

• BASIC RESEARCH •

Effect of actin microfilament on potassium current in guinea pig gastric myocytes

Xiang-Lan Li, Hai-Feng Zheng, Zheng-Yuan Jin, Meng Yang, Zai-Liu Li, Wen-Xie Xu

Xiang-Lan Li, Hai-Feng Zheng, Zheng-Yuan Jin, Meng Yang, Zai-Liu Li, Department of Physiology, Yanbian University College of Medicine, Yanji 133000, Jilin Province, China

Wen-Xie Xu, Department of Physiology, Shanghai Jiaotong University School of Medicine, 1954 Huashan Rd, Shanghai 200030, China

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Correspondence to: Professor Wen-Xie Xu, Department of Physiology, Shanghai Jiaotong University School of Medicine, 1954 Huashan Rd, Shanghai 200030, China. wenxiexu@sjtu.edu.cn

Telephone: +86-21-62932910 **Fax:** +86-21-62932528

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Abstract

AIM: To investigate the effect of actin microfilament on potassium current and hyposmotic membrane stretch-induced increase of potassium current in gastric antral circular myocytes of guinea pig.

METHODS: Whole-cell patch clamp technique was used to record potassium current in isolated gastric myocytes.

RESULTS: When the membrane potential was clamped at -60 mV, an actin microfilament disruptor, cytochalasin-B (Cyt-B, 20 μ mol/L in pipette) increased calcium-activated potassium current ($I_{K(Ca)}$) and delayed rectifier potassium current ($I_{K(V)}$) to $138.4 \pm 14.3\%$ and $142.1 \pm 13.1\%$ respectively at +60 mV. In the same condition, an actin microfilament stabilizer phalloidin (20 μ mol/L in pipette) inhibited $I_{K(Ca)}$ and $I_{K(V)}$ to $74.2 \pm 7.1\%$ and $75.4 \pm 9.9\%$ respectively. At the holding potential of -60 mV, hyposmotic membrane stretch increased $I_{K(Ca)}$ and $I_{K(V)}$ by $50.6 \pm 9.7\%$ and $24.9 \pm 3.3\%$ at +60 mV respectively. In the presence of cytochalasin-B and phalloidin (20 μ mol/L, in the pipette) condition, hyposmotic membrane stretch also increased $I_{K(Ca)}$ by $44.5 \pm 7.9\%$ and $55.7 \pm 9.8\%$ at +60 mV respectively. In the same condition, cytochalasin-B and phalloidin also increased $I_{K(V)}$ by $23.0 \pm 5.5\%$ and $30.3 \pm 4.5\%$ respectively. However, Cyt-B and phalloidin did not affect the amplitude of hyposmotic membrane stretch-induced increase of $I_{K(Ca)}$ and $I_{K(V)}$.

CONCLUSION: Actin microfilaments regulate the activities of potassium channels, but they are not involved in the process of hyposmotic membrane stretch-induced increase of potassium currents in gastric antral circular myocytes of guinea pig.

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INTRODUCTION

Cytoskeleton is an intracellular superstructure that consists of microfilaments of actin and associated proteins, microtubules,

and intermediate filaments. Actin microfilaments of the cytoskeleton form a complex network, providing the structural basis for simultaneous interactions between multiple cellular structures. It is well established that many ion channels and transporters are anchored in the membrane by either direct or indirect association with the cytoskeleton. In addition, there is growing evidence that altering the integrity of cytoskeletal elements, in particular actin microfilaments, could modulate the activity of a variety of ion channels^[1] and receptors^[2]. Many previous studies have demonstrated that actin microfilament could mediate different types of potassium channels of a variety of cells such as those in rat collecting duct^[3], smooth muscle cell line DDT1 MF-2^[4], cardiac myocytes of guinea pig^[5], human meningioma cells^[6], rat hippocampal CA1 pyramidal neurons^[7], rat ventricular myocytes^[8] and xenopus oocytes^[9]. Wang *et al.*^[10] reported that neither the microfilaments nor the microtubules were involved in the enhancement of $I_{K(V)}$ induced by cell distension in ventricular muscle cells of guinea pig. However, Ribeiro *et al.*^[11] showed that microtubule was involved in the cell volume-induced changes in K⁺ transport across the rat colon epithelial cells. Our previous study demonstrated that main outward current was carried by calcium-activated potassium channel and delayed rectifier potassium channel in gastric antral circular myocytes of guinea pig and hyposmotic cell swelling enhanced the activity of that two kinds of potassium channel^[12,13]. In order to investigate the mechanism of hyposmotic membrane stretch-induced increase of potassium current, in the present study, the effect of actin microfilament on potassium currents was observed and the possibility whether actin microfilament was involved in the process of hyposmotic membrane stretch-induced increase of potassium currents was examined.

MATERIALS AND METHODS

Single cell preparation and electrophysiological recording

Fresh, single smooth muscle cells (SMCs) were isolated enzymatically from the circular layer of guinea pig antrum as previously described^[14]. Isolated SMCs were stored at 4 °C KBS until the time of use. All experiments were performed within 12 h after cell dispersion. The isolated cells were transferred to a small chamber (0.1 mL) on the stage of an inverted microscope (IX-70 Olympus, Japan) for 10-15 min to settle down. Solution was perfused at a speed of 0.9-1.0 mL/min through the chamber by gravity from the 8-channel perfusion system (L/M-sps-8; list electronics, Germany). Glass pipette with a resistance of 3-5 M Ω was used to make a giga seal of 5-10 G Ω the whole-cell currents were recorded with an Axopatch 1-D patch-clamp amplifier (Axon Instrument, USA).

Drugs and solution

Tyrod solution containing (mmol/L) NaCl 147, KCl 4, MgCl₂·6H₂O 1.05, CaCl₂·2H₂O 0.42, Na₂PO₄·2H₂O 1.81, and 5.5 mmol/L glucose was used. Ca²⁺-free PSS containing (mmol/L) NaCl 134.8, KCl 4.5, glucose 5, and N-[2-hydroxyethyl] piperazine- N-[2-ethanesulphonic acid] (HEPES) 10 was adjusted to pH 7.4 with Tris [hydroxymethyl] aminomethane (TRIZMA). Modified K-B solution containing (mmol/L) L-glutamate 50, KCl 50, taurine 20, KH₂PO₄ 20, MgCl₂·6H₂O 3, glucose 10, HEPES 10 and egtazic

acid 0.5 was adjusted to pH 7.40 with KOH. Isosmotic solution (290 mOsm/L) containing (mmol/L) NaCl 80, KCl 4.5, HEPES 10, $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ 1, $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ 2, Glucose 5, Sucrose 110, was adjusted to pH 7.4 with Tris. Hypoosmotic solution (200 mOsm/L) contained (in mmol/L) sucrose 30, and other ingredients was the same as the isosmotic solution. Pipette solution recording $I_{\text{K(Ca)}}$ contained (mmol/L) potassium-aspartic acid 110, Mg-ATP 5, HEPES 5, $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ 1.0, KCl 20, egtazic acid 0.1, di-tris-creatine phosphate 2.5, disodium-creatine phosphate 2.5 and its pH was adjusted to 7.3 with KOH. Pipette solution recording $I_{\text{K(V)}}$ contained (mmol/L) EGTA 10, and other ingredients was the same as the pipette solution recording $I_{\text{K(Ca)}}$. Cytochalasin-B was dissolved in dimethyl sulphoxide (DMSO, 20 mmol/L) and phalloidin was dissolved in alcohol (1 mmol/L). The same amount of DMSO or alcohol as the final experimental solution was added to the pipette solution. All the chemicals in this experiment were purchased from Sigma (USA).

Data analysis

All data were expressed as mean \pm SD. Statistical significance was evaluated by a *t*-test. Differences were considered to be

significant when *P* value was less than 0.05.

RESULTS

Effect of Cyt-B and phalloidin on $I_{\text{K(Ca)}}$

Under the whole cell configuration, the membrane potential was clamped at -60 mV, $I_{\text{K(Ca)}}$ was elicited by step voltage command pulse from -40 mV to +100 mV for 440 ms with a 20 mV increment at 10 s intervals. An actin microfilament disruptor, Cyt-B (20 $\mu\text{mol/L}$ in pipette) markedly increased $I_{\text{K(Ca)}}$ to $138.4 \pm 14.3\%$ at +60 mV ($n = 15$, Figures 1B, C). In the same condition, an actin microfilament stabilizer, phalloidin (20 $\mu\text{mol/L}$ in pipette) inhibited $I_{\text{K(Ca)}}$ to $74.2 \pm 7.1\%$ at +60 mV ($n = 15$, Figures 2B, C).

Effect of Cyt-B and phalloidin on $I_{\text{K(V)}}$

Under the whole cell configuration, the membrane potential was clamped at -60 mV, $I_{\text{K(V)}}$ was elicited by step voltage command pulse from -40 mV to +80 mV for 440 ms with a 20 mV increment at 10 s intervals. Cyt-B (20 $\mu\text{mol/L}$ in pipette) markedly increased $I_{\text{K(V)}}$ to $142.1 \pm 13.1\%$ at +60 mV ($n = 12$, Figures 3B, C). In the same condition, phalloidin (20 $\mu\text{mol/L}$ in pipette) inhibited $I_{\text{K(V)}}$ to $75.4 \pm 9.9\%$ at +60 mV ($n = 12$, Figures 4B, C).

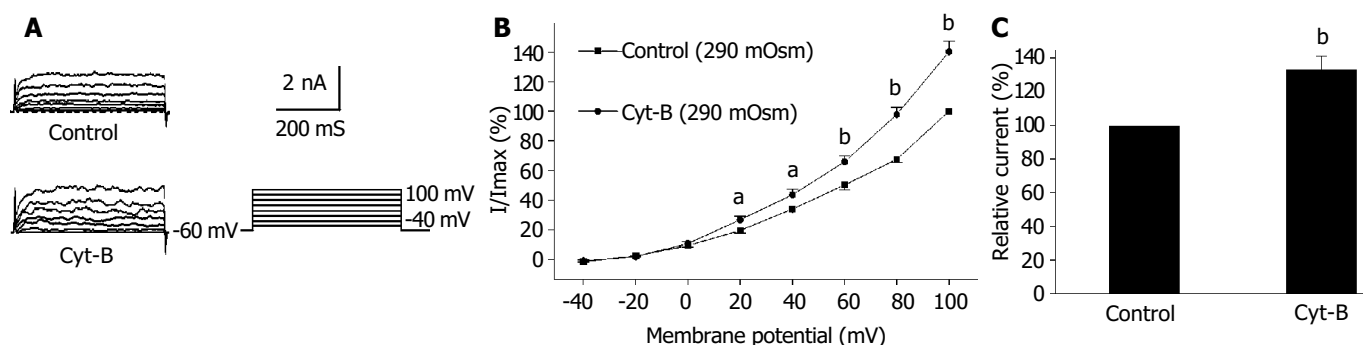


Figure 1 Effect of Cyt-B on $I_{\text{K(Ca)}}$. A: Representative current trace of $I_{\text{K(Ca)}}$. B: I/V relationship of $I_{\text{K(Ca)}}$. C: Cyt-B enhanced $I_{\text{K(Ca)}}$. In isosmotic condition. ($n = 15$, ^a $P < 0.05$, ^b $P < 0.01$ vs control).

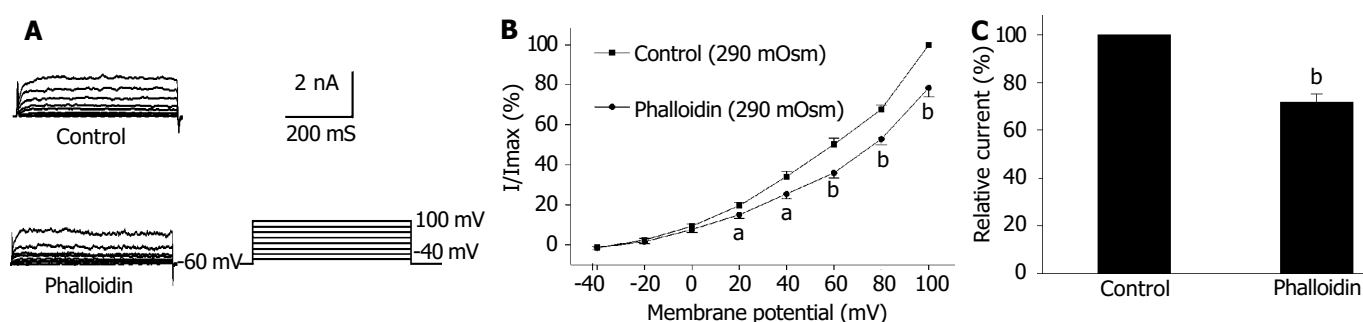


Figure 2 Effect of phalloidin on $I_{\text{K(Ca)}}$. A: Representative current trace of $I_{\text{K(Ca)}}$. B: I/V relationship of $I_{\text{K(Ca)}}$. C: Phalloidin inhibited $I_{\text{K(Ca)}}$ in isosmotic condition. ($n = 15$, ^a $P < 0.05$, ^b $P < 0.01$ vs control).

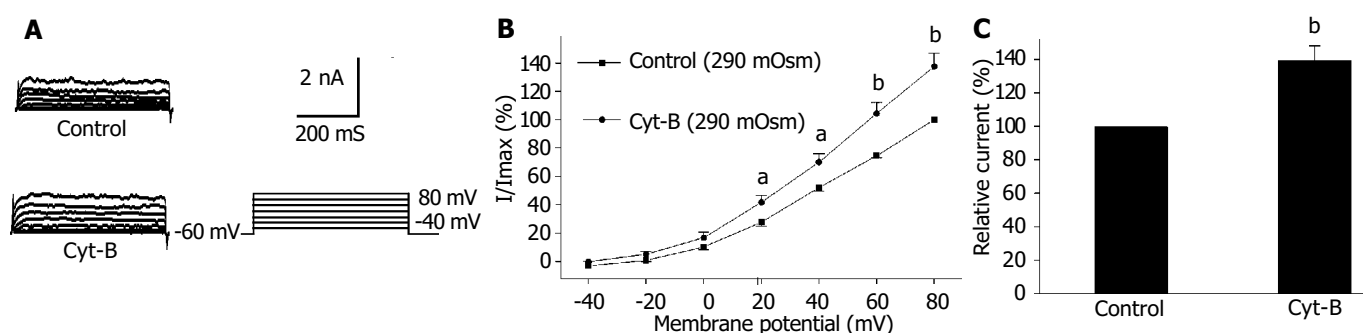


Figure 3 Effect of Cyt-B on $I_{\text{K(V)}}$. A: Representative current trace of $I_{\text{K(V)}}$. B: I/V relationship of $I_{\text{K(V)}}$. C: Cyt-B enhanced $I_{\text{K(V)}}$ in isosmotic condition. ($n = 15$, ^a $P < 0.05$, ^b $P < 0.01$ vs control).

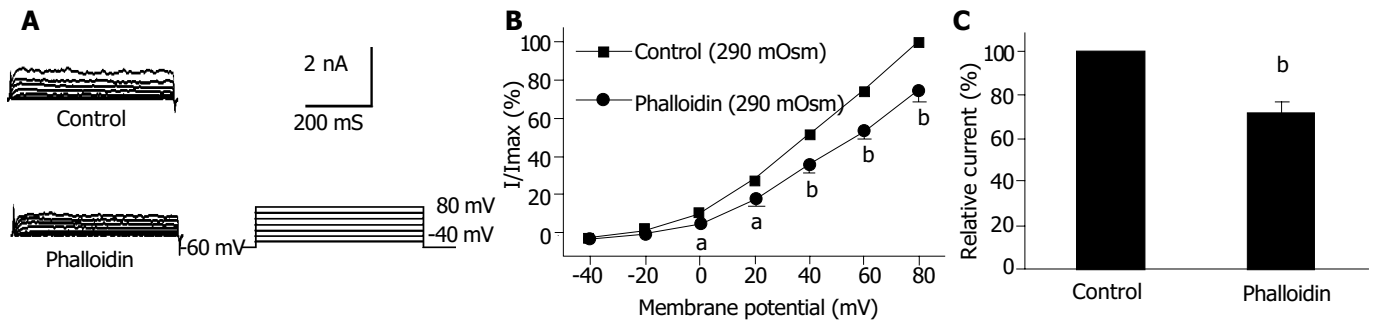


Figure 4 Effect of phalloidin on $I_{K(Ca)}$. A: Representative current trace of $I_{K(Ca)}$. B: I/V relationship of $I_{K(Ca)}$. C: Phalloidin inhibited $I_{K(Ca)}$ in isosmotic condition. ($n = 15$, ^a $P < 0.05$, ^b $P < 0.01$ vs control).

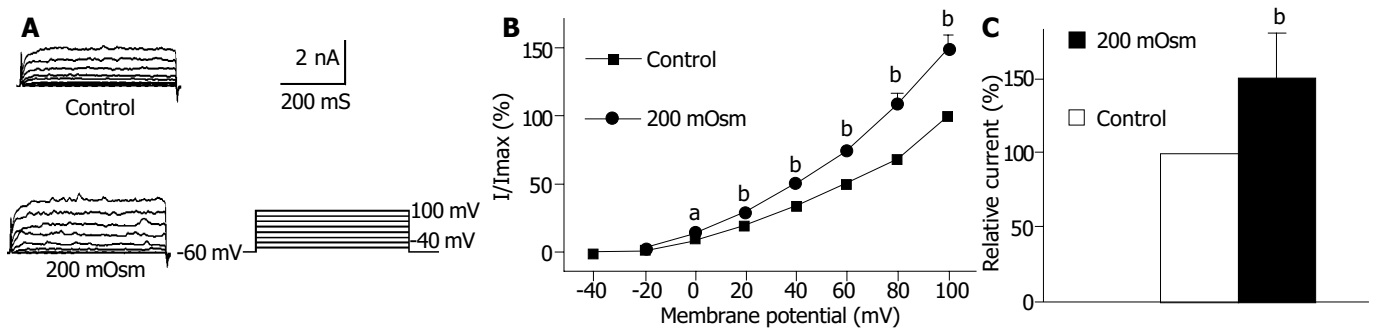


Figure 5 Effect of hyposmotic membrane stretch on $I_{K(Ca)}$. A: Representative current trace of $I_{K(Ca)}$. B: I/V relationship of $I_{K(Ca)}$. C: Hyposmotic membrane stretch increased $I_{K(Ca)}$. ($n = 15$, ^a $P < 0.05$, ^b $P < 0.01$ vs control).

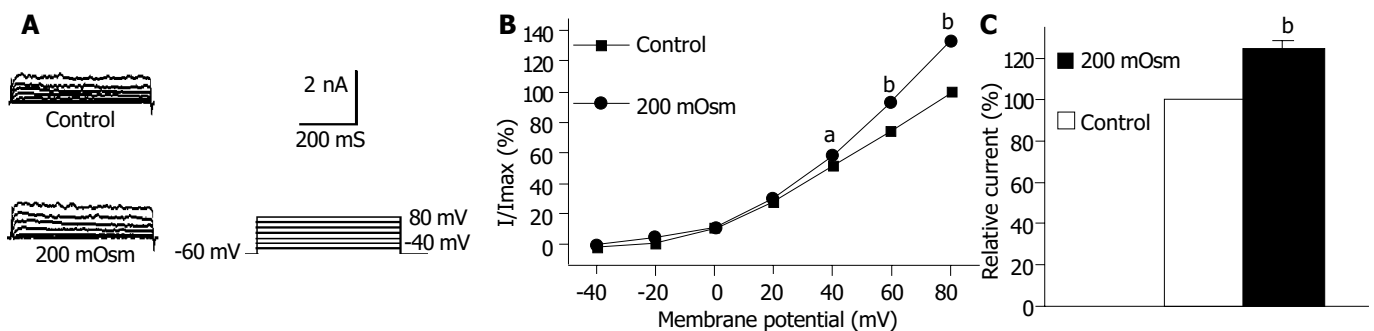


Figure 6 Effect of hyposmotic membrane stretch on $I_{K(V)}$. A: Representative current trace of $I_{K(V)}$. B: I/V relationship of $I_{K(V)}$. C: Hyposmotic membrane stretch increased $I_{K(V)}$. ($n = 15$, ^a $P < 0.05$, ^b $P < 0.01$ vs control).

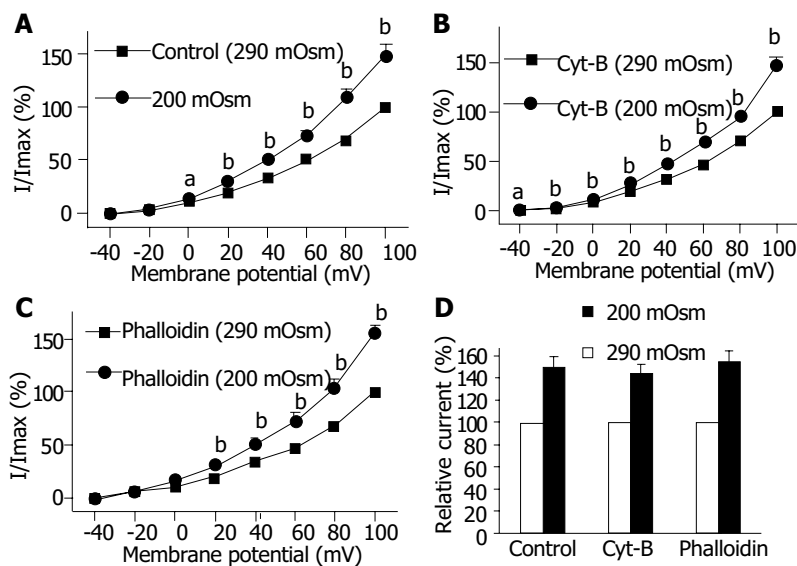


Figure 7 Effect of Cyt-B and phalloidin on hyposmotic membrane stretch-increase of $I_{K(Ca)}$. A, B and C: I/V relationship of $I_{K(Ca)}$. ($n = 15$, ^a $P < 0.05$, ^b $P < 0.01$ vs 290 mOsm). D: No effect of Cyt-B and phalloidin on the increased of $I_{K(Ca)}$ induced by hyposmotic membrane stretch ($n = 15$).

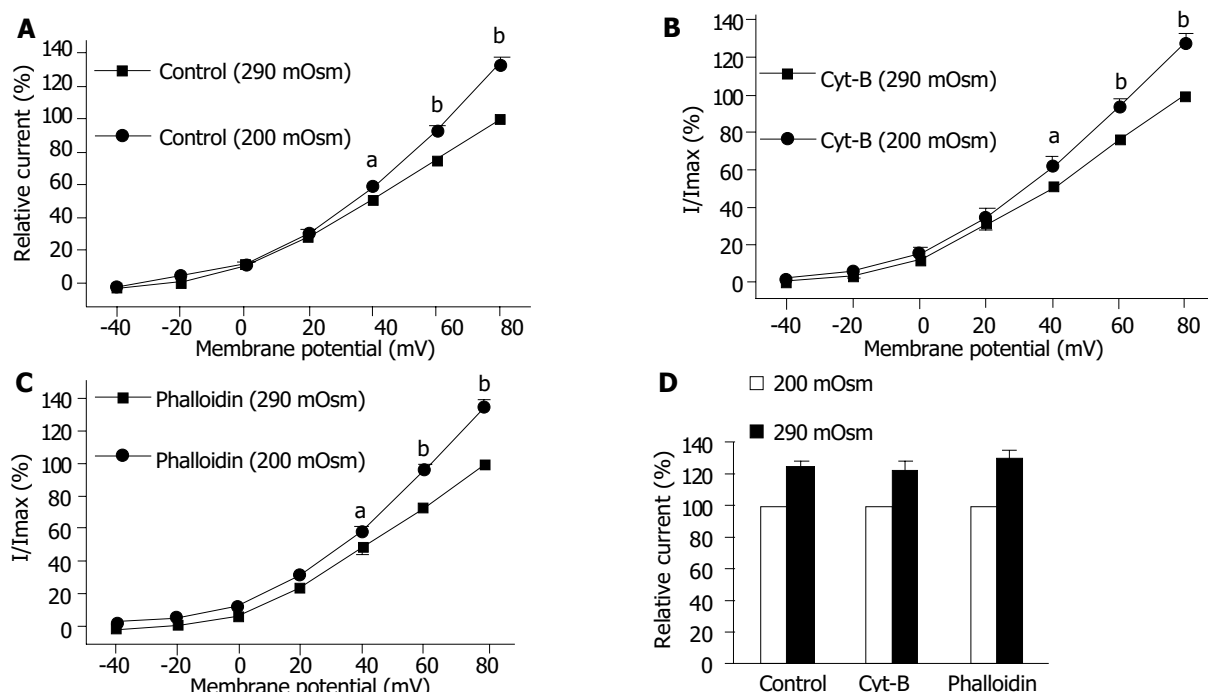


Figure 8 Effect of Cyt-B and phalloidin on hypotonic membrane stretch-increase of $I_{K(V)}$. A, B and C: I/V relationship of $I_{K(V)}$. ($n = 12$, $^aP < 0.05$, $^bP < 0.01$ vs 290 mOsm). D: No effect of Cyt-B and phalloidin on the increased of $I_{K(V)}$ induced by hypotonic membrane stretch ($n = 12$).

Effect of hypotonic membrane stretch on $I_{K(Ca)}$ and $I_{K(V)}$

Using the same pulse protocol, the effect of hypotonic membrane stretch on $I_{K(Ca)}$ and $I_{K(V)}$ was observed. When the cells were superfused with hypotonic solution (200 mOsm/L), step command pulse-induced $I_{K(Ca)}$ increased from 0 mV (Figure 5B) and the increasing amplitude was $50.6 \pm 9.7\%$ at +60 mV ($n = 15$, Figure 5C). In the same condition, hypotonic superfusing increased step command pulse-induced $I_{K(V)}$ from +40 mV (Figure 6B) and the increasing amplitude was $24.9 \pm 3.3\%$ at +60 mV ($n = 12$, Figure 6C).

Effect of Cyt-B and phalloidin on hypotonic membrane stretch-induced increase of $I_{K(Ca)}$

To determine the possibility of actin microfilament involved in hypotonic membrane stretch-induced increase of $I_{K(Ca)}$, the effects of Cyt-B and phalloidin on $I_{K(Ca)}$ in which cells were perfused with isosmotic and hypoosmotic solutions were observed respectively. Hypotonic membrane stretch increased $I_{K(Ca)}$ from 0 mV (Figure 7A) and the increasing amplitude was $50.6 \pm 9.7\%$ at 60 mV in the control group ($n = 15$, Figures 7A, 8D). In the presence of Cyt-B and phalloidin (20 $\mu\text{mol/L}$ in pipette) hypotonic membrane stretch also increased $I_{K(Ca)}$ by $44.5 \pm 7.9\%$ ($n = 15$, Figures 7B, D) and $55.7 \pm 9.8\%$ ($n = 15$, Figures 7C, D) at +60 mV respectively. There was no significant difference between control group and Cyt-B group or phalloidin group.

Effect of Cyt-B and phalloidin on hypotonic membrane stretch-induced increase of $I_{K(V)}$

Hypotonic membrane stretch increased $I_{K(V)}$ by $24.9 \pm 3.3\%$ at +60 mV in the control group ($n = 12$, Figures 8A, D). In the presence of Cyt-B and phalloidin (20 $\mu\text{mol/L}$ in pipette) hypotonic membrane stretch also increased $I_{K(V)}$ by $22.9 \pm 5.5\%$ ($n = 12$, Figures 8B, D) and $30.3 \pm 4.5\%$ ($n = 12$, Figures 8C, D) at +60 mV respectively. There was no significant difference between control group and Cyt-B group or phalloidin group.

DISCUSSION

Cytoskeleton is an intracellular superstructure that consists of

microfilaments of actin and associated proteins, microtubules, and intermediate filaments. Actin microfilament, in particular, are involved in structural support and a functional role in cell motility^[15]. Recent evidence indicated, however, actin-based cytoskeleton was involved in the control of ion channel activity across the plasma membranes of different cell types. For example, actin microfilaments were implicated in the regulation of sodium channels in human jejunal circular smooth muscle cells^[16] and ATP-sensitive potassium channel in ventricular myocytes^[5, 17]. Actin microfilaments could also regulate voltage-dependent channels, for example, actin microfilaments could mediate voltage-dependent epithelial sodium channels in neuron cells^[18].

It was proposed that cell surface proteins and extra cellular matrix were linked to the cytoskeleton by transmembrane proteins and modulate ion channels and enzymes by mechanical deformation under physiological conditions. In the present study, we observed that an actin microfilament disruptor, Cyt-B increased $I_{K(Ca)}$ and $I_{K(V)}$ significantly (Figures 1B, C, Figures 3B, C). However, an actin microfilament stabilizer, phalloidin inhibited $I_{K(Ca)}$ and $I_{K(V)}$ markedly (Figures 2B, C, Figures 4B, C) in gastric myocytes. These results suggested that when actin microfilaments were disrupted, $I_{K(Ca)}$ or $I_{K(V)}$ could be activated; while, when actin microfilaments were stabilized, $I_{K(Ca)}$ or $I_{K(V)}$ could be inhibited in gastric myocytes. Many previous studies also supported our experiment. For example, Cyt-D activated calcium-activated potassium channel in human meningioma cells^[6], Cyt-B activated K (ATP) channels in cardiac^[5].

Stretch is a physiological stimulation in gut smooth muscles. There are two kinds of potassium current, calcium-activated potassium current and delayed rectifier potassium current. In the present study, the two kinds of potassium current were activated by hypotonic swelling in gastric antral smooth muscle cells of guinea pigs (Figures 5-6). In order to investigate the mechanism of hypotonic membrane stretch-induced increase of $I_{K(Ca)}$ and $I_{K(V)}$, the relationship between potassium channel activity and actin microfilaments was observed. When actin microfilaments were disrupted by Cyt-B or stabilized by phalloidin, hypotonic membrane stretch-induced increase of

$I_{K(Ca)}$ and $I_{K(V)}$ was not affected (Figures 7-8). These results indicated that actin microfilaments were not involved in the increase of potassium current induced by hyposmotic cell swelling in gastric circular myocytes of guinea pig. Previous studies supported our results. For example, Wang *et al.*^[10] observed that neither the microfilaments nor the microtubules were involved in the enhancement of $I_{K(V)}$ induced by cell distension in ventricular myocytes of guinea pig. We also observed that unsaturated fatty acids, exogenous and endogenous, were involved in the increase of calcium-activated potassium current induced by hyposmotic membrane stretch (data not shown). So that hyposmotic membrane stretch-induced increase of potassium currents may be related to unsaturated fatty acids in cell membranes.

Our previous study demonstrated that actin microfilaments played an important role in the modulation of membrane stretch-induced calcium influx and hyposmotic membrane stretch-induced increase of muscarinic current in guinea-pig gastric myocytes^[19,20]. It is obvious that cytoskeleton plays a different role in different types of cells and different kinds of ion channels. In gastric smooth muscle actin microfilaments may be involved in the process of hyposmotic membrane stretch-induced depolarization of membrane potential. However, actin microfilaments would not be involved in the process of cell swelling-induced hyperpolarization of membrane potential.

In summary, actin microfilaments regulate potassium channel activities in normal condition. However, actin microfilaments are not involved in hyposmotic cell swelling-induced increase of potassium currents.

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