Volume Expansion Sensitivity of Swelling-Activated Cl^- Channel in Human Epithelial Cells

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Abstract: In human epithelial cell lines, whole-cell swelling-activated Cl^- current was not directly correlated to cell volume per se, membrane tension or hydrostatic pressure. The current density exhibited a relation described by a Boltzmann function to the square of the cell di-

ameter. Cytochalasin D enhanced the volume sensitivity. These results suggest that the activation mechanism of volume-sensitive Cl^- channel is related to cytoskeleton-dependent membrane spring energy in human epithelial cells. [Japanese Journal of Physiology, 50, 277–280, 2000]

Key words: Cl^- channel, volume sensor, cell volume regulation.

When cells are exposed to a hypotonic ambient solution or hypertonic intracellular solution, they immediately start swelling. Soon after, most mammalian cells tend to recover their original volume exhibiting a regulatory volume decrease (RVD), which is attained by KCl efflux due to the activation of channels and transporters [1, 2]. Swelling-activated Cl^- channels play an essential role in this volume regulation mechanism in most epithelial cells [2]. However, little is known how an increase in cell volume induces activation of the Cl^- channel. There have been some suppositions as to what energy in the cell is most precisely related to the cell volume definition and regulation; hydrostatic pressure, tonic membrane tension, linear tension of cytoskeleton, macromolecular crowding, and so on [2, 3]. To elucidate the volume-sensing mechanism of swelling-activated Cl^- channel, we examined the volume sensitivity of the Cl^- current in human epithelial cells on the whole-cell level. Brief accounts of this work have been reported in abstract form [4].

Human epithelial Intestine 407 and epidermoid KB cells were cultured as reported previously [5, 6]. Isolated single spherical cells were provided for experiments after suspension culturing, as described elsewhere [5]. Cell size was measured by cell image

recorded by time-lapse videomicroscopy, as described previously [6], or by a high-speed automatic image analyzing technique [7]. The isotonic NaCl solution contained (in mM): 100 NaCl , 5 KCl , 1 CaCl_2 , 1 MgCl_2 , 11 glucose, 70 mannitol, 2 NaHEPES and 3 HEPES (pH 7.4, osmolality 300 mosmol $\text{kgH}_2\text{O}^{-1}$). A variety of hypotonic NaCl solutions were prepared by diluting the isotonic solution with distilled water. Whole-cell Cl^- currents were recorded as reported previously [8]. Isotonic CsCl solutions contained (in mM): 110 CsCl , 12 HEPES , 5 MgSO_4 , 100 mannitol and 8 Tris (pH 7.5, 320 mosmol $\text{kgH}_2\text{O}^{-1}$). A variety of hypotonic CsCl solutions were prepared by reducing the mannitol concentration. Control or hypertonic pipette (intracellular) solutions consisted of (in mM): 110 CsCl , 2 MgSO_4 , 1 Na_2ATP , 15 NaHEPES , 10 HEPES , 1 EGTA , and 50 or 110 mannitol (pH 7.3, 300 or 360 mosmol $\text{kgH}_2\text{O}^{-1}$). In some experiments, cytochalasin D was added to the hypertonic pipette solution. Data are given as means $\pm$ SEM of observations. All experiments were performed at room temperature (23–26°C).

As shown in Fig. 1, high-speed cell size measurements demonstrated that the Intestine 407 cell responded to a hypotonic challenge with rapid swelling and gradual shrinkage thereafter, called a regulatory

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Fig. 1. Time course of volume change in single intact Intestine 407 cells after a hypotonic challenge with 25, 50 and 75% tonicity. Each curve is a representative of four similar experiments. The onset of regulatory volume decrease is indicated by arrows. V and V_0 represent the cell volume at a given time and the initial cell volume, respectively. **Inset:** Plot of relative cell volume vs. reciprocal of relative osmolality of bathing solution. The linear relation confirms that cell volume at the onset of RVD reached its theoretical maximum volume defined by the following equation: $(V-b)\Pi = (V_0-b)\Pi_0$, where Π and Π_0 denote the osmolality of isotonic and hypotonic solutions, respectively, and b is the water-inaccessible volume. Each symbol represents the mean value (with SEM) of 3 to 7 observations.

volume decrease (RVD). The peak values of cell volume after hypotonic challenges increased with decreases in osmolality of the bathing solution according to the van't Hoff relation (Fig. 1: inset). If hydrostatic pressure to the plasma membrane upon osmotic swelling is responsible for activation of ion channels during the RVD, the faster onset of RVD must be induced by the stronger hypotonic stress. Figure 1 showed, however, that rather slower onset (at arrows) of volume regulation was associated with stronger hypotonic stress in Intestine 407 cells. This result may rule out the involvement of hydrostatic pressure due to osmotic water inflow in the activation of volume-regulatory ion transport pathways, including volume-sensitive Cl^- channels.

Under the whole-cell configuration, sizable Cl^- currents were never observed until an increase in cell volume exceeded the threshold swelling level, as reported previously [2, 6], for several minutes after a challenge with 5% hypotonic (95% tonicity) solution in Intestine 407 cells (Fig. 2: top trace) and KB cells (data not shown). Swelling-activated whole-cell Cl^- currents, which showed phenotypic characteristics [2] such as inactivation kinetics at large positive potentials (Fig. 2: bottom traces), reached a saturated level around 20 min after a 5% hypotonic challenge (Fig. 2:

Fig. 2. Time course of swelling-induced activation of Cl^- current in a single Intestine 407 cell under the whole-cell configuration. The bath was perfused with isotonic or (5 or 10%) hypotonic CsCl solutions, and the cell was dialyzed with a control pipette solution. **Top:** Effects of changes in extracellular osmolality on currents recorded during the application of alternating pulses from 0 to ± 40 mV or of step pulses from -100 to $+100$ mV in 20-mV increments (at asterisks). When the current reached a saturated level (around 20 min after starting a 5% hypotonic challenge), the relative cell volume increased to 1.6 ± 0.1 ($n=6$). However, the relative cell volume still increased up to 2.0 ± 0.1 ($n=4$) 25 min after the hypotonic challenge. Thereafter, the cell volume again increased to 2.5 to 3 times within 10 min after a 10% hypotonic challenge. **Bottom:** Expanded traces of current responses (a and b) to step pulses (the protocol shown to the left). The data are representative of three similar experiments.

top trace) at around 1.6 times cell volume, while the cell volume was continuously increasing to around twofold during exposure to a 5% hypotonic solution. When a stronger (10%) hypotonic challenge was thereafter applied, the Cl^- current again increased (Fig. 2). These results indicate that the channel activity is not directly related to the cell volume per se.

The surface area ($4,180 \pm 114 \mu\text{m}^2$, if the specific capacitance is $1 \mu\text{F}/\text{cm}^2$) estimated from whole-cell membrane capacitance (41.8 ± 4.0 pF, $n=12$) was more than 4-fold larger than that measured by image analysis ($999 \pm 114 \mu\text{m}^2$, $n=37$) in Intestine 407 cells, indicating that the cells have many membrane infoldings. The membrane capacitance did not largely change upon osmotic swelling induced by the application of 5–10% hypotonic solution, although a stronger (45%) hypotonic challenge was previously observed to induce a small increase in membrane capacitance [9]. The same conclusion can be deduced for KB cells based on data reported previously [6]. Therefore, it appears that the Cl^- channel activation is associated with volume expansion without significant membrane tension in either epithelial cell line.

Volume expansion sensitivity of the Cl^- channel was reported to be assessed by plotting the current density against the relative surface area estimated by

Fig. 3. Relationship between the current density and relative surface area in an osmotically swollen Intestine 407 cell. The cell was dialyzed with a control pipette solution and exposed to a 10% hypotonic CsCl solution. Each curve represents a fitting with $n=1.0$, 1.9 , 2.0 or 3.0 to Eq. 1. The data were best fitted with $n=1.90$ (as well as $a=2.52$ and $q=0.37$), when n was set as a variable parameter. A fitting with $n=1.9$ or 2.0 (as well as $a=2.56$ and $q=0.37$) was significantly better than that with $n=1.0$ (as well as $a=1.60$ and $q=0.13$) and $n=3.0$ (as well as $a=4.23$ and $q=0.82$) ($p<0.05$ and <0.01 , respectively, by F -test). From seven different cells, essentially the same results were obtained.

cell contour morphology [2, 6]. In Intestine 407 cells, as shown in Fig. 3, the relation between the current density and relative surface area could be best fitted with $n=1.90$ to Boltzmann's equation:

$$I/I_{\max} = 1/[1 + \exp\{-(r^n - a)/q\}], \quad (1)$$

where I is the current density, r the cell diameter, a a constant (called the half activation energy), and q a slope factor. The current density could be better fitted with $n=2$ than with $n=3$ or $n=1$ (Fig. 3). This fact indicates that the channel activation mechanism is associated neither directly with cell volume per se nor with membrane tension, which is proportional to the cell diameter according to the law of Laplace. Instead, the fact of second-power best fitting implies that the potential energy responsible for gating of the channel is dependent on the internal energy of linear elastic cell material (such as actin filaments), namely the spring energy of cell membrane, because spring energy is defined by $1/2kx^2$, where k is the spring constant (which is inversely proportional to q) and x the displacement of spring (corresponding to a cell diameter change).

In KB cells, as shown in Fig. 4, volume expansion sensitivity of swelling-activated whole-cell Cl^- currents was also best fitted to Eq. 1, with the second power of the cell diameter in the absence (open symbols) and presence (filled symbols) of intracellular cytochalasin D ($5 \mu\text{M}$). Data presented in Fig. 4 show

Fig. 4. Effect of cytochalasin D on volume expansion sensitivity of whole-cell Cl^- currents in osmotically swollen KB cells. The cells were exposed to isotonic CsCl solution and dialyzed with hypertonic pipette solution without (open circles) or with (filled circles) cytochalasin D ($5 \mu\text{M}$). Each curve represents a fitting of the data collected from the same cell to Eq. 1 with $n=2$.

that cytochalasin D significantly enhanced volume expansion sensitivity of the Cl^- channel by reducing the half activation energy (from 2.39 ± 0.23 to 1.90 ± 0.15) and decreasing the slope factor (from 0.51 ± 0.07 to 0.26 ± 0.12). This would also explain previous observations of augmenting the effect of cytochalasin D or B on swelling-activated Cl^- channel currents in B lymphoma cells [10] and osteoclasts [11].

Taken together, it is concluded that cell swelling-induced activation of the volume-regulatory Cl^- channel is induced neither by membrane tension nor by hydrostatic pressure due to osmotic water inflow in the human epithelial cells. It is likely that activation energy of the Cl^- channel or its "volume sensor" is dependent on the spring energy of cell membrane, presumably associated with the cytoskeleton network.

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