

Regulation of swelling-activated chloride channels in embryonic chick heart cells

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ABSTRACT

Swelling-activated Cl⁻ currents, $I_{(Cl,swell)}$, were measured during hyposmotic shock in white Leghorn embryonic chick heart cells using the whole-cell recording of patch-clamp technique. Genistein, an inhibitor of protein tyrosine kinase (PTK), suppressed $I_{(Cl,swell)}$. Under isosmotic condition phorbol 12-myristate 13-acetate (PMA), an activator of PKC, elicited the Cl⁻ current similar to that in hyposmotic solution, whereas hyposmotic shock did not elicit $I_{(Cl,swell)}$ in chelerythrine chloride (an inhibitor of PKC)-treated cells. Confocal microscopy experiments using FITC-phalloidin as a fluorescent label of F-actin showed that the actin network was moved from cortical region of the cell to the center after hyposmotic shock as compared with the image under isosmotic condition. When the cells were treated with cytochalasin B (CB) or cytochalasin D (CD) under isosmotic condition the disruption of the F-actin integrity was observed, and $I_{(Cl,swell)}$ was not elicited. With combination treatment of CB with PMA, hyposmotic solution could not elicit $I_{(Cl,swell)}$. The results suggested that the role of PTK, probably receptor tyrosine kinase, for regulation of $I_{(Cl,swell)}$ appeared to be at upstream site related to the role of F-actin. Then PKC signal pathway was activated somehow and finally change in the polymerization state of cytoskeleton led to activate the swelling-activated Cl⁻ channels. These results demonstrate clearly that PTK, PKC and F-actin are important factors for regulation of $I_{(Cl,swell)}$ in embryonic chick heart cells as compared with often controversial results reported in different cell types.

Key words: *swelling activation, myocardium, chloride current, F-actin, phosphorylation.*

INTRODUCTION

The mechanosensitive processes in the cell after mechanical stimulation are implicated in a lot of physiological and pathological processes[1]. For example, exposing to hyposmotic media will induce most of cells to swell initially and then subsequently regulate their volume towards normal level by stimulating the efflux of osmotically active solutes[2]. The

mechanical force generated by blood flow can remodeling of the endothelial layer and plays an important role in inhibition of apoptosis of human endothelial cells[3]. Activation of the volume-regulated Cl⁻ channel during cell swelling has been suggested to be associated with cell volume regulatory processes. Besides, the swelling-activated Cl⁻ currents ($I_{(Cl,swell)}$) also affect the action potential in cardiac cells and thereby modulate the rhythmic cardiac electrical activity, and may contribute to the genesis of arrhythmia under pathological conditions[4].

Several articles reviewed the properties of volume-regulated Cl⁻ channel and the possible regulation of $I_{(Cl,swell)}$ [5,6]. The cytoskeleton, protein ty-

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rosine kinase (PTK), and protein kinase C (PKC) might involve in the regulation of this process. It has to be stressed that the effects of these factors on $I_{(Cl,swell)}$ are often controversial in different cell types. Disruption of F-actin, a specific component of the cytoskeleton, with cytochalasin B (CB), an inhibitor of actin polymerization, has been shown to abolish regulatory volume decrease (RVD) in PC12 cells[7] and gallbladder epithelial cell[8]. Therefore, an intact F-actin network was considered to be critical for normal volume regulatory response in these cells. However, $I_{(Cl,swell)}$ in human endothelial cells was not affected by F-actin disruption[9], and F-actin disassembly in B-lymphocyte was not essential for activation of $I_{(Cl,swell)}$ [10]. So, the functional relationship between F-actin and $I_{(Cl,swell)}$ is still not clear.

Phosphorylation has been shown to modulate $I_{(Cl,swell)}$ in some cell types[11], and cAMP-dependent protein phosphorylation is both necessary and sufficient to prevent development of $I_{(Cl,swell)}$ during chick heart cell swelling[12]. The role of PKC for activation of $I_{(Cl,swell)}$ is rather obscure. For example, PKC stimulated $I_{(Cl,swell)}$ in canine atrial myocytes, but inhibited $I_{(Cl,swell)}$ in rabbit myocardial cells[13], [14].

Tyrosine kinase was thought to be a candidate involved in activation of $I_{(Cl,swell)}$. It has been shown that hypotonic stimulation induced a rapid increase in tyrosine phosphorylation of several proteins[15]. In dog atrial myocytes, tyrosine phosphorylation inhibitor can prevent activation of $I_{(Cl,swell)}$ [16]. These data indicated that the activation of tyrosine kinase would be one of the early events in osmosensing.

We have demonstrated and characterized a swelling-activated Cl^- current in white Leghorn embryonic chick heart cells[17]. The current was suppressed by 4', 4'-diisothiocyanostibene-2, 2'-disulphonic acid (DIDS), a blocker of Cl^- channels. In present study, we examined the regulation effects of F-actin, PKC and protein tyrosine kinase on the activation of $I_{(Cl,swell)}$ in this cell type.

MATERIALS AND METHODS

Cell preparation

Fertilized eggs, SPF grade, were obtained from Shanghai Institute of Biological Products, Ministry of Public Health, China. Primary cultures of embryonic chick heart cells were performed

according to the method described previously[18]. Chick embryos of 14 or 16-day-old were extirpated from the eggs, the heart was mechanically minced and then digested at 37°C in sterilized D-Hanks' solution containing 0.20 %-0.25 % trypsin. The dispersed cells were pooled and centrifuged in the presence of 10% bovine serum at 100 × g for 5 min. The cells were resuspended in M-199 culture medium and centrifuged again to wash out the remaining trypsin. The cells were then placed in a 100 mm plastic culture dishes for 2 h to allow the fibroblasts to adhere on the plate. The heart cells that remained in suspension were plated in 35 mm plastic culture dishes at a density of $(2-5) \times 10^5$ cells/ml and then were kept in a CO₂ incubator at 37°C and over night.

Whole-cell current measurements

Current recording of $I_{(Cl,swell)}$ in embryonic chick heart cells cultured 24 h was performed using whole-cell configuration of the patch-clamp technique. Soft glass patch pipettes were prepared by pulling capillary tubes in two steps with a vertical puller (Narishige PP-83). Patch pipettes were filled with the solution containing (in mM): CsCl 30, caesium aspartate 110, MgCl₂ 2.0, CaCl₂ 0.5, egtazic acid 1.0, and HEPES 10 (adjusted to pH 7.2 with CsOH). The resistances of electrodes filled with the solution were 4-5 MW. Isotonic bath solution of 300 mOsm was a medium containing (in mM): NaCl 121.5, sodium aspartate 21.5, KCl 5.4, NaH₂PO₄ 0.8, HEPES 10, MgSO₄ 0.8, CaCl₂ 1.0, and dextrose 5.6 (adjusted to pH 7.4 with NaOH). Hypotonic solution of 270 mOsm was obtained just by omitting sodium aspartate from isotonic solution. The solution osmolarities were measured with a vapor pressure osmometer (Model 5520, Wescor Inc, Logan, UT, USA), and appropriate amount of sugar was used for adjustment of solution osmolarities. CB and cytochalasin D (CD) (Sigma) were dissolved in dimethyl sulfoxide (DMSO) as stock solution and were diluted to their final concentration of 30 mM. Final concentration of DMSO was less than 1%. Phalloidin-FITC (Sigma) was dissolved in phosphate-buffered saline (PBS, pH 7.2). Phorbol 12-myristate 13-acetate (PMA) and chelerythrine chloride were obtained from Sigma They were dissolved in DMSO as stock solution of 0.5 mM for late use.

All current signals were recorded with an EPC-7 patch-clamp amplifier (List Electronic, Germany) operated in the voltage-clamp mode. Step voltage commands, data acquisition and analysis were performed with pClamp 6.01 software (Axon Instruments, USA). No leakage subtraction was performed to the original recordings, and all cells with visible changes in leakage current during course of study were excluded from further analysis. Capacitive transients were compensated optimally. Data were filtered at 3 KHz.

Data were expressed as mean ± SEM and statistical analysis was performed using Student's t-test.

Confocal imaging

Confocal imaging of FITC-phalloidin was used to specifically show labeled F-actin network in the cell. For treatment of the cells with CB or CD, cultured embryonic chick heart cells were first incubated in PBS containing 30 μM CB or CD and 0.1 % Triton

X-100 for 1 h at 37 °C, then rinsed in PBS for 3 times at least. The cells were stabilized with 3.7 % formaldehyde in PBS for 20 min at least and rinsed again with PBS. Finally, the cells were incubated with 30 μ M FITC-phalloidin for 1 h at 37°C in CO₂ incubator. Fluorescent-labeled cells were examined using a Leica TCS NT confocal system with a 40 × objective lens fitted on a Leica DMRXA microscope.

RESULTS

Activation by osmotic swelling

In an isotonic solution, a depolarizing pulse from a holding potential of -40 mV to 80 mV elicited a basal outward Cl⁻ current. The currents increased from 452 ± 60 pA to 849 ± 112 pA ($n = 11$ cells, $P < 0.05$) after perfusion with hyposmotic solution within 2 min and recovered to nearly initial control value when the osmolarity of solution was returned to isotonic level. The current-voltage relationship indicated the characterization of outwardly rectifying current which we have characterized previously[17]. The outward current was more effectively activated than inward current, which was similar to the Cl⁻ current demonstrated by Hall et al[19]. We have demonstrated that this current is swelling-activated Cl⁻ current, and its behavior of response to chlorpromazine, a kind of amphipathic drug which can mimic the mechanical force, is different from that of mechanosensitive ion channels in *Escherichia coli*[17].

Inhibition by genistein

Genistein, an inhibitor of PTK, is often used to investigate the role of PTK in cell signal transduction[20]. In our experiment, when the cells were perfused with hyposmotic solution containing 50 mM genistein (Sigma) at room temperature no $I_{(Cl,swell)}$ was elicited. The currents before and after perfusion were recorded from the same cell. The currents for the cells under isotonic condition and in hyposmotic solution containing genistein were 438 ± 31 pA and 414 ± 34 pA, respectively ($n = 10$ cells, $P > 0.05$) (Fig 1). The $I_{(Cl,swell)}$ was elicited again after application of hyposmotic solution without genistein (Fig 1C). It is suggested that PTK was involved in the activation of $I_{(Cl,swell)}$ in white Leghorn chick heart cells.

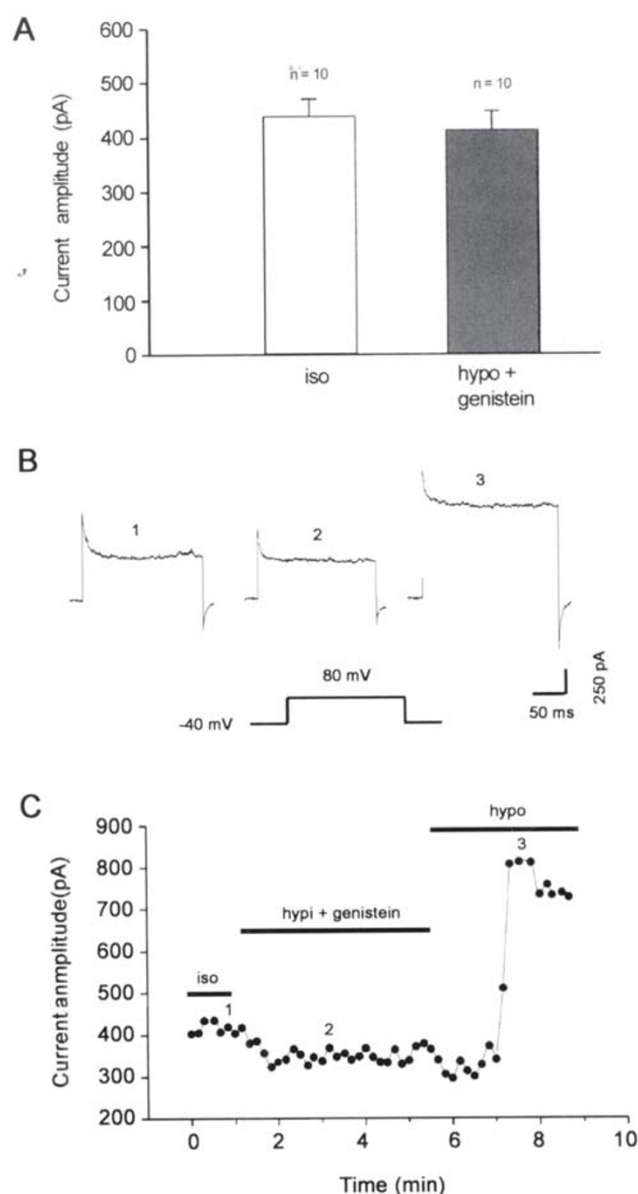


Fig 1. Suppression of $I_{(Cl,swell)}$ by genistein. Currents were evoked by 150 ms constant depolarizing pulse from a holding potential of -40 mV to 80 mV. **A.** The current recorded from the cells after perfusion with 50 mM genistein in hyposmotic solution (hypo) was not significantly different from the background level under isotonic condition (iso). Data were obtained from ten cells. **B.** The typical current traces under isotonic condition (curve 1), hyposmotic condition + genistein (curve 2), and hyposmotic condition alone after washing genistein out (curve 3). **C.** The time course of currents showed that $I_{(Cl,swell)}$ was inhibited in the presence of 50 μ M genistein in hyposmotic solution, and the current was activated again when genistein in the solution was washed out with hyposmotic solution.

Regulation by PKC

In order to understand the role of PKC in activation of $I_{(Cl,swell)}$, we investigated the effects of phorbol 12-myristate 13-acetate (PMA), an activator of PKC, and chelerythrin chloride, an inhibitor of PKC, on activation of $I_{(Cl,swell)}$. Under isosmotic conditions, 250 nM PMA in the pipette solution increased the current from 452 ± 60 pA ($n = 11$ cells) to 851 ± 59 pA ($n = 8$ cells) significantly ($P < 0.05$) (Fig 2). In other experiment, we observed that no significant additional current was elicited in hyposmotic solution when 10 mM chelerythrin chloride was in the pipette solution. The currents were 493 ± 20 pA ($n = 7$ cells) and 452 ± 60 pA ($n = 11$

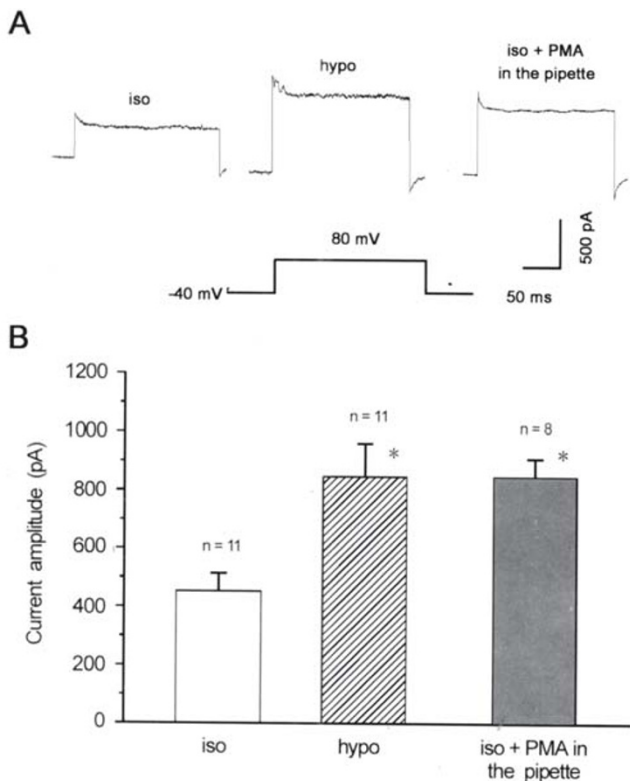


Fig 2. Elicitation of $I_{(Cl,swell)}$ by PMA under isosmotic condition. Currents were evoked by 150 ms constant depolarizing pulse from a holding potential of -40 mV to 80 mV. **A.** The typical current traces under isosmotic condition (left), hyposmotic condition (middle), and isosmotic condition + PMA in the pipette (right). **B.** The average currents recorded from the cells under isosmotic condition (eleven cells), hyposmotic condition (eleven cells), and isosmotic condition + PMA in the pipette (eight cells) showed that under isosmotic condition PMA could elicit the same Cl⁻ current as that activated by hyposmotic swelling. * $P < 0.05$ vs isosmotic control.

cells) for the cells treated by chelerythrin chloride and control, respectively (Fig 3). The results showed that PKC was essential in activation of $I_{(Cl,swell)}$ in white Leghorn chick heart cells.

Regulation by F-Actin

The cytoskeleton is involved in transduction of extracellular mechanical signals to intracellular chemical signal and transduces signals via changing its physical shape. In our experiments, the F-actin network, visualized by F-actin labeled with FITC-phalloidin, under isosmotic condition appeared as a shell underneath the plasma membrane (Fig 4).

After hyposmotic shock, labeled region moved from the cortical region of the cell to the center. When the cells were treated with 30 μ M CB or 30 μ M CD

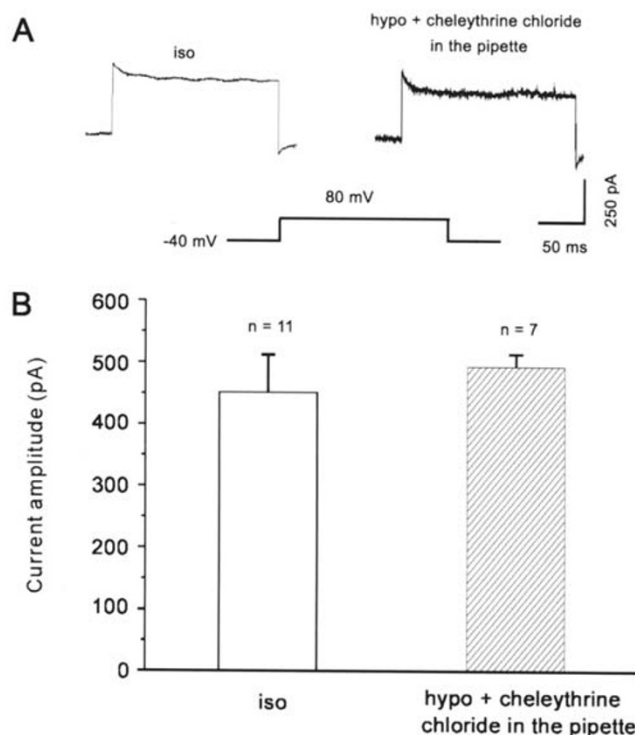


Fig 3. Suppression of $I_{(Cl,swell)}$ by chelerythrin chloride. Currents were evoked by 150 ms constant depolarizing pulse from a holding potential of -40 mV to 80 mV. **A.** The typical current traces under isosmotic condition (left) and hyposmotic condition + chelerythrin chloride in the pipette (right). **B.** The average currents recorded from the cells under isosmotic condition (eleven cells) and hyposmotic condition + chelerythrin chloride in the pipette (seven cells) showed that chelerythrin chloride could suppress $I_{(Cl,swell)}$ under hyposmotic condition.

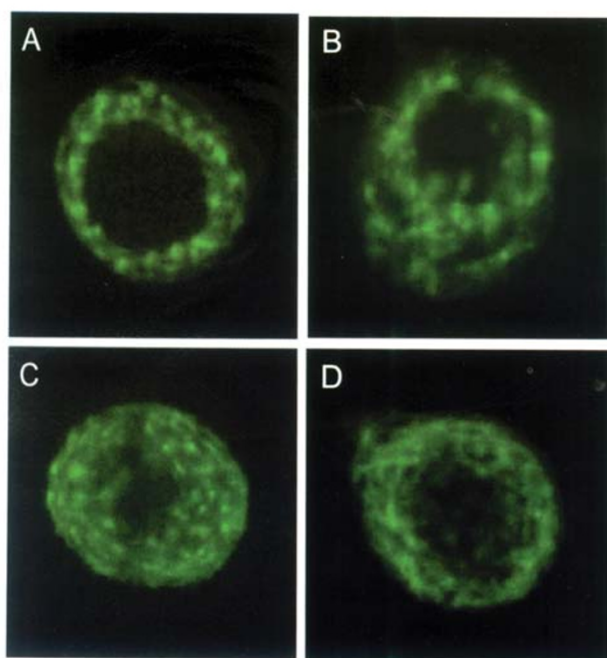


Fig 4. Morphological changes of F-actin by hyposmotic shock, CB or CD treatments. Panels A and B show the fluorescence confocal images of the control under isosmotic condition and the cells exposed to an hyposmotic solution, respectively. Panels C and D show the images of the cells treated with 30 mM CB and 30 μ M CD, respectively. 2 μ m

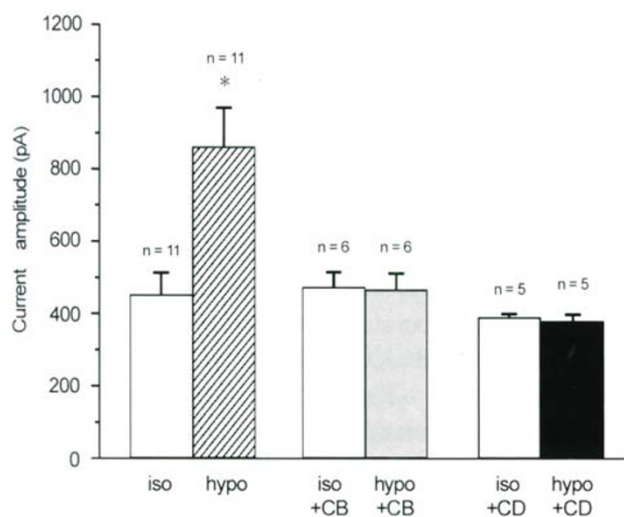


Fig 5. Suppression of $I_{(Cl,swell)}$ by CB and CD. Currents were evoked by 150 ms constant depolarizing pulse from a holding potential of -40 mV to 80 mV. The average currents recorded from the cells of control (eleven cells), CB treatment (six cells), and CD treatment (five cells) showed that CB or CD can not elicit $I_{(Cl,swell)}$ under both isosmotic and hyposmotic conditions. * $P < 0.05$ vs isosmotic control

under isosmotic condition and incubated for 1 h at 37 °C, disruption of the F-actin integrity was observed, and the picture was similar to that of cells treated by hyposmotic shock. This suggested that the state of depolymerization of F-actin or disruption of microfilaments would be involved in physiological response during cell swelling. Furthermore, it was observed that the hyposmotic solution could not elicit $I_{(Cl,swell)}$ after depolymerization of F-actin by using of CB or CD (Fig 5). In the CB treated cells, the currents were 475 ± 41 pA ($n = 6$ cells) and 468 ± 44 pA ($n = 6$ cells) in isosmotic and hyposmotic solution, respectively. In the CD treated cells, the currents were 391 ± 10 pA ($n = 5$ cells) and 377 ± 18 pA ($n = 5$ cells) in isosmotic and hyposmotic solution, respectively. There were no significant differences among the paired groups ($P > 0.05$). The results indicated that integrity of F-actin is necessary for activation of $I_{(Cl,swell)}$. Here the characterization of F-actin integrity also refers to the dynamic structure of assembly and disassembly of the microfilaments in the cells.

Relationship between F-actin and PKC

In order to understand clearly which of F-actin and PKC is the earlier or later response factor in the current activation, CB and PMA were applied to the cells simultaneously. The cells were pretreated with 30 μ M CB for 1 h in the isosmotic solution, and then the currents were recorded using 250 nM PMA-containing pipettes. It was found that PMA-induced increase in the current was not observed (Fig 6), implying that the role of cytoskeleton would be at the downstream site related to the role of PKC during the activation of $I_{(Cl,swell)}$.

DISCUSSION

Activation of the swelling-activated Cl channels during hyposmotic shock is critical to RVD. Such Cl channels were already partially activated under isosmotic condition. This basal Cl conductance contributes to the resting potential of the cell[5]. Although it is now known that many important physiological processes in the cell are related to the activation of $I_{(Cl,swell)}$, what hyposmotic sensor is in the cell's response to hyposmotic shock is still an interesting question. Some special molecules which sense mechanical

stimulation are well known in hair cells or on skin [21],[22]. The mechanism of activation of $I_{(Cl,swell)}$ in myocardial cells, however, is not yet clear.

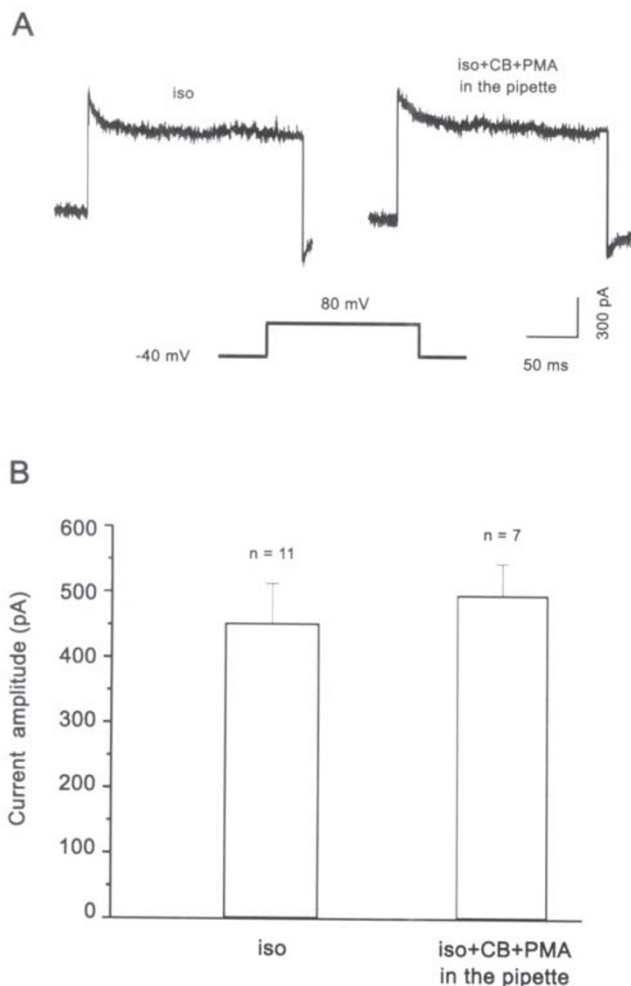


Fig 6. Suppression of $I_{(Cl,swell)}$ by combination of CB with PMA. Currents were evoked by 150 ms constant depolarizing pulse from a holding potential of -40 mV to 80 mV. **A.** The typical current traces were recorded for the control (left) and combination treatment of 30 μ M CB with 250 nM PMA in the pipette (right) under isosmotic conditions. **B.** The average currents recorded from the cells of control (eleven cells) and CB + PMA in the pipette (seven cells) showed that PMA can not elicit $I_{(Cl,swell)}$ in the presence of CB under isosmotic condition (as compared with Fig 2).

Although many signal molecules were activated during hyposmotic stress, it is not clear which molecule is the initially activated and is hyposmotic sensor. Our result showed that $I_{(Cl,swell)}$ was suppressed by genistein under hyposmotic condition (Fig 1), showing that protein tyrosine phosphoryla-

tion was required in activation of $I_{(Cl,swell)}$ of white Leghorn chick cardiac myocytes. It has been reported that hyposmotic stress activated PTK within 5 s in mouse cardiac myocytes, and while activation of other kinases peaked from 5 min to 15 min[15]. It was proposed that the molecules sensitive to hyposmotic stress must be transmembrane molecules which can sense membrane tension caused by hyposmotic stress. So we can make a presumption that a certain receptor tyrosine kinase would be the earliest cellular response molecule in the cardiac myocytes.

The role of PKC in regulation of $I_{(Cl,swell)}$ is obvious in our experiment. PMA, an activator of PKC, mimicked the $I_{(Cl,swell)}$ under isosmotic condition (Fig 2), whereas chelerythrine chloride, an inhibitor of PKC, blocked the $I_{(Cl,swell)}$ (Fig 3). This means that PKC is necessary in activation of $I_{(Cl,swell)}$ in white Leghorn chick heart cells. It can be inferred that PKC phosphorylated either the Cl⁻ channels or the regulatory proteins in the heart cells, which then induced gating of the swelling-activated Cl⁻ channels.

The change in assembly state of the cytoskeleton network was required for RVD in many cells[23]. However, the effects of various cytoskeletal modifiers on $I_{(Cl,swell)}$ gating are heterogeneous and controversial[5]. By means of confocal microscopy, we observed that the F-actin network of chick heart cells was dispersed from cortical region of the cells to the center under hyposmotic condition (Fig 4A, B). That is, a shell-like labeled F-actin network underneath the plasma membrane appears to be broken by hyposmotic shock. Similar pictures were also observed after treatment of the cells with CB or CD under isosmotic conditions, which depolymerized the F-actin and disrupted microfilament of the cytoskeleton (Fig 4C, D). These results suggested that hyposmotic stress can change the assembly state of F-actin and disruption of the microfilaments to a certain extent, which might be associated with $I_{(Cl,swell)}$. However, no $I_{(Cl,swell)}$ of white Leghorn heart cells was activated after treatment with CB and CD (Fig 5). So it would be reasonable to make a suggestion that cytoskeleton integrity is essential for activation of $I_{(Cl,swell)}$. The similar results in other cells have been reported. In B-lymphocyte, no $I_{(Cl,swell)}$ was recorded after F-actin integrity was disrupted by application of CB[9]. Sup-

pression of RVD by CB was also observed in cultured PC12 cells[24], *Necturus maculosus* gallbladder epithelial cells[8] and isolated axons of the green crab *Carcinus maenas*[25]. It was also reported that the either stabilization or depolymerization of F-actin by chemical reagents suppressed $I_{(Cl,swell)}$ in chick heart cells[26]. Together with these observation, it can be deduced that the activation of $I_{(Cl,swell)}$ would be accompanied by the dynamic transition of the “polymerization \leftrightarrow depolymerization” state of F-actin. The another possibility for interpretation of the morphological changes shown in Fig 4 after hyposmotic shock would be due to a redistribution of the cytoskeleton. But both the cases the integrity of F-actin was different from the control, and lack of F-actin integrity suppresses $I_{(Cl,swell)}$.

The pathway of signal transduction of activation of $I_{(Cl,swell)}$ is still unknown. Our results showed PTK, PKC and cytoskeleton were involved in the activation of $I_{(Cl,swell)}$ in white Leghorn embryonic chick heart cells. In order to study the relationship between PKC and F-actin in the activation pathway we examined the effect of PMA together with CB on the activation of Cl^- current under isosmotic condition. The result showed that no significant current was activated as compared with the control (Fig 6), which was different from the activation effect of PMA alone under isosmotic condition (Fig 2). This means that activation of PKC by PMA under the condition of depolymerization of F-actin can't elicit Cl^- current any longer. The result suggested that the role of F-actin for regulation of $I_{(Cl,swell)}$ in the signaling pathway would be at downstream site related to the role of PKC. For stress-activated or mechanically gated channels several studies have shown that the cytoskeleton directly interacts with the channel protein and thus can intrinsically sense the cell stretch[1],[27]. Swelling-activated Cl^- channels would be also like this.

In conclusion, the activation of PTK, probably receptor tyrosine kinase, appeared to be an earlier cellular response to hyposmotic shock in white Leghorn embryonic chick heart cells. PKC and F-actin are also important factors for regulation of the process. The change in the polymerization state of cytoskeleton would finally led to activate the swelling-activated Cl^- channels.

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REFERENCES

- [1] Hamill OP, Martinac B. Molecular basis of mechanotransduction in living cells. *Physiol Rev* 2001; **81**:685-740.
- [2] Manolopoulos V, Voets T, Declercq R, Droogmans G, Nilius B. Swelling-activated anion channels mediate efflux of taurine and other organic osmolytes in endothelial cells. *Am J Physiol* 1997; **273**:C214-22.
- [3] Geng YJ. Molecular signal transduction in vascular cell apoptosis. *Cell Res* 2001; **11**:253-64.
- [4] Harvey RD. Cardiac chloride channels. *News Physiol Sci* 1994; **11**:175-80.
- [5] Nilus B, Eggermont J, Voets T, Buyse G, Manolopoulos V, Droogmans G. Properties of volume-regulated anion channels in mammalian cells. *Prog Biophys Molec Biol* 1997; **68**:69-119.
- [6] Weber WM. Endogenous ion channels in oocytes of *Xenopus laevis*: recent developments. *J Membrane Biol* 1999; **170**:1-12.
- [7] Cornet M, Ubl J, Kolb HA. Cytoskeleton and ion movements during volume regulation in cultured PC 12 cells. *J Membrane Biol* 1993; **133**:161-70.
- [8] Foskett JK, Spring KR. Involvement of calcium and cytoskeleton in gallbladder epithelial cell volume regulation. *Am J Physiol* 1985; **248**:C27-36.
- [9] Oike M, Schwarz G, Seherer J et al. Cytoskeletal modulation of the response to mechanical stimulation in human vascular endothelial cells. *Pflügers Arch* 1994; **428**:569-74.
- [10] Levitan I, Almonte C, Mollard P, Garber SS. Modulation of a volume-regulated chloride current by F-actin. *J Membrane Biol* 1995; **147**:283-94.
- [11] Valverde MA, Bond TD, Hardy SP et al. The multidrug resistance P-glycoprotein modulates cell regulatory volume decrease. *EMBO J* 1996; **15**:4460-8.
- [12] Sadoshima J, Izumo S. Mechanical stretch rapidly activates multiple signal transduction pathways in cardiac myocytes: potential involvement of an autocrine/paracrine mechanism. *EMBO J* 1993; **12**:1681-92.
- [13] Du XY, Sorota S. Protein kinase C stimulates swelling-induced chloride current in canine atrial cells. *Pflügers Arch* 1999; **437**:227-34.
- [14] Dunn D, Fermini B, Nattel S. Alpha-adrenergic control of volume-regulated Cl^- currents in rabbit atrial myocytes. Characterization of a novel ionic regulatory mechanism. *Circ Res* 1995; **77**:379-93.
- [15] Sadoshima J, Qiu Z, Morgan J P, Izumo S. Tyrosine kinase activation is an immediate and essential step in hypotonic cell swelling-induced ERK activation and *c-fos* gene expression in cardiac myocytes. *EMBO J* 1996; **15**:5535-46.
- [16] Sorota S. Tyrosine protein kinase inhibitors prevent

- activation of cardiac swelling-induced chloride current. *Pflugers Arch* 1995; **431**:178-85.
- [17] Wei H, Mei YA, Wu MM, Sun JT, Zhou HQ, Zhang ZH. Swelling-activated chloride currents in embryonic chick heart cells. *Acta Pharmacol Sin* 2000; **21**:986-90.
- [18] Mei YA, Huang LX, Wei H, Sun JT, Zhou HQ, Zhang ZH. Characterization of outward potassium current in embryonic chick heart cells. *Acta Pharmacol Sin* 1999; **20**:303-7.
- [19] Hall SK, Zhang J, Lieberman M. Cyclic AMP prevents activation of a swelling-induced chloride-sensitive conductance in chick heart cells. *J Physiol* 1995; **488**:359-69.
- [20] Valerie C, William P, Melanie EMK, Brian AM. Mitogen-activated protein and tyrosine kinases in the activation of astrocyte volume-activated chloride current. *J Neurosci* 1998; **18**:1196-206.
- [21] Carcia-Anoveros J, David PC. The molecules of mechanosensation. *Annu Rev Neurosci* 1997; **20**:567-94.
- [22] Gillespie PG, Walker RG. Molecular basis of mechanosensory transduction. *Nature* 2001; **413**:194-202.
- [23] Schwiebert EM, Mills JW, Stanton BA. Actin-based cytoskeleton regulates a chloride channel and cell volume in a renal cortical collecting duct cell line. *J Biol Chem* 1994; **269**:7081-9.
- [24] Cornet M, Lambert IH, Hoffmann EK. Relation between cytoskeleton, hypo-osmotic treatment and volume regulation in Ehrlich ascites tumor cells. *J Membrane Biol* 1993; **131**:55-66.
- [25] Gilles R, Delpire E, Duchene C, Cornet M, Pequeux A. The effect of cytochalasin B on the volume regulation response of isolated axons of the green crab *Carcinus maenas* submitted to hypo-osmotic media. *Comp Biochem Physiol* 1986; **85A**:523-5.
- [26] Zhang J, Larsen TH, Lieberman M. F-actin modulates swelling-activated chloride current in cultured chick cardiac myocytes. *Am J Physiol* 1997; **273**:C1215-24.
- [27] Ingber DE. Tensegrity: The architectural basis of cellular mechanotransduction. *Annu Rev Physiol* 1997; **59**:575-99.