasting reduction in osmolality on basolateral K^+ conductance in an additional set of A6. Basolateral K^+ conductance was calculated from g_b and the apparent transference numbers for K^+ (from Eqns. 3 and 4). The three tested A6 layers showed a significant increase in g_K. Mean value of total basolateral conductance, g_b, either during standard (200 mosmol/kg) or lower (170 mosmol/kg) osmolality were 802 ± 261 and 1887 ± 766 $\mu S/cm^2$, respectively. Only part of this gain was due to the increase in g_K. Average values of g_K were 205 ± 64 and 391 ± 96 $\mu S/cm^2$ during standard, respectively hyposmotic perfusion.

The permeability patterns of the basolateral conductive pathway during hyposmotic conditions (170 mosmol/kg) are different from those at higher osmolality. This is illustrated by representative I/V plots for the same A6 layer during control and hyposmotic conditions in Fig. 3. Apical perfusion with stepwise increasing concentrations of amiloride was used to gradually decrease I_{sc}. Under control conditions, the relationship between corresponding values of I_{sc} and V_{sc} displayed the linear slope reported previously, which would be consistent with the presence of inward rectification in K^+-selective channels [6]. After a switch to the hyposmotic medium, the slope changed and the I/V relationship became slightly concave. The present data cannot reveal whether previously existing channels are modified or whether new K^+-channels with different characteristics and/or other ionic pathways were opened. The increase in K^+ conductance together with outward rectification of g_b would agree with previous observations that cell swelling results mainly in the appearance of voltage-insensitive K^+-channels [7,8]. A similar conclusion has been made for A6 preparations, in which cell swelling was presumably induced by permeabilization of the apical membrane [9]. Our data demonstrate that the main part of increase in g_b was due to another ionic leak conductance. Interestingly, in preliminary experiments, this leak conductance disappeared in the presence of the Cl^--channel blocker, NPPB. Accordingly, the increase in g_b could play a role in mediating the loss of KCl during a regulatory volume decrease. Patch clamp analysis indicate that MDCK cells respond to a hyposmotic swelling by an early activation of highly selective potassium conductances and a delayed activation of anionic conductances with outward rectification pattern [10]. Their data agree very well with the changes of membrane potential measured during regulatory volume decrease (RVD) [11]. Since we never observed a quick transient hyperpolarization, we might speculate that both K^+ and other leak conductances arise at the same time. Since increase in cell height was larger than expected for an ideal osmometer [2], RVD appears to be non-existing in the A6 preparation. However as noted by Crowe and Wills [2], the RVD might be masked by the increase in I_{sc} as a result of stimulated Na^+ transport. Swelling was remarkably reduced during inhibition of the Na^+ transport with amiloride [2].

The onset of increase in g_a was not so clear and pronounced as that of g_b and ensued continously



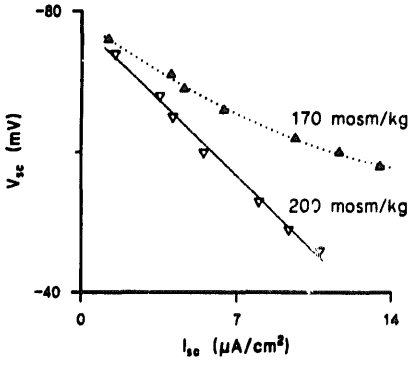


Fig. 2. Effect of reduced basolateral osmolality on basolateral K^+ conductance, g_K, in three A6 layers.

Fig. 3. I/V relationships of the basolateral membrane of an A6 layer induced by stepwise increasing concentrations of amiloride at the apical side. Open triangles and continuous line refer to control (200 mosmol/kg) conditions. Filled triangles and dashed line represent the hyposmotic (170 mosmol/kg) basolateral medium.

during the time (20 min) of observation. The magnitude of increase in g_a after 10 min (1.2-fold higher) was considerably smaller than the change in g_b. The maximal increase in I_{sc} (after 20 min) agrees very well with values reported by Crowe and Wills [2]. In relative terms, g_a increased to the same extent as the I_{sc}, which indicates that the observed stimulation of transport was essentially dependent on the apical membrane response. This is not surprising, since the apical membrane was the main resistive barrier under both conditions. Although increase in g_b should affect the driving force for apical Na^+ entry, the hyperpolarization by 6 mV might be too small for detectable responses. The mechanism underlying the increase in g_a is not clear. As noted previously by Crowe and Wills [2], the difference between time courses of change in cell volume and g_a excludes a direct mechanical influence of cellular volume. Since the permeability change of the basolateral membrane is not limited to K^+, intracellular electrolyte composition could be affected. This might influence actual ion concentrations in the nucleoplasm and lead to activation of genes [12–16] and thus g_a. Up- and downregulation of g_a, as proposed by Wills [3], in response to basolateral osmolality variations might be an excellent mechanism to protect the aquatic toad, *Xenopus* against desiccation without direct interference of hormones.

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