IK channels are involved in the regulatory volume decrease in human epithelial cells

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Wang, Jun, Shigeru Morishima, and Yasunobu Okada. IK channels are involved in the regulatory volume decrease in human epithelial cells. Am J Physiol Cell Physiol 284: C77-C84, 2003. First published September 11, 2002; 10.1152/ajpcell.00132.2002.—Parallel activation of Ca2+-dependent K+ channels and volume-sensitive Cl- channels is known to be responsible for KCl efflux during regulatory volume decrease (RVD) in human epithelial Intestine 407 cells. The present study was performed to identify the K+ channel type. RT-PCR demonstrated mRNA expression of Ca2+-activated, intermediate conductance K+ (IK), but not small conductance K+ (SK1) or large conductance K+ (BK) channels in this cell line. Whole cell recordings showed that ionomycin or hypotonic stress activated inwardly rectifying K+ currents that were reversibly blocked by IK channel blockers [clotrimazole (CLT) and charybdotoxin] but not by SK and BK channel blockers (apamin and iberiotoxin). Inside-out recordings revealed the existence of CLT-sensitive single K+-channel activity, which exhibited an intermediate unitary conductance (30 pS at −100 mV). The channel was activated by cytosolic Ca2+ in inside-out patches and by a hypotonic challenge in cell-attached patches. The RVD was suppressed by CLT, but not by apamin or iberiotoxin. Thus we conclude that the IK channel is involved in the RVD process in these human epithelial cells.

Ca2+-activated K+ channel; patch clamp; clotrimazole; osmotic swelling

CELL VOLUME REGULATION is an essential function for animal cells because osmotic perturbation is coupled to a variety of physiological and pathological processes, such as cell proliferation, cell differentiation, and apoptosis (23, 37). Under hypoosmotic conditions, a regulatory volume decrease (RVD) is accomplished by efflux of K+ and Cl−, and organic osmolytes, which results in the extrusion of osmotically obliged water in a variety of cell types (15, 35, 36).

Osmotic cell swelling has been reported to be associated with the activation of different types of K+-channels, including Ca2+-activated K+ channels (5, 21, 27, 39, 50, 52, 57, 58), stretch-activated K+ channels (8, 31, 42, 54), Ca2+- and stretch-activated K+ channels (20, 38), voltage-gated K+ channels (1, 6, 9, 43, 46, 50), and two-pore (2P) domain TASK channels (33). Summarizing previous observations, Pasantes-Morales and Morales-Mulia (40) recently suggested that the RVD in most types of epithelial cells involves Ca2+-dependent K+ channels, whereas in nonepithelial cells involves Ca2+-independent K+ channels.

Ca2+-activated K+ channels are ubiquitously distributed in mammalian cells, and these channels play important roles in many different cell functions. On the basis of their electrophysiological characteristics, three major classes of Ca2+-activated K+ channels have been described (24): voltage-dependent, large-conductance K+ channels (BK or hSlo for its α-subunit); voltage-independent, small-conductance K+ channels (SK); and inwardly rectifying, intermediate-conductance K+ channels (IK). It is well known that the BK, IK, and SK channel proteins are products of three different genes (10, 56). Although there is ample evidence that the RVD process involves Ca2+-activated K+ channels in Intestine 407 cells (13, 14), it is not known which type of Ca2+-activated K+ channel protein is involved. Thus, in the present study, we had the aim of determining the molecular identity of this volume-regulatory K+ channel.

MATERIALS AND METHODS

Cell culture. A human epithelial cell line, Intestine 407, was cultured in monolayer in Fischer’s medium supplemented with 10% newborn bovine serum, as described previously (13). For patch-clamp and volume measurements, cells were detached from the plastic substrate and suspended, as described previously (22). After cell culture was suspended with agitation, the cells were placed in a chamber (0.3 ml) and, after they had attached to the glass bottom, perfused with bath solution at about 3 ml/min by gravity feed from reservoirs. Cells used for some cell-attached recordings were first loaded with 1,2-bis(2-aminophenoxy)ethane-N,N,N′,N′-tetraacetic acid (BAPTA) by preincubation with 50 µM BAPTA-AM for 15 min at 37°C.

RT-PCR. Poly(A)+ RNA was extracted from Intestine 407 cells by using the Direct mRNA purification kit with magnetic porous glass (MPG; CPG, Lincoln Park, NJ). Briefly, the
cells were detached from culture flasks and homogenized in a buffer containing LiDs and ethylene glycol-bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid (EGTA). Poly(A)'+ RNA was extracted with MPG-bound oligo(dT)20-35. The isolated poly(A)'+ RNA was reverse transcribed by using the SuperScript preamplification system (Invitrogen, Carlsbad, CA). The resultant first strand cDNA was used for PCR. Primers were designed and synthesized according to the published sequences of cDNA encoding human SK1 (hSK1), human IK (hIK) (18), and human BK α-subunit (hSlo) (51), as summarized in Table 1. As a positive control, we also amplified mRNA of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) using the primers listed in Table 1.

PCR was performed in a total volume of 50 μl of a buffer solution containing (in mM) 20 Tris-HCl (pH 8.4), 50 KCl, 1.5 MgCl2, 0.2 dNTP, and 250 units Ampli Taq Gold (Perkin-Elmer, Norwalk, NC). The thermal cycle protocol used was 94°C for 1 cycle, 55°C for 2 min, and 72°C for 3 min for 40 cycles in a programmable thermocycler (GeneAmp PCR System 9600; Perkin Elmer Life Sciences, Boston, MA). A negative control experiment was performed using primers and RNA that had not been reverse transcribed. The products of RT-PCR were electrophoresed and sized separately on a 2% agarose gel. Positive bands were cut out of the gel, and the cDNA was extracted and purified using the GENE CLEAN kit (Bio101, Carlsbad, CA), after which it was immediately subjected to sequencing with the ABI PRISM Big Dye Terminator Cycle Sequencing Ready Reaction kit and an ABI PRISM 310 genetic analyzer (Applied Biosystems, Foster City, CA).

**Cell volume measurements.** Cell volume was measured at room temperature (22–26°C) or 37°C by an electronic sizing technique with a Coulter-type cell size analyzer (CDA-500; Sysmex, Kobe, Japan), as previously described (15). The mean volume of the cell population was calculated from the cell volume distribution after the machine was calibrated with latex beads of known volume.

Isotonic (310 mosmol/kgH2O) or hypotonic (200 mosmol/kgH2O) solution consisted of (in mM) 147 K-gluconate, 1 MgCl2, 2 Mg-ATP, 0.08 EGTA, 0.03 CaCl2, 10 mannitol, and 10 HEPES/KOH (pH 7.3). The free Cl−/Ca2+ selectivity of the channel observed, K-gluconate in the bath solution was replaced by Na+Cl−-agar bridge. When Cl−-free bath solution was used, a 3 M KCl-agar bridge was used.

In whole cell recordings, series resistance (<6 MΩ) was compensated (to 60–70%) to minimize voltage errors. The time course of whole cell current activation and recovery was monitored by repetitively applying (every 15) ramp pulses (1-s duration) from −100 to +100 mV from a holding potential of −40 mV. To obtain whole cell I-V relations, step pulses (1-s duration) from −60 to +60 mV were used. To test the Cl−/Ca2+ selectivity of the channel observed, K-gluconate in the bath solution was replaced by NaCl in an equimolar basis.

Single-channel recordings were made in the cell-attached mode or the excised, inside-out mode. When patches contained only one active channel, single-channel analysis was

<table>
<thead>
<tr>
<th>Target</th>
<th>mRNA</th>
<th>Primer Sequence</th>
<th>Product Size, bp</th>
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| hSK1   | Pair 1 | Forward: 5'TCTCGTGTTCCTCTACCATGC-3'  
Reverse: 5'AGTGCTTCTGACGGACGCG-3' | 365 |
|        | Pair 2 | Forward: 5'CTTCTCTCTCTTACTGCACTCC-3'  
Reverse: 5'TGCTCAGTCTTACTGCACTCC-3' | 351 |
| hIK    | Pair 1 | Forward: 5'TACAAAGGCAGAAGACGACGC-3'  
Reverse: 5'CATCTTGTGAGAAGACGACGC-3' | 241 |
|        | Pair 2 | Forward: 5'TCTCAATCAAGTCCGCTTC-3'  
Reverse: 5'AGCATGAGACTGCTTCTGC-3' | 457 |
| hSlo   | Pair 1 | Forward: 5'TATGGCTTCTTCTCACAGCTCG-3'  
Reverse: 5'CTTATTTGGCTACGCTTCTGC-3' | 415 |
|        | Pair 2 | Forward: 5'TACCATGAGACTGCTTCTGC-3'  
Reverse: 5'ATTGCTGAGACTGCTTCTGC-3' | 306 |
| GAPDH  |       | Forward: 5'ACCCACGTCATCCATCCATAC-3'  
Reverse: 5'TCCACACCCCGCCTGTA-3' | 452 |

hSK1, human small-conductance K+ channel; hIK, human intermediate-conductance K+ channel; hSlo, human large-conductance K+ α-subunit channel.
performed for events, the closed level of which was flat and stable. Amplitude histograms were plotted from data samples of 30- to 60-s duration from at least eight patches and were fitted by two Gaussian curves using a Peak Fitting module in Origin 6.1. Single-channel conductance was determined by the difference of these two Gaussian's center values, and open probability ($P_o$) was by the area under the curve of each Gaussian. For inside-out recordings, the intracellular side was perfused with Cl$^-$-free bath solution (285 mosmol/kgH$_2$O) consisting of (in mM) 144 K-gluconate, 1 MgSO$_4$, 2 EGTA, 10 HEPES/NaOH (pH 7.4), and CaSO$_4$ at concentrations calculated by CaBuf software (provided by Dr. G. Droogmans, KUL, Belgium) to give [Ca$^{2+}$] of 0.01, 0.1, 1, and 100 $\mu$M. To test the $K^+$ selectivity of the channel, the K-gluconate in the bath solution was replaced with equimolar Na-gluconate. The pipette was filled with extracellular solution (285 mosmol/kgH$_2$O) composed of (in mM) 144 K-gluconate, 2 CaCl$_2$, 1 MgCl$_2$, and 10 HEPES/KOH (pH 7.4). At the end of experiments, patches were perfused with 5 $\mu$M clotrimazole (CLT) to confirm that the current was a CLT-sensitive one. For on-cell patches, high-K$^+$ bath solution was used to nullify the membrane potential. High-K$^+$-Cl$^-$-free isotonic (290 mosmol/kgH$_2$O) or hypotonic (230 mosmol/kgH$_2$O) consisting of (in mM) 144 K-gluconate, 1 CaCl$_2$, 1 MgCl$_2$, and 10 HEPES/KOH (pH 7.4). The pipette solution (285 mosmol/kgH$_2$O) contained in (mM) 144 K-gluconate, 2 CaCl$_2$, 1 MgCl$_2$, and 10 HEPES/KOH (pH 7.4).

Chemicals. EGTA, BAPTA, BAPTA-AM, and Na-gluconate were purchased from Wako Pure Chemical Industries (Osaka, Japan), and clotrimazole was from Research Biochemicals (Natick, MA). All other reagents were obtained from Sigma-Aldrich Japan (Tokyo, Japan). Stock solutions of 5 mM CLT, 50 mM NPPB, and 1 mM ionomycin were prepared in dimethyl sulfoxide (DMSO). Tetraethylammonium (TEA), charybdotoxin (ChTX), iberiotoxin, and apamin were directly added to the appropriate solution before use.

**Statistical analysis.** Data are presented as means ± SE of $n$ observations. Statistical differences in data were evaluated by one-dimensional ANOVA and Scheffe’s post hoc multiple comparison tests. Data were considered to be significant at $P < 0.01$.

**RESULTS**

**Molecular expression of IK channels.** RT-PCR was performed on RNA isolated from Intestine 407 cells to examine the expression of five types of Ca$^{2+}$-activated K$^+$ channels. As shown in Fig. 1 (lanes 2 and 4), DNA fragments of expected size at 241 and 457 bp were amplified by hIK-specific primers (Table 1, pairs 1 and 2) from reverse transcribed cDNA. The nucleotide sequence of these PCR products was completely identical to the corresponding sequence in the hIK channel (18). However, no PCR product was amplified when reverse transcriptase was omitted from the reaction (Fig. 1, lanes 3 and 5).

In contrast, as shown in Fig. 1 (lanes 6 and 7), no specific DNA products were amplified by primers (Table 1, pair 1) specific for SK1 and hSlo. The same results were obtained using another set of primers (Table 1, pair 2). However, GAPDH mRNA was consistently detected (Fig. 1, lane 8).

**Functional expression of IK channels.** When Intestine 407 cells were dialyzed with low-Cl$^-$-pipette solution and exposed to Cl$^-$-free bath solution containing a Cl$^-$ channel blocker, NPPB (50 $\mu$M), addition of ionomycin (1 $\mu$M) increased whole cell currents, as observed previously (17). As shown in Fig. 2A, the ionomycin-activated current exhibited slight inward rectification. Time-dependent activation was observed at large positive potentials (upper inset). When the extracellular K$^+$ concentration ($[K^+]_o$) was increased, the reversal potential ($E_{rev}$) shifted in the positive direction. The $E_{rev}$ shift per 10-fold increase in $[K^+]_o$ was $53$ mV (lower inset), indicating high selectivity of K$^+$ ($P_{Na}/P_{K} < 0.01$). A wide spectrum K$^+$ channel blocker (24, 56), TEA (20 mM), partially suppressed the currents (Fig. 2Ba). The current was largely abolished by 200 nM CLT (Fig. 2Bb), which is a blocker specific to IK (53). The ionomycin-activated current was also sensitive to 20 nM ChTX (Fig. 2Bc), which is known to block not only BK channels (32) but also IK channels (11, 45). The $E_{rev}$ values for TEA-, CLT-, and ChTX-sensitive currents were $-80.6 \pm 5.3$, $-82.8 \pm 4.2$, and $-83.6 \pm 3.0$ mV ($n = 6$), respectively, and these values are close to that of the equilibrium potential for K$^+$ ($-90$ mV). In contrast, the ionomycin-activated current was insensitive to 100 nM apamin (Fig. 2Bd) and 100 nM iberiotoxin (data not shown, $n = 3$), blockers that are specific to SK and BK (24, 56), respectively. When single-channel recordings were performed in inside-out patches under symmetrical K$^+$ conditions, unitary events were found to be induced by increases in the intracellular Ca$^{2+}$ concentration (Fig. 3A). The amplitude histogram was well fitted by two Gaussian curves. The $P_o$ value increased from 0 to 0.17 ± 0.04 and 0.45 ± 0.03 ($n = 8$) at $-100$ mV, when [Ca$^{2+}$] was increased from 0.01 to 0.1 and 1 $\mu$M, respectively. This channel activity showed no obvious voltage dependence. The $P_o$ values at $-100$, $-40$, $+40$, and $+100$ mV were 0.46 ± 0.06, 0.41 ± 0.05, 0.42 ± 0.04, and 0.44 ± 0.03 ($n = 8$), respectively, at 100 $\mu$M [Ca$^{2+}$]. As shown in Fig. 3B, the single-channel activity at 100 $\mu$M [Ca$^{2+}$]...
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Fig. 2. Ionomycin-induced whole cell K+ channel currents in Intestine 407 cells and their sensitivity to K+ channel blockers. Arrowheads indicate zero current level. A: current-voltage relations. Data represent the mean values of 8 experiments. SE values are presented as vertical bars, where the values exceed the symbol size. Upper inset: representative traces of the current response to step pulses from −100 to 100 mV in 20-mV increments. Lower inset: relation between the reversal potential (Erev) and the logarithm of the extracellular K+ concentration ([K+]o). The slope value given in the figure was determined by regression fitting. B: representative traces of the current response to step pulses 3–5 min after application of 20 mM tetraethylammonium (TEA) (a), 200 mM clotrimazole (CLT) (b), 20 nM charybdotoxin (ChTX) (c), and 100 nM apamin (d). Data represent 8 similar experiments.

was sensitive to 200 nM CLT in a reversible manner. The current-voltage curve exhibited slight inward rectification (Fig. 3C). The mean unitary conductance was 30 ± 2 pS at −100 mV and 18 ± 4 pS (n = 6) at +100 mV. When the intracellular K+ concentration was decreased from 144 to 4.2 mM, the Erev value shifted from 0 to +69 mV, indicating K+ selectivity. Both BK-like larger conductance and SK-like smaller conductance events were never observed in inside-out patches in the tested [Ca2+]o range.

Combining the results of the whole cell and single-channel recordings, we conclude that Intestine 407 cells functionally express CLT-sensitive, Ca2+-dependent IK channels.

Swelling-induced activation of IK channels. A hypotonic challenge reversibly induced increases in cell size and membrane currents in Intestine 407 cells under whole cell clamp in which low-Cl− pipette solution and Cl−-free bath solution containing 50 μM NPPB were used (Fig. 4A). The profile of the swelling-induced current (Fig. 4Aa) and the I-V relation (Fig. 4Ac) were similar to those of ionomycin-induced current (Fig. 2A).

The swelling-induced current was very sensitive to CLT (200 nM; Fig. 4Ab). The Erev for CLT-sensitive current was −84.3 ± 7.2 mV (Fig. 4Ac). When intracellular Ca2+ was chelated with 5 mM BAPTA introduced to the pipette solution, a hypotonic challenge induced cell swelling but activated whole cell currents very little, as shown in Fig. 4B.

Single-channel events were observed only rarely in cell-attached patches on Intestine 407 cells exposed to isotonic solution (Fig. 5). Osmotic swelling induced by hypotonic stress was always associated with a prominent increase in single-channel activity with an intermediate unitary current of 3.1 ± 0.2 pA (n = 10) at a pipette potential of +100 mV (intracellular potential of around −100 mV), under high K+ conditions (Fig. 5A). Unitary events with SK-like smaller or BK-like larger single-channel conductance were never observed. The P0 value of intermediate type single-channel events increased from around 0 to 0.43 ± 0.04 (n = 10) after induction of osmotic swelling. For BAPTA-loaded swollen cells, however, swelling-induced activation of intermediate-conductance single-channel events were far less prominent (Fig. 5B), and the P0 value was 0.004 ± 0.001 (n = 10) under hypotonic conditions. Also, swelling-induced activation of single-channel events was largely prevented by 200 nM CLT when it was added to

Fig. 3. Ca2+-activated single K+ channel currents in inside-out patches excised from Intestine 407 cells and their sensitivity to CLT. Arrowheads indicate the closed-state level. A: effects of Ca2+ concentration on the single-channel activity recorded at −100 mV. B: effect of CLT on the single-channel activity recorded at −100 mV and [Ca2+] = 100 μM. The middle and bottom records were obtained around 3 min after application and wash-out, respectively, of CLT. C: current-voltage curves for single-channel currents recorded at [Ca2+] = 100 μM under symmetrical K+ conditions ([K+]o = 144 mM) and asymmetrical K+ conditions ([K+]o = 4.2 mM). Data represent the mean of 6 experiments (vertical bar: SE).

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the pipette solution (Fig. 5C). The $P_o$ value was $0.010 \pm 0.005$ ($n = 8$) during osmotic swelling in the presence of 200 nM CLT.

Both these whole cell and single-channel data indicate that activation of CLT-sensitive, Ca$^{2+}$-dependent, IK channels is associated with osmotic swelling in Intestine 407 cells.

**Involvement of IK channel in the RVD process.** After Intestine 407 cells were exposed to hypotonic solution at room temperature, the mean cell volume promptly increased and then gradually recovered (Fig. 6), as observed previously (13). The RVD was inhibited by the application of 200 nM CLT (Fig. 6A). However, application of SK and BK channel blockers, apamin (100 nM) and iberiotoxin (100 nM), failed to significantly affect the RVD (Fig. 6A). Also, the RVD was not inhibited by another K$^+$ channel blocker, clofilium (100 $\mu$M; Fig. 6B), which is known to block both KCNQ (MinK) (2) and KCNK5 (TASK) channels (33). CLT sensitivity and clofilium insensitivity of the RVD that were essentially the same were observed at 37°C (data not shown, $n = 6$ each). In light of these data, we conclude that the K$^+$ channel type involved in the RVD of Intestine 407 cells is the CLT-sensitive IK channel.

**DISCUSSION**

A number of types of K$^+$ channels have been demonstrated to be involved in K$^+$ efflux during volume regulation after osmotic swelling (40). In human epithelial Intestine 407 cells, we previously concluded that the volume-regulatory K$^+$ channel is classified into a Ca$^{2+}$-activated one, based on the following observations: the RVD was inhibited by chelation of cytosolic Ca$^{2+}$ and by application of a K$^+$ channel blocker Ba$^{2+}$ (13), osmotic cell swelling brought about activation of Ca$^{2+}$-dependent K$^+$ conductance under voltage-clamp (13), and swelling-induced K$^+$ conductance acti-

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**Fig. 4.** Swelling-induced whole cell K$^+$ currents in Intestine 407 cells and their sensitivity to CLT and cytosolic Ca$^{2+}$ chelation. Arrowheads represent zero current level. A: representative record showing currents before, during, and after exposure to hypotonic solution in the absence or presence of CLT (200 nM) during application of ramp pulses (from $-100$ to $+100$ mV) and step pulses of $-100$ to $+100$ mV in 20-mV increments. Expanded traces of the record under isotonic and hypotonic conditions are shown in a and b, and the current-voltage relationships before (•) and during exposure to hypotonic solution in the absence (○) and presence (△) of CLT are shown in c. Each symbol represents the mean instantaneous current of 7 observations ± SE (vertical bar). B: representative record showing currents during application of ramp pulses and step pulses before, during, and after exposure to hypotonic solution under conditions in which cytosolic Ca$^{2+}$ was chelated with 5 mM BAPTA added to the pipette solution. Data represent 8 similar experiments.

**Fig. 5.** Swelling-induced single K$^+$ channel events in cell-attached patches on Intestine 407 cells and their sensitivity to cytosolic Ca$^{2+}$ chelation and extracellular CLT. Arrowheads indicate the closed-state level. A: representative record showing currents at the pipette potential of $+100$ mV before and after exposure to hypotonic solution. Bottom panels a and b represent expanded traces. B: representative record of currents before and during exposure to hypotonic solution from an on-cell patch of a cell preincubated with 50 $\mu$M BAPTA-AM. C: representative record of currents before and during exposure to hypotonic solution with CLT (200 nM) present in the pipette solution.
Maxi-K⁺ or BK channels, which exhibit striking voltage dependence (24), have been found to be activated during osmotic swelling in a number of other cell types (4, 7, 19, 44, 49). In human osteoblast-like C1 cells, BK channels, together with IK channels, were shown to be involved in the RVD (58). In Intestine 407 cells, however, a BK channel blocker, iberiotoxin, failed to inhibit the RVD and Ca²⁺-activated whole cell K⁺ currents. Also, swelling- or Ca²⁺-activated K⁺ channel events exhibited weak voltage dependence and never exhibited Maxi unitary conductance. Moreover, mRNA of the BK channel α-subunit hSlo was never detected by RT-PCR. Involvement of apamin-sensitive SK channels in the RVD of Intestine 407 cells could also be excluded by the observation that there was no RT-PCR signal for SK1 and that the RVD and the Ca²⁺-activated whole cell K⁺ currents were not sensitive to apamin.

Recently, Niemeyer et al. (33) provided clear evidence for the involvement of the TASK-2 type of 2P domain K⁺ channels in the RVD of Ehrlich ascites tumor cells. It is known that 2P domain K⁺ channels are independent of cytosolic Ca²⁺ and are insensitive to Ba²⁺ (41). Thus, in Intestine 407 cells, TASK-2 channels may not play a role in the RVD, which was found to be dependent on cytosolic Ca²⁺ (13, 14) and sensitive to Ba²⁺ (13). In the present study, in fact, the RVD of Intestine 407 cells was found to be totally insensitive to clofilium (Fig. 6B), which is known to block TASK-2 (33). Clofilium insensitivity may also rule out the involvement of the MinK channel, which is sensitive to this class III antiarrhythmic drug (2).

The involvement of IK channels in the RVD process has been reported in mouse erythroid cells (32), human T lymphocytes (21), and human tracheal cells (25). Vázquez et al. (55) reported that CFTR expression is a prerequisite to swelling-induced IK channel activation in tracheal cells. However, the present study provided an example of the involvement of IK channel activation in Intestine 407 cells, which do not express CFTR (12). In Intestine 407 cells, IK channels could be activated by a cytosolic Ca²⁺ rise due to swelling, as well as due to stimulation of phospholipase C-linked P2Y₂ receptor by ATP released from swollen cells (37).

The RVD mechanism is of essential importance to some cell types, such as enterocytes, in which the swelling-inducing osmotic gradient across the cell membrane is produced by active solute uptake (29, 34, 47). K⁺ channel activation may play an important role in volume regulation during Na⁺-coupled absorption of organic solutes in small intestine, as Ba²⁺-sensitive K⁺ conductance activation was observed in Necturus small intestinal enterocytes during exposure to galactose (25, 26). Single-channel recordings demonstrated that L-alanine application activated Ca²⁺-dependent K⁺ channels with an intermediate unitary conductance in Necturus enterocytes (48). In guinea pig jejunum enterocytes, the RVD that takes place after osmotic swelling due to Na⁺-coupled solute absorption was found to be inhibited by ChTX (29, 30). Our results suggest that IK channels play, at least in part, a volume-regulatory role in the RVD of Intestine 407 cells.
role in small intestinal epithelial cells during Na$^{2+}$-dependent organic solute absorption in vivo.

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