Potassium channels in basolateral membrane vesicles from Necturus enterocytes: stretch and ATP sensitivity

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EXPOSURE OF EPITHELIAL CELLS to hypotonic solutions results in cell swelling and concomitant increases in the K+ and, often, the Cl− conductances of the basolateral membrane (14, 20). These conductance changes permit the efflux of KCl, accompanied by water, which either limits the degree of swelling or, if swelling is marked, actually serves to restore the swollen cell toward normal size; the latter is referred to as “regulatory volume decrease” (14). Cell swelling can also occur isotonically secondary to increased Na+ conductance. The increase in K+ conductance not only prevents excessive accumulation of intracellular K+ and volume secondary to increased Na+–K+ pump activity, but also serves to restore the electrical driving force for the entry of these Na+–coupled solutes across the apical membrane (15, 21). The mechanism(s) responsible for the increases in basolateral membrane K+ channel activity in response to hypotonically or isotonically induced cell swelling are not entirely resolved.

We have previously reported that K+ channels in vesicles derived from the basolateral membranes of Necturus small intestinal epithelial cells that are essentially devoid of soluble intracellular contents, but possess cytoskeletal elements, respond to exposure to anisotonic solutions, as do the intact cells (8). Thus exposure to a solution that is hypotonic to the intravesicular solution (“hypotonic shock”) increases the activity of these channels, whereas exposure to a hypertonic solution decreases, and may abolish, this activity. Further, these “volume regulatory responses” are dependent upon an intact cytoskeleton inasmuch as treatment with cytochalasin D or depolymerization of actin without employing pharmacological agents (8) abolishes these responses.

In the present study, we examine the effects of apparent vesicle swelling on K+ channel activity under osmotically active solutes. We also demonstrate that swelling of these vesicles reduces inhibition of channel activity by ATP. The results suggest that the activity of these basolateral membrane ATP-inhibitable, stretch-activated K+ channels is modulated by the effect of membrane stretch on ATP sensitivity and that these channels may be responsible for the increase in K+ conductance associated with sugar and amino acid absorption by the intact small intestine.

METHODS

The method for isolating a basolateral membrane fraction from Necturus enterocytes has been described in detail (6). Briefly, a membrane fraction enriched in Na+–K+–ATPase activity was isolated from mucosal scrapings of Necturus small intestine by differential centrifugation without the use of cytochalasin D or depolymerization of actin.

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of enzymes. This method results in a >20-fold enrichment of Na$^-$-K$^+$-ATPase activity over that in the crude homogenate with minimal contamination by enzyme markers for membranes other than the basolateral membranes. The membranes were frozen, stored in liquid N$_2$, and thawed immediately before use.

K$^+$ channel activity of the vesicles was assayed using $^{86}$Rb$^+$ as a tracer for K$^+$, according to the method of Garty et al. (10), as described previously (7). This method permits determination of the time course of equilibration of the tracer with its electrochemical potential difference across the vesicle membrane over the course of minutes, thereby obviating the need for rapid-sampling techniques. A decrease in channel activity is reflected by a decrease in the initial rate of uptake as well as in the steady-state level achieved (10). Vesicles were loaded by addition of 200 µl of membranes (1.5–4 mg protein/ml) to 50 µl of 0.5 M K$_2$SO$_4$ and 10 mM K-HEPES, pH 7.0, and other reagents as indicated. The osmolarity of the loading solution was adjusted with sucrose as indicated in the text. The mixture was frozen in liquid N$_2$ and thawed; during the freeze-thaw cycle, the intravesicular compartment equilibrates with the loading solution, and the cytoplasmic contents retained during the isolation procedure were washed out. Columns were prepared from DOWEX 50W-X-8 (Tris form), poured into glass Pasteur pipettes, and pretreated with three drops of 30% bovine serum albumin.

The columns were washed with 4 ml of a solution of sucrose, 10 mM Tris-HEPES, pH 7.6 adjusted to the osmolarity of the loading solution. Two hundred microliters of the vesicle suspension were pipetted onto the DOWEX column to remove extravesicular K$^+$ and is eluted with 2 ml of sucrose, 10 mM Tris-HEPES, pH 7.0 buffer under mild vacuum; the sucrose wash is adjusted to the test osmolarity and contains other reagents as indicated. Thus the vesicles were eluted into a buffer that is isotonic, hypertonic, or hypotonic relative to the intravesicular solution, or isotonic but containing 40 mM glucose or alanine in place of the osmotic equivalent of sucrose as specified in the text and/or legends. After the vesicles were collected, a 10-µl aliquot of $^{86}$Rb$^+$ (1–4 µCi) was added to initiate uptake. At timed intervals, starting immediately (~5 s) after the addition of tracer (nominally “zero time”), 200-µl aliquots were withdrawn and placed on a second DOWEX column to remove all extravesicular tracer. The vesicles were eluted from the column with 2 ml of the sucrose buffer directly into scintillation vials and assayed for $^{86}$Rb$^+$ content. Intravesicular $^{86}$Rb$^+$ was expressed as the percent of total radioactivity in a 200-µl aliquot of reaction mixture normalized to the protein content of the vesicle suspension. As discussed previously (7), because the intravesicular compartment is markedly electrically negative with respect to the external compartment, only channels oriented so that the intravesicular compartment corresponds to the intracellular compartment will be active.

RESULTS

Effects of glucose or alanine. The results of a series of experiments in which K$^+$ channel activity was determined in vesicles after exposure to an isotonic sucrose solution, a hypotonic sucrose solution, and isotonic solutions in which 40 mM D-glucose or L-alanine replaced their osmotic equivalent of sucrose are shown in Fig. 1; the average measured osmolarities of these solutions are given in parentheses. The vesicles that were exposed to isotonic solutions containing D-glucose or L-alanine displayed increases in $^{86}$Rb$^+$ uptake that...
were indistinguishable from those that were exposed to the hypotonic sucrose solution.

**Effect of stretch on ATP inhibition.** Van Wagoner (23) has reported that the $K_{\text{ATP}}$ channels found in rat atrial myocytes are mechanosensitive inasmuch as inhibition by ATP can be overcome by cell swelling or suction applied to an excised membrane patch. As shown in Figs. 2 and 3, the same appears to be true for the $K^+$ channel activity in the basolateral membranes from *Necturus* enterocytes. Figure 2 shows that loading the vesicles with 1 mM ATP-$\gamma$-S abolished $^{86}\text{Rb}^+$ uptake by vesicles exposed to isotonic or hypertonic sucrose solutions but had no significant effect on $^{86}\text{Rb}^+$ uptake by vesicles swollen by exposure to a hypotonic solution. The data shown in Fig. 3 indicate that the same is true for isotonic swelling that resulted from the presence of 40 mM alanine or glucose in the suspension. As shown in Fig. 4, exposure of the vesicles to increasingly hypotonic sucrose solutions resulted in a graded reversal of the inhibitory effect of ATP on channel activity.

To examine the possibility that the reversal of inhibition observed after vesicle swelling is simply due to a dilution of intravesicular ATP, a series of experiments was carried out to examine the effect of varied ATP concentrations on $K^+$ channel activity, and the results of these studies are shown in Fig. 5. Although these data are admittedly qualitative, comparison of the findings reported in Figs. 2–4 with those reported in Fig. 5 leads to the conclusion that a decrease in intravesicular ATP concentration cannot entirely account for the reversal of inhibition that results from vesicle swelling. Thus the inhibition by ATP is completely reversed by exposure to a solution that is only 15–20% hypotonic to the control but is only partially reversed by a 50% decrease in intravesicular ATP concentration.

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**Fig. 3.** Vesicles were preloaded with isotonic sucrose solutions with or without 1 mM ATP-$\gamma$-S. $^{86}\text{Rb}^+$ was determined after suspension in an isotonic sucrose solution or in isotonic solutions containing 40 mM D-glucose or L-alanine. ATP-$\gamma$-S did not inhibit $^{86}\text{Rb}^+$ uptake by vesicles suspended in the latter solutions. *Uptake by isotonic vesicles preloaded with ATP-$\gamma$-S was significantly lower than in all other 3 conditions; $P < 0.01$.

**Fig. 4.** $^{86}\text{Rb}^+$ uptake by vesicles preloaded with an isotonic (426 mosM) sucrose solution containing 1 mM ATP-$\gamma$-S after suspension in increasingly hypotonic solutions as indicated in the figure.

**Fig. 5.** $^{86}\text{Rb}^+$ uptake by vesicles preloaded with an isotonic (426 mosM) sucrose solution containing graded concentrations of ATP-$\gamma$-S as indicated in the figure.
DISCUSSION

The basolateral membranes of enterocytes possess carrier mechanisms that “facilitate” the Na\(^+\)-independent equilibration of sugars and amino acids across that barrier. In the present study, we have demonstrated that the suspension of basolateral membrane vesicles derived from Necturus small intestinal cells, preloaded with a sucrose solution, in isotonic solutions containing 40 mM D-glucose or L-alanine, results in increases in K\(^{+}\) channel activity (measured as \(^{86}\)Rb\(^{+}\) uptake) that closely resemble those observed after exposure to hypotonic solutions of the same magnitude. This is undoubtably due to swelling under isotonic conditions secondary to the equilibration of these solutes within the intravesicular space accompanied by their isotonic equivalent of water. Breton et al. (4) have demonstrated the same phenomenon after exposure of the peritubular (basolateral) surface of collapsed, non-transporting segments of rabbit proximal tubule to glucose and alanine. This qualitatively resembles the events that accompany the absorption of these solutes by intact small intestine and renal proximal tubule when \(-5\) mM glucose or alanine is present in the mucosal solution (4, 15).

Na\(^+\)-coupled absorption of sugars or amino acids by small intestinal and renal proximal tubule epithelial cells is accompanied by: 1) an increase in Na\(^+\)-K\(^{+}\) pump activity at the basolateral membrane and hence a decrease in bulk and/or local ATP activity, and 2) cell swelling. These effects have been best documented for rabbit proximal tubule. Thus Beck et al. (3) have reported a decrease in cell ATP from 4.44 to 2.69 mM after stimulation of Na\(^+\) absorption by the addition of 5.5 mM glucose and 6 mM alanine to the luminal perfusate and a 9% increase in cell volume; a similar increase in cell volume has been reported by Beck et al. (2) in a different study under similar conditions. Both a decrease in cell ATP and an increase in cell volume have been, individually, implicated in the increase in basolateral membrane K\(^{+}\) conductance observed during the absorptive process (20, 21, 25). Thus in epithelia where K\(_{\text{ATP}}\) channels have been identified in the basolateral membranes, such as rabbit (22, 25) and Ambystoma (17) proximal tubule, the local decrease in ATP activity is postulated to be directly responsible for the increase in K\(^{+}\) conductance. In addition, in all epithelia studied to date, cell swelling that results from exposure to hypotonic solutions, and presumably unrelated to increases in transepithelial transport, is followed by increases in basolateral membrane K\(^{+}\) conductance; the mechanism(s) responsible for this phenomenon remain unresolved.

The results of the present study suggest that in Necturus small intestinal cells, the response to ATP and membrane stretch may be closely entwined. Thus an increase in Na\(^-\)coupled solute entry into the cell across the apical membrane with subsequent activation of the Na\(^-\)K\(^{+}\) basolateral membrane pump will result in a decrease in local ATP activity. At the same time, cell swelling, due to an accumulation of osmotically active solutes, would be expected not only to activate stretch-activated K\(^{+}\) (SAK) channels, but also to dilute cell ATP and decrease its inhibitory effect on these channels. All of these mechanisms would act synergistically to increase basolateral membrane K\(^{+}\) conductance. These results are consistent with the findings of Lau et al. (16) that perfusion of Necturus small intestine, with a galactose solution that is 20% hypertonic with respect to control, prevents the increase in basolateral membrane K\(^{+}\) conductance that is seen when the galactose-containing solution is isotonic to the control perfusate.

Van Wagoner (23) has reported that the sensitivity of K\(_{\text{ATP}}\) channels in atrial myocytes to ATP is reduced, and can be abolished, by a stretch of patches of excised membrane resulting from applied pressure or from hypotonic swelling of whole cells. Kim et al. (13) have also presented evidence for mechanosensitivity of atrial K\(_{\text{ATP}}\) channels. The mechanism(s) underlying this interaction is obscure. It should be noted that these K\(_{\text{ATP}}\) channels, which Ashcroft and Ashcroft (1) classify as Type I, differ from those found in Necturus enterocytes and other epithelia.

Finally, it is of interest that SAK channels have now been identified in the basolateral membranes of Necturus proximal renal tubule cells (9, 18, 19), Necturus gallbladder (24), and Necturus small intestine (8). They have also been identified by a number of investigators in the basolateral membranes of frog proximal tubule (5, 11, 12) where evidence has been presented for their involvement in the increase in the K\(^{+}\) conductance of that barrier in response to swelling accompanying Na\(^+\)-coupled solute absorption (5).

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