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Swelling-activated chloride currents in embryonic chick heart cells[1]

WEI Hua, MEI Yan-Ai, WU Ming-Ming, SUN Jia-Ting, ZHOU Han-Qing, ZHANG Zhi-Hong[2]
(*Department of Physiology and Biophysics, Liren Laboratory, Fudan University, Shanghai 200433, China*)

KEY WORDS swelling activation; chick embryo; myocardium; chloride channels; patch-clamp techniques; chlorpromazine

ABSTRACT

AIM: To characterize a swelling-activated chloride current, $I_{(Cl, swell)}$, in white *Leghorn* chick heart cells and the effects of chlorpromazine (CPZ) effects. **METHODS**: The patch-clamp technique in the whole-cell configuration was used. **RESULTS**: Hyposmotic swelling elicited $I_{(Cl, swell)}$ in white *Leghorn* chick heart cells. The current amplitude increased from (452 ± 200) pA to (849 ± 373) pA with a reduction of osmolarity from 300 mmol · L^{-1} to 270 mmol · L^{-1}. 4′, 4-Diisothiocyanostilbene-2, 2′-disulphonic acid (DIDS) 100 μmol · L^{-1} decreased $I_{(Cl, swell)}$ from (1196 ± 505) pA to (830 ± 328) pA in hyposmotic solution. In white Leghorn chick heart cells $I_{(Cl, swell)}$ was not induced by CPZ 30 μmol · L^{-1}, which is different from the case of *E coli* spheroplast. **CONCLUSION**: Swelling-activated chloride current was activated by hyposmotic swelling in white *Leghorn* chick heart cells. The mechanism for activating the current is different from that of mechanosensitive ion channels of *E coli*.

INTRODUCTION

Cell volume homeostasis is fundamentally important to maintain the functional and structural integrity of all living cells[1]. Having been exposed to hyposmotic media most of cells swell initially and then subsequently regulate their volume towards normal level by stimulating the efflux of osmotically active solutes[2]. Activation of chloride channels during cell swelling has been suggested to be associated with volume regulatory processes[3]. This volume-regulated anion channels in mammalian cells involve a lot of important physiological processes such as intracellular pH regulation, vectorial transport, exocytosis, and modulation of the driving force for Ca^{2+} entry[4]. Inhibition of swelling-activated Cl^- currents, $I_{(Cl, swell)}$, suppresses cell proliferation in many cell types[5]. Besides, a role of swelling-activated Cl^- channels has been proposed for maintaining the normal lens hydration and transmittance[6]. $I_{(Cl, swell)}$ also affects the repolarization of the action potential in cardiac cells and modulates thereby the rhythmic cardiac electrical activity, and under pathological conditions may contribute to the genesis of arrhythmia[7].

Several observations pointed to a link between activation of $I_{(Cl, swell)}$ and dephosphorylation in chick heart cells[3]. But a little is known about the relationship between $I_{(Cl, swell)}$ and membrane deformation. In *E coli* spheroplasts, chlorpromazine (CPZ), a cationic amphipath, and trinitrophenol (TNP), an anionic amphipath, were able to increase the open probability of a single mechanosensitive channel by changing membrane curvature[8]. In the present study, we examined $I_{(Cl, swell)}$ in white *Leghorn* embryonic chick heart cells and CPZ effect on this current.

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 of Mei *et al*[9]. Chick embryos 14- or 16-d-old were extirpated from the eggs, the heart was mechanically minced and then was digested at 37 ℃ in sterilized D-Hanks' solution containing 0.20 % – 0.25 % trypsin. The dispersed cells were pooled and

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[1] Project supported by the National Natural Science Foundation of China (№ 39730150).
[2] Correspondence to Prof ZHANG Zhi-Hong.
Phn 86-21-6564-3673. Fax 86-21-6565-0149.
E-mail zhzhang@fudan.edu.cn
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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 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 $2 \times 10^8 - 5 \times 10^8$ cells·L^{-1} and then were kept in a CO_2 incubator at 37 ℃ and over night. All chemicals used were of analytical reagent grade.

Current recording and analysis Current recording of $I_{(Cl, swell)}$ in embryonic chick heart cells cultured for 24 h was performed by patch-clamp technique in the whole-cell configuration. 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 CsCl 30, caesium aspartate 110, $MgCl_2$ 2.0, $CaCl_2$ 0.5, egtazic acid 1.0, and HEPES 10 mmol·L^{-1} (pH 7.2 with CsOH). The resistances of electrodes filled with the solution were 4 – 5 MΩ. Isotonic bath solution with osmolarity 300 mmol·L^{-1} was a medium containing NaCl 121.5, sodium aspartate 21.5, KCl 5.4, NaH_2PO_4 0.8, HEPES 10, $MgSO_4$ 0.8, $CaCl_2$ 1.0, and dextrose 5.6 mmol·L^{-1} (pH 7.4 with NaOH). Hyposmotic solution with osmolarity 270 mmol·L^{-1} without change in the concentration of [Cl^-] was obtained just by omission of sodium aspartate. The solution osmolarities were measured with a vapour pressure osmometer (model 5520, Wescor Inc, Logan, UT, USA).

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). Cell membrane capacitance was estimated from the integral of the transient current response to a 5-mV hyperpolarizing clamp step.

Statistics Data were expressed as $\bar{x} \pm s$ and analyzed by paried *t*-test or unpaired *t*-test.

RESULTS

Swelling-activated chloride currents $I_{(Cl, swell)}$ was measured using whole-cell patch-clamp technique. In osmotic conditions, a depolarizing plus from a holding potential of –40 mV to 80 mV elicited a small outward current. The current began to increase from (452 ± 200) pA to (849 ± 373) pA ($n = 11$ cells, $P < 0.05$) after perfusing with hyposmotic solution and recovered nearly to initial control value when the osmolarity of solution was returned back to isotonic level. On an average, the swelling-activated outward current increased by about (95 ± 27) % over the control. An example for current traces from a typical cell exposed to hyposmotic solutions is shown in Fig 1.

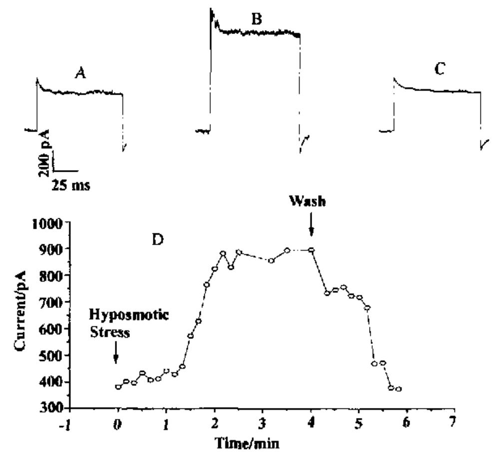

Fig 1. Activation of $I_{(Cl, swell)}$ by hyposmotic swelling. $I_{(Cl, swell)}$ was elicited by applying 150 ms 80 mV testing potential from holding potential of – 40 mV. A) whole-cell currents under isotonic conditions. B) $I_{(Cl, swell)}$ under hyposmotic conditions. C) whole-cell currents after wash-out. D) time course of $I_{(Cl, swell)}$ elicited by hyposmotic solution and washed out by isosmotic solution.

The current-voltage relations for $I_{(Cl, swell)}$ indicate the characterization of outward rectifying current and no time-dependence during the 150 ms voltage step (Fig 2). The outward current was more effectively activated than inward current, which was similar to the Cl^- current demonstrated by Hall *et al*[3].

Effect of DIDS on $I_{(Cl, swell)}$ DIDS is one of blockers of Cl^- channel and usually used to confirm the existence of Cl^- channel[10,11]. In our experiments, DIDS 100 μmol·L^{-1} decreased the $I_{(Cl, swell)}$ from the control value of (1196 ± 505) pA to (830 ± 328) pA ($n = 9$, $P < 0.05$). The current was decreased to about 30 % of the control, and was partly reversible after DIDS was washed out (Fig 3). The results supported that the

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hyposmotically activated current was mainly carried by Cl^- ions. However, $I_{(Cl, swell)}$ in white *Leghorn* embryonic chick heart cells was less sensitive to DIDS as compared with the *Xenopus* oocytes and rat osteoblast-like cells[10,11].

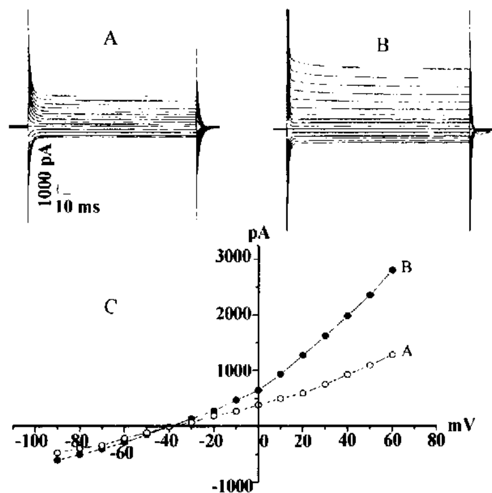

Fig 2. Current-voltage relations under isotonic and hyposmotic conditions. $I_{(Cl, swell)}$ was elicited by a series of 150 ms voltage steps from − 90 mV to 60 mV at 10 mV increment. A) Whole-cell currents under isotonic conditions. B) $I_{(Cl, swell)}$ under hyposmotic conditions. C) Current-voltage relationships of $I_{(Cl, swell)}$.

CPZ effect on $I_{(Cl,swell)}$ CPZ is a kind of amphipathic drug, which can insert into the inner leaflet of the membrane lipid bilayer and cause membrane deformation. It was reported that the insertion of CPZ resulted in activation of mechanosensitive channels of *E coli* and hereby mimicked the effect of hyposmotic stress[8]. In our experiment, no significant increase in current was activated although the cells incubated with CPZ 30 μmol·L^{-1} in isotonic bath solution at 37 ℃ for 45 min did undergo membrane deformation as appeared in electronic micrographs (not shown). The chloride currents were changed from (36 ± 11) pA/pF in control (n = 11 cells) to (10 ± 4) pA/pF in present of CPZ (n = 12 cells, $P < 0.05$). In addition, for the cells incubated with CPZ 30 μmol·L^{-1} in isotonic solution $I_{(Cl,swell)}$ was also elicited after perfusion with hyposmotic solution containing the same concentration of CPZ. The chloride currents were increased from (899 ± 434) pA to (1377 ± 383) pA (n = 5 cells, $P < 0.05$). On an average, the current was increased by (66 ± 34)%. There was no significant difference between the rate of current increasing elicited by hyposmotic solution without CPZ and that elicited by hyposmotic solution with CPZ (n = 5 cells, $P > 0.05$). Fig 4 shows the typical current traces. The results suggest that CPZ can not mimic the effect of hyposmotic conditions in cultured embryonic white *Leghorn* chick heart cells as in *E coli*.

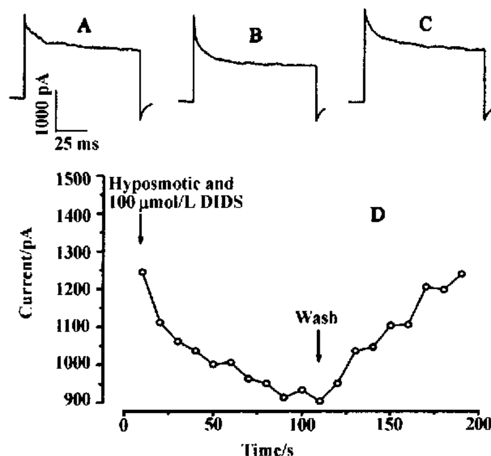

Fig 3. Effect of 100 μmol·L^{-1} DIDS on $I_{(Cl, swell)}$. A) $I_{(Cl, swell)}$ under hyposmotic conditions. B) $I_{(Cl, swell)}$ after perfusion with 100 μmol·L^{-1} DIDS. C) $I_{(Cl, swell)}$ after wash-out. D) time course of $I_{(Cl, swell)}$ suppressed by 100 μmol·L^{-1} DIDS and elicited again by hyposmotic solution.

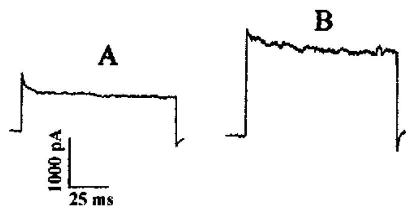

Fig 4. Whole-cell currents under isotonic and hyposmotic conditions with CPZ 30 μmol·L^{-1}. Currents were elicited by applying a 150 ms 80 mV testing potential from holding potential of − 40 mV. A) Whole-cell currents under isotonic conditions with CPZ 30 μmol·L^{-1}. B) Whole-cell currents under hyposmotic conditions with CPZ 30 μmol·L^{-1}.

DISCUSSION

Our results obtained by the whole-cell recording techniques demonstrated that there was $I_{(Cl,swell)}$, in

white *Leghorn* chick heart cells and its properties were similar to those $I_{(Cl,swell)}$, described previously[12], it was outward rectifying Cl^- current and voltage-dependent. DIDS is an specific inhibitor of the chloride transport, which can block $I_{(Cl,swell)}$ in *Xenopus* oocytes[10], rat osteoblast-like[11] and endothelial cells[4]. In these cells 75 % of $I_{(Cl,swell)}$ was suppressed by DIDS 100 μmol/L. In our experiments, $I_{(Cl,swell)}$ was also sensitive to DIDS although the inhibition was not so strong, DIDS 100 μmol/L suppressed about 30 % of $I_{(Cl,swell)}$ (Fig 3). It seems that the sensitivity of $I_{(Cl,swell)}$ to DIDS would not be the same for different type of cells. Some amphipathic compounds can insert into inner or outer leaflet of lipid bilayer and cause a change in membrane curvature, resulting in appearance of convex or concave patches on the membrane surface[8]. CPZ molecules, insert into inner leaflet of the membrane and activate the mechanosensitive channel in *E coli*[8]. It was reported that mechanical force directly opened the mechanosensitive ion channel in *E coli*[14]. CPZ can mimic the mechanical force, so it is able to active the mechanosensitive ion channel in *E coli*. It has also been observed that CPZ could induce hypotonic stress-induced c-*fos* expression in rat heart cells[13]. In the present study, although electronic micrographs showed the membrane deformation after treatment with CPZ 30 $\mu mol \cdot L^{-1}$, no significant increase of $I_{(Cl,swell)}$ was observed in white *Leghorn* heart cells. This result was different from that in *E coli*. It is reasonable to suggest that the mechanism for activation of $I_{(Cl,swell)}$ in white *Leghorn* heart cells was not the same as that of mechanosensitive ion channel in *E coli*. It has been proved that $I_{(Cl,swell)}$ of embryonic chick heart cells was caused by membrane distension[12]. The result that no additional current was elicited by CPZ suggested that more complex mechanism should exit in activation of $I_{(Cl,swell)}$ in white Leghorn chick heart cells. The detail of the mechanism is needed to be further studied.

In conclusion, hyposmotic condition activated swelling-activated chloride currents similar to those described previously. CPZ does not mimic the hyposmotic activation of $I_{(Cl,swell)}$ in white *Leghorn* heart cells. Mechanism of activation of $I_{(Cl,swell)}$ in white *Leghorn* heart cells is more complex than that of activation of mechanosensitive ion channel in *E coli* spheroplast.

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鸡胚心肌细胞的膨胀激活氯电流[1]

魏 华，梅岩艾，吴明明，孙家庭，周汉清，张志鸿[2] (复旦大学生理学和生物物理学系，立人实验室，上海 200433，中国)

关键词 膨胀激活；鸡胚；心肌；氯通道；膜片箝技术；氯丙嗪

目的：研究白羽鸡胚心肌细胞的膨胀激活氯电流及氯丙嗪对其的模拟作用. **方法**：膜片箝技术的全细胞记录模式. **结果**：低渗膨胀在白羽鸡胚心肌上诱发出一个可逆变化的氯电流，渗透压从 300 mmol·L^{-1}减小至 270 mmol·L^{-1}时，该电流从(452±200) pA 增加到(849±373) pA. DIDS 100 μmol·L^{-1}使氯电流从(1196±505) pA 减至(830±328) pA. $I_{(\mathrm{Cl,\ swell})}$不能被 CPZ 30 μmol·L^{-1}模拟出来，该结果不同于大肠杆菌原生质体的结果. **结论**：白羽鸡胚心肌细胞的膨胀激活氯通道能被低渗激活，其激活的机制与大肠杆菌的机制敏感性离子通道激活的机制不同.

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