ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 2001 Jun; 22 (6): 566 - 572
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Increment of calcium-activated and delayed rectifier potassium current by hyposmotic swelling in gastric antral circular myocytes of guinea pig[1]

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KEY WORDS potassium channels; pyloric antrum; guinea pigs; patch-clamp techniques

ABSTRACT

AIM: To observe the effect of hyposmotic swelling on calcium-activated potassium current [$I_{K(Ca)}$] and delayed rectifier potassium current [$I_{K(V)}$] in gastric antral circular myocytes of guinea pig. **METHODS**: The whole cell patch-clamp technique was used, and the myocytes were isolated by collagenase. Cells were swelled by the hyposmotic solution (200 Osmmol/kg). **RESULTS**: The hyposmotic solution markedly increased $I_{K(Ca)}$ and $I_{K(V)}$. The increase of $I_{K(Ca)}$ was markedly inhibited by tetrathylammonium (TEA) 4 mmol·L^{-1} and charybdotoxin (ChTX) 200 nmol·L^{-1}. The increase of $I_{K(V)}$ was incompletely blocked by TEA and completely blocked by 4-aminopyridine (4-AP) 10 mmol·L^{-1}. There was no significant difference between the amplitudes of the increase of $I_{K(Ca)}$ and $I_{K(V)}$ ($P > 0.05$). $I_{K(Ca)}$ increased (17.0 ± 4.8) s after the cells were perfused with the hyposmotic solution, whereas $I_{K(V)}$ increased (30.7 ± 13.7) s after the cells exposed to the hyposmotic solution. There was significant difference between the latency of $I_{K(Ca)}$ and $I_{K(V)}$ ($P < 0.05$). **CONCLUSION**: Hyposmotic swelling increased both $I_{K(Ca)}$ and $I_{K(V)}$, and the increment was likely related to the cell volume regulation.

INTRODUCTION

When subjected to hyposmotic solutions, cells initially swell by osmotic water equilibration but subsequently regain near normal size through loss of solutes such as K^+, Cl^-, amino acid, and osmotically obliged water[1-3]. The mechanism underlying this process, known as regulatory volume decrease (RVD), varies in different cell types. K^+ and Cl^- channels have been proposed to play an important role in RVD in various kinds of cells, such as amphibian urinary bladder[4], human platelets[5], frog skin[6], and thick ascending limb[7].

The important role of Ca^{2+} during RVD process has been known for many years. Many patch-clamp studies have indicated that activation of volume-regulatory K^+ channels are controlled by Ca^{2+}[8-12]. However, the calcium-independent cell volume regulation was also found in human lymphocytes[13] and guinea pig hepatocytes[14]. In the previous study, we have reported that the voltage-operated calcium current (I_{Ca})[15] and volume-sensitive chloride current (I_{Cl}) were activated by hyposmotic swelling[16] in gastric antral circular myocytes of guinea pig. In the present study, we investigated the effect of hyposmotic swelling on $I_{K(Ca)}$ and $I_{K(V)}$ in gastric antral circular myocytes of guinea pig.

MATERIALS AND METHODS

Preparation of cells EWG/B guinea pigs (obtained from the Experimental Animal Department of Norman Bethune University, Certificate No 10-6004) of either sex, weighing 250 - 350 g, were euthanized by lethal dose of intravenous pentobarbital sodium (50 mg/kg). The antral part of the stomach was rapidly cut. First, the muscosal layer was separated from the muscle layer, dissected from the longitudinal layer using fine scissors, and cut into small segments (1 mm × 4 mm). These segments were kept in a modified Kraft-Bruhe (K-B) medium at 4 ℃ for 15 min. Next, they were incubated at 36 ℃ in 4 mL of digestion medium [Ca^{2+}-free normal saline (Ca^{2+}-free NS)] containing 0.1 % colla-

[1] Project supported by the National Natural Science Foundation of China, No 39860031.
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Received 2000-11-03 Accepted 2001-02-09

genase (Ⅱ), 0.1 % dithioerythreitol, 0.15 % trypsin inhibitor, and 0.2 % bovine serum albumin for 25 – 35 min. Then, the softened muscle segments were transferred into the modified K-B medium, and cells were individually dispersed by gentle trituration with a wide-bore fire-polished glass pipette. Isolated gastric myocytes were kept in modified K-B medium at 4 ℃ until use.

Electrophysiologic recording 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. The cells were continuously superfused with isoosmotic PSS by gravity (0.9 – 1.0 mL·min^{-1}). An 8-channel perfusion system (L/M-sps-8, List Electronics, Germany) was used to change solution. Experiments were performed at 20 – 25 ℃ and the whole-cell configuration of the patch-clamp technique was used[17]. Patch-clamp pipettes were manufactured from borosilicate glass capillaries (GC 150T-7.5, Clark Electromedical Instruments, UK) using a two-stage puller (PP-83, Narishige, Japan). The resistance of the patch pipette was 3 – 5 MΩ when filled with pipette solution. Liquid junction potentials were canceled prior to seal formation. Whole-cell currents were recorded with an Axopatch 1-D patch-clamp amplifier (Axon Instrument, USA) and command pulses were applied by using the IBM-compatible 486-grade computer and pCLAMP software (Version 6.02).

Drugs and solutions All drugs were purchased from Sigma Chemical Co, USA. Tyrode's solution contained NaCl 147, KCl 4, $MgCl_2 \cdot 6H_2O$ 1.05, $CaCl_2 \cdot 2H_2O$ 0.42, $Na_2HPO_4 \cdot 2H_2O$ 1.81, and glucose 5.5 mmol·L^{-1}, and the pH was adjusted to 7.35 with NaOH. The pH of Ca^{2+}-free solution containing NaCl 134.8, KCl 4.5, glucose 5, and HEPES 10 mmol·L^{-1}, was adjusted to pH 7.4 with Tris. The isosmotic solution (290 Osmmol/kg) contained NaCl 80, KCl 4.5, $MgCl_2 \cdot 6H_2O$ 1, $CaCl_2 \cdot 2H_2O$ 2, glucose 5, HEPES 10, and sucrose 110 mmol·L^{-1}, and the pH was adjusted to 7.4 with Tris. Hyposmotic solution (200 Osmmol/kg) contained sucrose 30 mmol·L^{-1}, and the others were the same as the isoosmotic solution. Modified K-B solution containing *L*-glutamate 50, KCl 50, taurine 20, KH_2PO_4 20, $MgCl_2 \cdot 6H_2O$ 3, glucose 10, HEPES 10, and egtazic acid 0.5 mmol·L^{-1}, had the pH adjusted to 7.4 with KOH. The pipette solution contained K-aspartic acid 110, Mg-ATP 5, $MgCl_2 \cdot 6H_2O$ 1, KCl 20, egtazic acid 0.1 or 10, di-tris-creatine phosphate 2.5, and disodium-creatine phosphate 2.5 mmol·L^{-1}, and the pH was adjusted to 7.3 with KOH. 4-AP, TEA, and ChTX were prepared as aqueous stock solutions (1 mol·L^{-1}, 1 mol·L^{-1}, and 10 mmol·L^{-1}, respectively).

Data analysis Data were expressed as $\bar{x} \pm s$. Statistical significance was evaluated by *t*-test.

RESULTS

$I_{K(Ca)}$ and $I_{K(V)}$ of gastric antral circular myocytes Under the whole-cell configuration, the membrane potential was clamped at −60 mV. When the pipette solution contained egtazic acid 0.1 mmol·L^{-1}, $I_{K(Ca)}$ was elicited by step voltage command pulse from −40 mV to 80 mV for 400 ms (with a 20 mV increment, 10 s intervals). The mean amplitude $I_{K(Ca)}$ was (766 ± 63) pA at 60 mV (n = 50). TEA 4 mmol·L^{-1}, a nonselective potassium channel blocker, markedly blocked $I_{K(Ca)}$ about 76 % ± 7 %. ChTX 200 nmol·L^{-1}, a selective blocker of $I_{K(Ca)}$, inhibited $I_{K(Ca)}$ about 66 % ± 10 % at 60 mV (Fig 1).

$I_{K(V)}$ was elicited by the step depolarization with the pipette solution containing egtazic acid 10 mmol·L^{-1} using the same mode. The mean amplitude of $I_{K(Ca)}$ was (707 ± 60) pA at 60 mV. Because the current was not affected by the release of intracellular calcium, $I_{K(V)}$ was smoother than $I_{K(Ca)}$[18]. TEA inhibited $I_{K(V)}$ about 40 % ± 10 % at 60 mV, and the inhibition was less than that of $I_{K(Ca)}$. 4-AP 10 mmol·L^{-1}, a kind of selective inhibitor of $I_{K(V)}$[19], inhibited the current about 40 % ± 10 % at 60 mV (Fig 2).

Effect of hyposmotic swelling on $I_{K(Ca)}$ and $I_{K(V)}$ Using the same pulse protocol, we studied the effect of hyposmotic swelling on $I_{K(Ca)}$ and $I_{K(V)}$. When the cells were superfused with hyposmotic solution (200 Osmmol/kg), both $I_{K(Ca)}$ and $I_{K(V)}$ increased reversibly at 60 mV (Fig 3A and Fig 4A). To study the durations of the amplifications of hyposmotic swelling-induced currents, membrane potential was clamped at −60 mV, and the two currents were elicited by a single depolarizing step pulse (depolarized to +60 mV, 10 s intervals) for 400 ms. Bath solution 200 Osmmol/kg sharply increased $I_{K(Ca)}$ about 59 % ± 4 % at 60 mV. $I_{K(Ca)}$ increased (17 ± 5) s after the cells were perfused with the hyposmotic bath solution (Fig 3B). Whereas $I_{K(V)}$ increased about 66 % ± 14 % at 60 mV and increased (31 ± 14) s after the cells were exposed to 200 Osmmol/kg bath solution (Fig 4B). The latency of $I_{K(Ca)}$ and $I_{K(V)}$ increased by hyposmotic swelling were significantly different

ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 2001 Jun; 22 (6)
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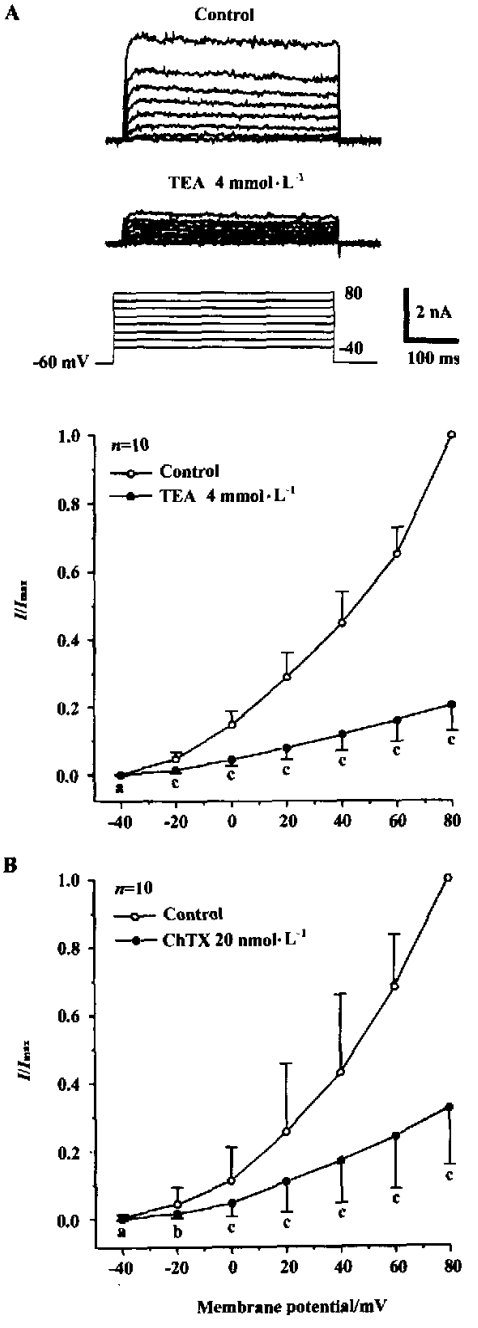

Fig 1. Effect of TEA (A) and ChTX (B) on Ca^{2+}-activated potassium current in gastric antral circular myocytes of guinea pig. $\bar{x} \pm s$. $^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ *vs* control.

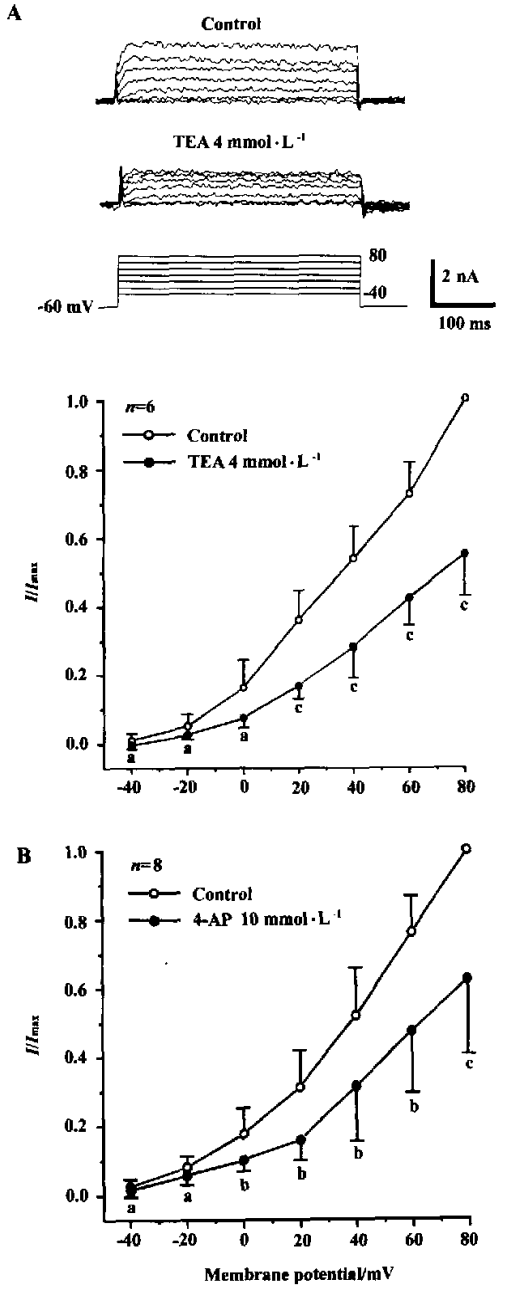

Fig 2. Effect of TEA (A) and 4-AP (B) on delayed rectifier potassium current in gastric antral circular myocytes of guinea pig. $\bar{x} \pm s$. $^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ *vs* control.

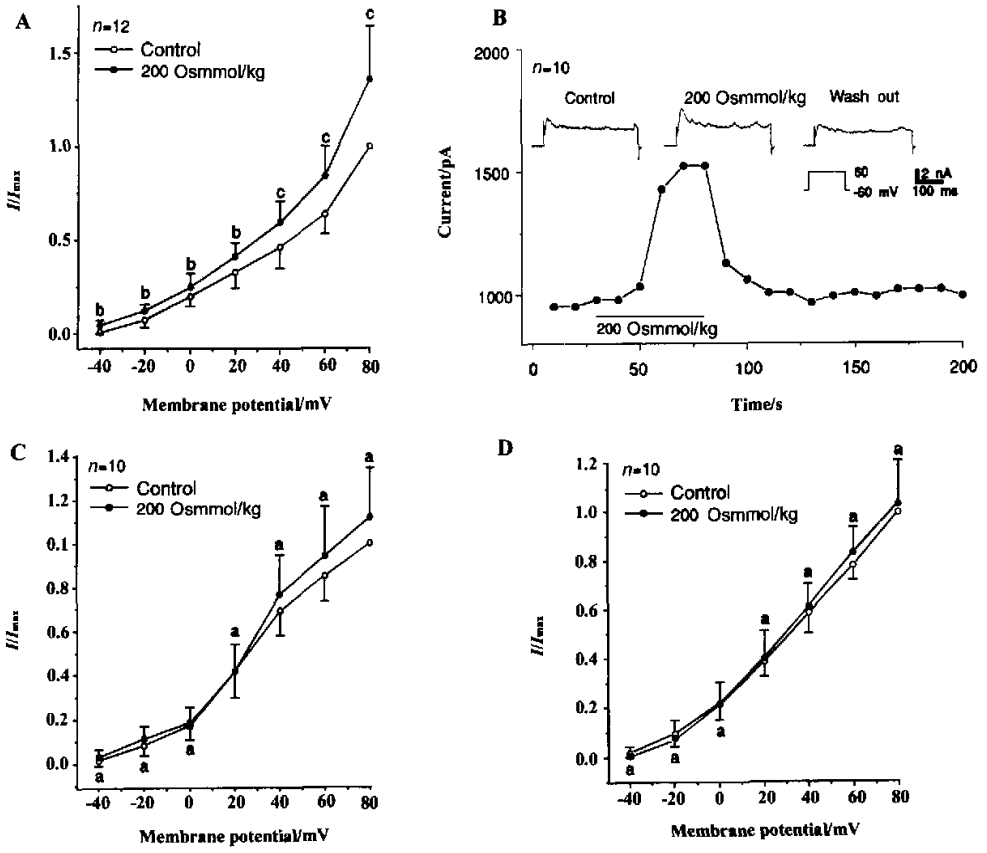

Fig 3. Effect of hyposmotic swelling on Ca^{2+}-activated potassium current [$I_{K(Ca)}$]. A) I-V relationship of $I_{K(Ca)}$; B) The latency course of $I_{K(Ca)}$ increased by hyposmotic swelling; C) 4 mmol·L^{-1} TEA-pretreated; D) 200 nmol·L^{-1} ChTX-pretreated. $\bar{x} \pm s$. $^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ *vs* control.

($P < 0.05$), but the amplitudes were not significantly different ($P > 0.05$).

Then the pharmacological features of the effects on $I_{K(Ca)}$ and $I_{K(V)}$ were observed. When hyposmotic solution containing TEA 4 mmol·L^{-1} was used, $I_{K(Ca)}$ did not increase, and ChTX 200 nmol·L^{-1} also blocked the increase of $I_{K(Ca)}$ induced by hyposmotic swelling (Fig 3 C and D). $I_{K(V)}$ still increased when TEA 4 mmol·L^{-1} was added into the hyposmotic solution (Fig 4C), but 4-AP 10 mmol·L^{-1} completely blocked the effect of hyposmotic swelling on $I_{K(V)}$ (Fig 4D).

DISCUSSION

In gastric antral circular myocytes of guinea pig, the step depolarization activated two types of K^+ currents, calcium-activated potassium currents [$I_{K(Ca)}$] and delayed rectified potassium current [$I_{K(V)}$]. We observed that both $I_{K(Ca)}$ and $I_{K(V)}$ were inhibited by TEA 4 mmol·L^{-1}, but TEA inhibited $I_{K(Ca)}$ more markedly than $I_{K(V)}$. The similar features have been indicated previously by Bolton *et al*[18]. They indicated that in smooth muscle cells, although TEA blocked various of K^+ channels at concentrations above 5 mmol·L^{-1}, it seemed to markedly affect only $I_{K(Ca)}$ below this concentration. $I_{K(Ca)}$ was also efficiently blocked by ChTX, a specific inhibitor of $I_{K(Ca)}$. The property was already reported in many smooth muscle cells[19,20]. Both TEA and 4-AP reduced $I_{K(V)}$, but neither TEA nor 4-AP was capable of inhibiting the current completely. These pharmacological characteristics are similar to the 4-AP-sensitive current identified in portal veins of rabbit[21],

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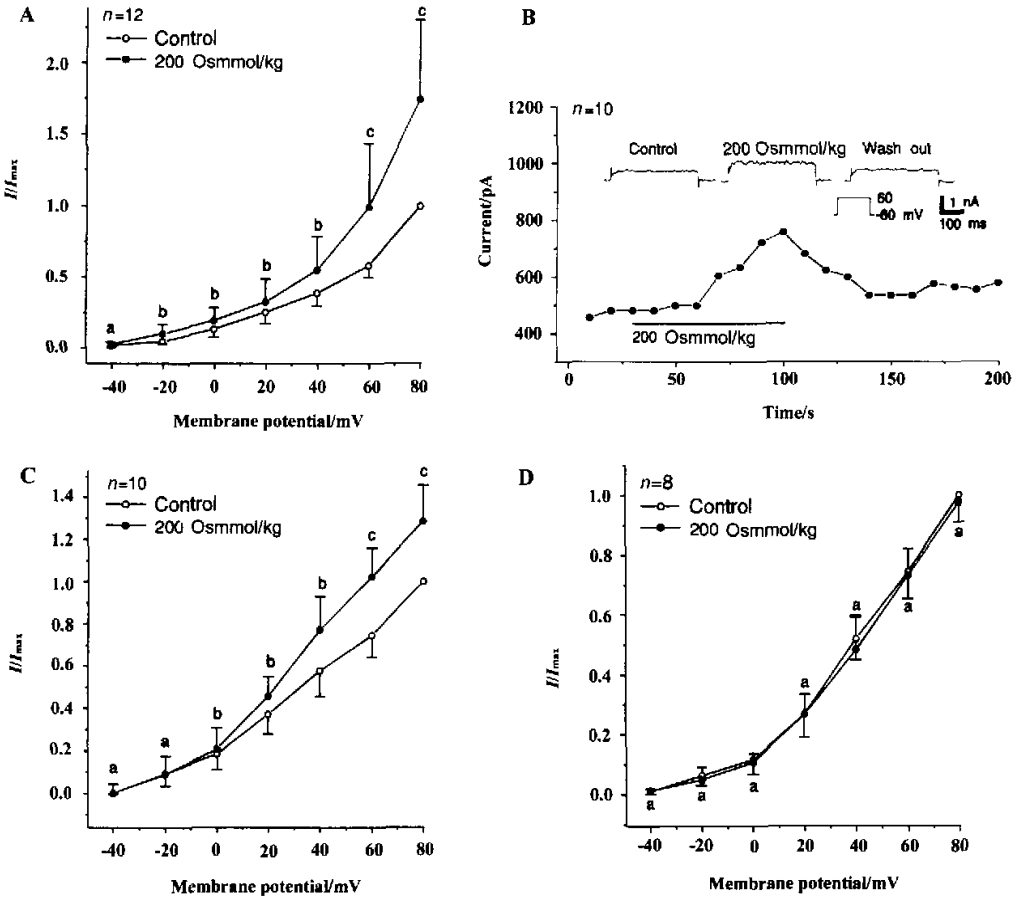

Fig 4. Effect of hyposmotic swelling on delayed rectifier potassium current [$I_{K(V)}$]. A) I-V relationship of $I_{K(V)}$; B) The latency of $I_{K(V)}$ increased by hyposmotic swelling; C) 4 mmol·L^{-1} TEA-pretreated; D) 10 mmol·L^{-1} 4-AP-pretreated. $\bar{x} \pm s$. $^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ *vs* control.

cerebral arteries ofcat[22], and coronary arteries of rabbit[23].

The main point of the present study was to determine the effect of hyposmotic swelling on $I_{K(Ca)}$ and $I_{K(V)}$ in gastric antral circular myocytes of guinea pig. The increment of both $I_{K(Ca)}$ and $I_{K(V)}$ caused by hyposmotic swelling was observed. As an important component of cell volume regulation, Ca^{2+} signaling was already described by scientists. Majority of cells have shown a Ca^{2+}-dependent RVD process[9,11]. During hyposmotic perfusion, an influx of calcium was responsible for the increase of $I_{K(Ca)}$ in many kinds of cells[7,8,10,12,24]. In the present study, we confirmed that $I_{K(Ca)}$ increased by hyposmotic swelling in gastric antral circular myocytes, and the increment was ChTX- and TEA-sensitive. We also observed that $I_{K(V)}$ increased by hyposmotic swelling, and the increment was TEA-sensitive and 4-AP-insensitive. Because of the important roles of Ca^{2+} signal and $I_{K(Ca)}$ during RVD processes, $I_{K(V)}$ was paid less attention to than $I_{K(Ca)}$. But in human lymphocytes[13] and guinea pig hepatocytes[14], Ca^{2+}-insenstive potassium currents were raised when the cells exposed to hyposmotic solution, and the authors indicated that the currents involved in the RVD process. In the present study, the comparison between $I_{K(Ca)}$ and $I_{K(V)}$ increased by hyposmotic swelling had shown that the amplitudes of $I_{K(Ca)}$ and $I_{K(V)}$ were not significantly different and the latencies of $I_{K(Ca)}$ and $I_{K(V)}$ were significantly different. When hyposmotic solutions were used, $I_{K(Ca)}$ was increased at first, and then $I_{K(V)}$ was raised. Results

showed that both $I_{K(Ca)}$ and $I_{K(V)}$ involved in the cell volume regulation. $I_{K(Ca)}$ involved in the RVD process at first, and $I_{K(V)}$ played its roles subsequently in RVD during the cell swelling. Several questions remain for further study. Perhaps the most important point is the mechanism of $I_{K(V)}$ increased by hyposmotic swelling, since we have observed that $I_{K(Ca)}$ was activated by Ca^{2+} released from stores (data not shown) during hyposmotic swelling.

In summary, hyposmotic swelling increased both $I_{K(Ca)}$ and $I_{K(V)}$ in gastric antral circular myocytes of guinea pig. The increased currents may be involved in cell volume regulation.

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低渗膨胀增加豚鼠胃窦环行肌细胞钙激活钾电流和延迟整流型钾电流[1]

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关键词 钾通道; 幽门窦; 豚鼠; 膜片箝技术

目的: 观察低渗膨胀对豚鼠胃窦环行肌细胞钙激活钾电流和延迟整流型钾电流的影响. **方法**: 采用传统全细胞膜片箝技术, 对以胶原酶急性分离的单细胞进行低渗灌流, 观察钾电流的变化. **结果**: 低渗

ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 2001 Jun; 22 (6)
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灌流液(200 Osmmol/kg)可增加钙激活钾电流和延迟整流型钾电流，钙激活钾电流的增加可被四乙基胺 4 mmol·L^{-1}和 Charybdotoxin 200 nmol·L^{-1}完全抑制；延迟整流型钾电流的增加可被四乙基胺 4 mmol·L^{-1}部分抑制，可被 4-氨基吡啶 10 mmol·L^{-1}完全抑制. 两种钾电流的增加幅度无显著性差异($P > 0.05$). 钙激活钾电流在施加低渗灌流(17.0 ± 4.8) s 后增加；延迟整流型钾电流在(30.7 ± 13.7) s 后增加，两者的潜伏期存在显著性差异($P < 0.05$). **结论**：低渗膨胀可增加钙激活钾电流和延迟整流型钾电流，这种增加效应可能与细胞容积调节有关.

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