Intracellular ionic strength regulates the volume sensitivity of a swelling-activated anion channel

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Cannon, Carolyn L., Srisaila Basavappa, and Kevin Strange. Intracellular ionic strength regulates the volume sensitivity of a swelling-activated anion channel. Am. J. Physiol. 275 (Cell Physiol. 44): C416–C422, 1998.—Cell swelling activates an outwardly rectifying anion channel termed VSOAC (volume-sensitive organic osmolyte/anion channel). Regulation of VSOAC by intracellular electrolytes was characterized in Chinese hamster ovary cells by whole-cell patch clamp. Elevation of intracellular CsCl concentration from 40 to 180 mM resulted in a concentration-dependent decrease in channel activation. Activation of VSOAC was insensitive to the salt gradient across the plasma membrane, the intracellular concentration of specific anions or cations, and the total intracellular concentration of cations, anions, or electrolytes. Comparison of cells dialyzed with either CsCl or Na2SO4 solutions demonstrated directly that VSOAC activation is modulated by intracellular ionic strength (µi). The relative cell volume at which VSOAC current activation was triggered, termed the channel volume set point, decreased with decreasing ionic strength. At µi = 0.04, VSOAC activation occurred spontaneously in shrunken cells. The rate of VSOAC activation was nearly 50-fold higher in cells with µi = 0.04 vs. those with µi = 0.18. We propose that µi modulates the volume sensor responsible for channel activation.

MATERIALS AND METHODS

Cell culture.

CHO cells were grown in Ham's F-12 (GIBCO) with 10% fetal bovine serum and penicillin-streptomycin. After growth to 25–50% confluence, cells were acclimated to 400 mosmol/kgH2O Ham's F-12 (60 mM NaCl addition) for 24–48 h before experiments. Hypertonic growth medium was used so that cells could be patch clamped with pipette solutions containing high salt concentrations that were isotonic to the control extracellular bath. The effects of ionic strength described in this paper were also observed in cells grown in normotonic (i.e., 300 mosmol/kgH2O) medium (21).

Patch-clamp recordings.

CHO cells were grown in 35-mm culture dishes and dissociated by brief treatment with Ca2+- and Mg2+-free modified Hanks' solution. Dissociated cells were allowed to reattach to the poly-L-lysine-coated coverslip bottom of a bath chamber (model R-26G; Warner Instrument, Hamden, CT), which was mounted onto the stage of a Nikon Diaphot or TE 300 inverted microscope. Patch electrodes were pulled from 1.5-mm outer diameter borosilicate glass microhematocrit tubes (Fisher Scientific, St. Louis, MO) that had been silanized with dimethylchlorosilane (Sigma Chemical, St. Louis, MO). Electrodes were not fire polished before use.

The standard bath solution contained (in mM) 140 NaCl, 5 MgSO4, 12 HEPES, 8 Tris, 5 glucose, 2 glutamine, and 100 sucrose (pH 7.4; osmolality = 400 mosmol/kgH2O). Cells were patch clamped with a pH 7.2 pipette solution that contained...
Effect of intracellular salt concentration on current activation. Figure 1 illustrates the effect of intracellular electrolyte concentration on cell swelling and VSOAC current activation. The predominant salt in the pipette solutions used in these experiments was CsCl. CsCl concentration was altered by isosmotic replacement with sucrose. Once the whole cell configuration had been attained and whole cell parameters adjusted, cells were dialyzed for 1.5–2 min with various pipette solutions before swelling was initiated. However, cells dialyzed with 40 mM CsCl exhibited spontaneous current activation in the absence of apparent swelling (discussed in more detail below). For these cells, therefore, bath osmolality was reduced as soon as current activation was observed (typically 15–30 s after obtaining the whole cell configuration).

As shown in Fig. 1, reduction of CsCl levels enhanced current activation in a concentration-dependent manner. The initial rates of swelling in all four groups of cells were similar (Fig. 1, inset). Thus intracellular electrolyte concentration exerts its effect on the mechanism responsible for channel activation rather than altering the stimulus (i.e., cell swelling).

Intracellular ionic strength alters VSOAC activation. When intracellular CsCl concentration is altered, eight solution physicochemical parameters are changed simultaneously: 1) nonelectrolyte (i.e., sucrose) concentration, 2) transmembrane ion gradients, 3) Cl\(^-\) concentration, 4) Cs\(^+\) concentration, 5) total anion concentration, 6) total cation concentration, 7) total electrolyte concent-

**RESULTS**

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tration (i.e., the sum of both cation and anion concentrations), and 8) ionic strength. Thus it is possible that any one or any combination of these parameters controls channel activation. We have shown previously that channel activation is not altered by the sucrose concentration of the patch pipette solution and therefore carried out experiments to assess the effects of electrolytes on swelling-induced current activation.

The effect of reductions in intracellular electrolyte levels on swelling-induced current activation may have been due to the imposition of a transmembrane electrolyte gradient. Two experiments were performed to test this possibility. First, cells were dialyzed with an 80 mM CsCl solution, and current activation was monitored in the presence of a bath solution containing 140 or 80 mM NaCl. As shown in Fig. 2A, swelling-induced current activation was the same in the presence and absence of a transmembrane electrolyte gradient. Cells were also dialyzed with 140 mM CsCl and then swollen in a hypotonic bathing medium containing either 140 or 40 mM NaCl. Reducing the bath electrolyte concentration did not enhance the rate of current activation.

Our earlier studies on skate hepatocytes suggested that VSOAC activation was modified by intracellular Cl<sup>−</sup> levels. To test this possibility, cells were dialyzed with an 80 mM salt solution containing 5 mM CsCl and 75 mM NaCl, 75 mM NaBr, or 75 mM sodium gluconate. As shown in Fig. 2B, current activation was unaffected by replacement of Cl<sup>−</sup> with either Br<sup>−</sup> or gluconate. Replacement of Cl<sup>−</sup> with SO<sub>4</sub><sup>2−</sup> also had no effect on current activation (Fig. 3).

VSOAC activation may be modulated by the concentration of specific cations. This possibility was tested by dialyzing cells with 80 mM salt solutions containing 5 mM CsCl and 75 mM NaCl, 75 mM KCl, or 75 mM LiCl. Current activation was similar with all three cations (Fig. 2C).

The results discussed above demonstrate that VSOAC activation is modified by total anion concentration, total cation concentration, total electrolyte concentration, and/or ionic strength. It is not possible to experimentally isolate each of these variables, since a change in one also alters the other three. However, it is possible to hold ionic strength constant and simultaneously vary total anion, cation, and electrolyte concentration. Cells were therefore dialyzed with pipette solutions having an ionic strength of either 0.08 or 0.14. The 0.08 ionic strength solutions contained 80 mM CsCl and 5 mM CsCl plus 25 mM Na<sub>2</sub>SO<sub>4</sub>, whereas the 0.14 ionic...
strength solutions contained 140 mM CsCl or 5 mM CsCl plus 45 mM Na$_2$SO$_4$. As shown in Fig. 3, current activation occurred more slowly in the 0.14 ionic strength solutions. Importantly, current activation was similar in the pure Cl$^-$ and mixed Cl$^-$ and SO$_4^{2-}$ solutions with the same ionic strength despite the fact that total anion, cation, and electrolyte concentrations were considerably lower in the mixed solutions. These results demonstrate that VSOAC activation is modulated by intracellular ionic strength rather than other parameters that vary with changes in the electrolyte concentrations of the pipette solutions.

Ionic strength regulates VSOAC by altering volume set point and rate of activation. Analysis of the data in Fig. 1 revealed that decreases in intracellular ionic strength increased the rate of swelling-induced current activation (Fig. 4A). Reduction of ionic strength also decreased the channel volume set point, which is defined as the relative cell volume at which VSOAC activation is triggered (Fig. 4B).

As noted earlier, cells dialyzed with a 40 mM salt solution exhibited spontaneous current activation that occurred within 30 s after membrane rupture and was not associated with observable cell swelling. The solid line in Fig. 4B is a linear regression through the volume set points obtained from cells dialyzed with 180, 140, and 80 mM salt solutions. With the assumption that the relationship between volume set point and ionic strength is linear below an ionic strength of 0.08, the regression predicts that VSOAC will activate in cells shrunk to a relative volume of 0.83 when they are dialyzed with 40 mM salt solution. We tested this prediction by performing the following experiment. The osmolality of a pipette solution containing 40 mM CsCl was reduced from ~380 to 360 mosmol/kg$_2$H$_2$O by removal of sucrose. When the plasma membrane was ruptured, the cells shrank spontaneously due to the outwardly directed osmotic gradient (Fig. 5). In many cells, the shrinkage was followed by a slow reswelling and current activation. However, in eight cells, we observed that current activation occurred over a period of 1–3 min during which no reswelling was observed (Fig. 5). The relative volume (mean ± SE) of these cells 1 min after current recordings were started was 0.84 ± 0.03.

It is possible that the spontaneously activating and swelling-induced currents are due to the activity of different channels. We therefore examined the biophysical and pharmacological characteristics of the two currents (Table 1). Both currents had similar rectification and voltage sensitivity and were inhibited in a voltage-dependent manner and to the same extent by 100 µM DIDS. The channel responsible for the currents had similar relative anion permeabilities. Reversal potentials ($E_{rev}$) for both currents were approximately −16 mV. With the assumption that relative cation permeability ($P_{cation}/P_{Cl}$) was 0.03, which we and others have estimated previously for VSOAC, $E_{rev}$ should have been approximately −30 mV. With an $E_{rev}$ of −16 mV, the $P_{cation}/P_{Cl}$ estimated from the Goldman-Hodgkin-Katz equation of the spontaneously activating and swelling-activated current in cells dialyzed with 40 mM CsCl is −0.24. Interestingly, these results are consistent with our recent studies of VSOAC in skate hepatocytes in which we observed that reduction in intracellular ionic strength substantially increased the relative cation permeability of the channel (6).

Taken together, the data in Table 1 indicate that both the spontaneously activating and swelling-induced currents are due to the activity of the same channel. We conclude, therefore, that reduction of intracellular ionic

![Fig. 4. VSOAC volume set point and rate of activation are modulated by intracellular ionic strength. A: reducing intracellular µ from 0.18 to 0.04 increases rate of current activation by nearly 50-fold. Rates were obtained from data shown in Fig. 1 by performing linear regression analysis on the first 5–6 time points after current values began to rise above the baseline. B: reductions in intracellular ionic strength reduce VSOAC volume set point. Volume set point is defined as the relative cell volume at which VSOAC activation is triggered. Values illustrated by ● were obtained from data in Fig. 1 and Fig. 1, inset. Solid line is a linear regression through these points ($y = 6.98x + 0.555$). It was not possible to obtain a volume set point for cells dialyzed with 40 mM CsCl ($µ = 0.04$) using the experimental protocol defined in Fig. 1. Under these conditions, whole cell current activated without apparent cell swelling 15–30 s after membrane rupture. □, Estimated volume set point obtained from the experiment shown in Fig. 5. This value falls on the line predicted by linear regression.]
characteristics of Cl− currents activated spontaneously or by swelling in cells dialyzed with a pipette solution containing 40 mM CsCl

Table 1. Characteristics of Cl− currents activated spontaneously or by swelling in cells dialyzed with a pipette solution containing 40 mM CsCl

<table>
<thead>
<tr>
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<th>Spontaneously Activating Cl− Current</th>
<th>Swelling-Induced Cl− Current</th>
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<tbody>
<tr>
<td>Reversal potential, mV</td>
<td>15.8 ± 0.9</td>
<td>15.4 ± 0.4</td>
</tr>
<tr>
<td>Rectification ratio</td>
<td>2.93 ± 0.36</td>
<td>3.30 ± 0.17</td>
</tr>
<tr>
<td>Time constant (t) for inactivation at +120 mV, ms</td>
<td>588 ± 86</td>
<td>481 ± 46</td>
</tr>
<tr>
<td>Inhibition at +60 mV by 100 μM DIDS, %</td>
<td>58.7 ± 1.2</td>
<td>64.2 ± 2.4</td>
</tr>
<tr>
<td>Anion permeability</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PCl/P&lt;sup&gt;-&lt;/sup&gt;</td>
<td>1.35 ± 0.06</td>
<td>1.31 ± 0.02</td>
</tr>
<tr>
<td>PNO3/P&lt;sup&gt;-&lt;/sup&gt;</td>
<td>1.19 ± 0.05</td>
<td>1.12 ± 0.01</td>
</tr>
<tr>
<td>Pgluconate/P&lt;sup&gt;-&lt;/sup&gt;</td>
<td>0.22 ± 0.03</td>
<td>0.13 ± 0.01</td>
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Values are means ± SE; n = no. of experiments. Rectification ratio is the ratio of currents measured at +60 and −60 mV. Relative anion permeabilities (PCl/P<sup>-</sup>) were calculated using the Goldman-Hodgkin-Katz equation and measured changes in reversal potential induced by complete replacement of bath Cl<sup>-</sup> with test anion. Reversal potentials were corrected for changes in liquid junction potential induced by Cl<sup>-</sup> substitution. Liquid junction potentials were measured with an open-tip microelectrode filled with 3 M KCl.
Parker et al. (19) demonstrated recently that the volume set point for the swelling-activated KCl cotransporter in dog red blood cells is reduced by increased intracellular salt concentration. In other words, as cytoplasmic salt levels rise, less swelling is required to trigger activation of the cotransporter. This effect is opposite to that observed for VSOAC activation; elevation of salt concentration (i.e., ionic strength) renders the channel less sensitive to swelling.

Motais et al. (13) concluded that intracellular ionic strength plays a key role in controlling volume regulatory amino acid and KCl efflux from trout red blood cells. These investigators manipulated cytoplasmic ionic strength by swelling cells in the presence of 145 mM urea, 50 mM NH4Cl, or mixtures of urea and NH4Cl. Swelling-induced amino acid loss was an inverse function of cytoplasmic ionic strength. At high ionic strength, there was little or no swelling-induced amino efflux and volume regulation was instead mediated by coupled K+ and Cl− loss, presumably via the KCl cotransporter. Similarly, volume regulation occurs normally in C6 glioma cells that have elevated cytoplasmic ionic strength despite the fact that there is little or no VSOAC activation (2). This implies the existence of transport pathways, such as the KCl cotransporter, that selectively mediate volume regulatory efflux of electrolytes.

The differential effect of intracellular salt concentration on volume regulatory electrolyte and organic osmolyte transport pathways may have important physiological implications (2, 21). When cell swelling occurs concomitantly with elevated cytoplasmic ionic strength, it is advantageous for cells to use electrolytes selectively for RVD via activation of an “electrolyte-selective” transport pathway such as the KCl cotransporter. The loss of organic osmolytes under such conditions would mediate RVD but would also further concentrate intracellular electrolytes as cells undergo volume regulatory water loss and concomitant shrinkage. Changes in intracellular ionic strength may therefore play an important role in coordinating the activities of various volume regulatory transport pathways. This postulated coordinated regulation could in turn contribute to the long-term maintenance of cytoplasmic ionic strength.

There are at least two physiologically relevant conditions under which coordinated regulation of volume regulatory electrolyte and organic osmolyte efflux pathways might be beneficial for cell function. First, when cells are exposed to hypertonicity, they shrink and undergo an RVI response mediated initially by salt uptake (4), which increases intracellular ionic strength. If cells with elevated cytoplasmic ionic strength swell and then volume regulate by losing organic osmolytes, ionic strength will remain elevated. The use of organic osmolytes for RVD by cells that experience repetitive periods of shrinking and swelling could conceivably cause electrolytes to rise to damaging levels. Intertidal organisms and cells in the renal medulla, for example, may be exposed to repetitive, short-term changes in extracellular osmolality during tidal shifts and during changes in urinary concentrating ability, respectively.

Coordinated regulation of electrolyte and organic osmolyte efflux pathways might also be beneficial during RVD following cell swelling brought about by net salt influx, which is referred to as “isotonic swelling.” Swelling induced by intracellular salt accumulation occurs during abrupt shifts in transmembrane ion transport and can occur under normal and pathophysiological conditions. Loss of organic osmolytes following isotonic swelling induced by inorganic ion accumulation would cause cytoplasmic ionic strength to rise further. Therefore, a mechanism that allows cells to monitor electrolyte levels and control the relative amounts of organic osmolytes and inorganic ions lost during RVD would allow the simultaneous regulation of volume and intracellular ionic strength.

In addition to regulating organic osmolyte efflux, ionic strength may also regulate organic osmolyte accumulation. Cell shrinkage increases the transcription of genes coding for organic osmolyte transporters and enzymes involved in their synthesis (1, 3). The rise in ionic strength brought about by shrinkage-induced water loss and/or by activation of RVI electrolyte accumulation pathways has been proposed by Burg and co-workers (1, 3) to play a key role in regulating the expression of these genes. Thus changes in cytoplasmic ionic strength may coordinate the activities of organic osmolyte accumulation and loss pathways.

Parker and co-workers (18, 19) have proposed that there is a common volume sensor and signaling system that coordinates the activity of several different volume-sensitive transporters in dog red blood cells. This hypothesis is based on observations that the volume sensitivity of three different ion transport pathways is modulated in a coordinated fashion by a variety of different experimental manipulations (18). Parker and co-workers have also postulated that the volume signal is a change in intracellular macromolecular crowding (11, 17, 19) and has suggested that ionic strength alters the association of a putative regulatory protein with various transport pathways (19). The mechanism by which ionic strength modulates VSOAC volume set point is unknown. Indeed, there is little understanding of how VSOAC senses cell volume and how the volume signal is transduced into channel activation (15, 21). It is important to emphasize here that channel activation is not triggered by a swelling-induced reduction in intracellular ionic strength. As shown in Fig. 6, VSOAC can be activated in the absence of osmotically driven water influx simply by placing positive pressure on the patch pipette and forcing fluid into the cytoplasm, thereby expanding cell volume. Clearly, under such conditions, there is no change in cytoplasmic ionic strength. Although swelling-induced reductions in ionic strength may modulate VSOAC, other events associated with cell swelling must be involved in channel activation.

Mechanosensitivity has been invoked as playing an important role in regulating volume-sensitive channels (5, 15) and the KCl cotransporter (7). As described by
Hamill and McBride (5), two broad classes of mechanisms have been implicated in conferring mechanosen-
sitivity. These mechanisms are referred to as the “tethered model” and the “bilayer model.” The tethered model envisions direct interactions between cytoskele-
tal proteins and proteins that comprise the transport pathway or its regulatory machinery. Changes in cyto-
skeletal tension or cytoskeletal-protein interactions induced by cell swelling could activate transport path-
ways directly or indirectly via changes in the activity of signal transduction pathways. The bilayer model arose from studies with purified and recombinant proteins reconstituted into artificial lipid membranes (16, 23). Cell swelling may induce changes in bilayer tension that activate the transport pathway through tension-
induced changes in protein conformation (5).

Our ability to activate VSOAC in shrunken cells (Figs. 4 and 5) argues against a role for swelling-
induced changes in bilayer mechanical properties. How-
ever, changes in cytoskeletal mechanical properties could be involved. It has been suggested that VSOAC activation is modulated by F-actin. Levitán et al. (9) demonstrated that disruption of F-actin with cytochala-
sin B or stabilization with phalloidin increased and decreased, respectively, the rate of VSOAC activation when cells were swollen slowly in the presence of small osmotic gradients. At higher rates of swelling, these compounds had no effect on channel activity. Using a different experimental protocol, Zhang et al. (24) have shown that cytochalasin B and phalloidin both inhibit VSOAC activation (10, 24). Cell swelling, via unknown mechanisms, has been shown to disrupt F-actin (9, 10, 15). Interestingly, F-actin can also be disrupted by reductions in ionic strength (12, 20). If a critical level of actin disassembly is required to trigger VSOAC activation, then it is possible to imagine that, in the presence of low intracellular ionic strength, less swelling is needed to reach that critical level. Under conditions of very low ionic strength, sufficient F-actin disassembly may occur to trigger VSOAC activation without swelling or even to trigger it in shrunken cells (see Fig. 5). Extensive studies using electrophysiological, biophysical, and molecular approaches will be needed to test this hypothesis.

This work was supported by National Institutes of Health Grants NS-30591 and DK-51610. C. L. Cannon was supported in part by National Institutes of Health training grant T32-HL-0763-3 and Grant CANNON 98DO from the Cystic Fibrosis Foundation. Address for reprints: K. Strange, Vanderbilt University Medical Center, Dept. of Anesthesiology, 504 Oxford House, 1313 21st Ave. South, Nashville, TN 37232-4125.

Received 2 March 1998; accepted in final form 6 May 1998.

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