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Effects of erysimin G on renal tubular function and 70-pS K^+ channel activity of thick ascending limb[1]

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KEY WORDS *Erysimum cheiranthoides* L.; kidney; patch-clamp techniques; potassium channels

ABSTRACT

AIM: To study the effect of erysimin G ($CH_{35}H_{52}O_{13}$) on the thick ascending limb (TAL) 70-pS K^+ channel of rat kidney and its effect on diuresis. **METHODS**: The patch-clamp cell-attached recording technique was used to record the single potassium channel current, and the urine volume (UV) was measured by urethal intubation to determine the diuretic effect. **RESULTS**: Erysimin G can increase the urine volume and decrease the 70-pS potassium channel activity of TAL. **CONCLUSION**: Erysimin G has a diuretic effect and its inhibition on the activity of apical 70-pS potassium channel may be the mechanism of its diuretic effect.

INTRODUCTION

Erysimum cheiranthoides is a biennial cruciferae herbage. There are many kinds of cardiotonic glucosides in its whole grass and seeds, so it has obvious cardiotonic activity. This study was to evaluate the diuretic effect of this Chinese traditional drug and delve further into the principles of its effect. It is well-known that the thick ascending limb (TAL) reabsorbs 20 % - 25 % of filtered Na^+ load and plays a key role in the urinary concentrating mechanism. The apical K^+ channel in TAL is important in maintaining the function of Na^+-K^+-$2Cl^-$ cotransport[1]. Three types of K^+ channels, the low-conductance (30 pS), intermediate-conductance (70 pS), and Ca^{2+}-activated K^+ channel with maximal conductance (127 pS) have been found in the apical membrane of the TAL. A previous study[2] has revealed that 70-pS K^+ channel contributes about 70 % - 80 % of the apical K^+ conductance, so 70-pS K^+ channel is involved in K^+ recycling across the apical membrane, a process which is essential for Na^+ reabsorption.

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MATERIALS AND METHODS

Animals Rabbit, male, weighing 2 - 3 kg, Grade Ⅱ, Certificate № 9-4-1; pathogen-free rat of either sex, weighing 80 - 100 g, Grade Ⅱ, Certificate № 09-2-1 were purchased from Experimental Animal Center, Harbin Medical University.

Solutions HEPES-buffered NaCl Ringer solution contained (in mmol·L^{-1}): NaCl 140, KCl 5, $MgCl_2$ 1.8, $CaCl_2$ 1.8, glucose 5, HEPES 10, pH adjusted to 7.4 with NaOH; the pipette solution contained (in mmol·L^{-1}): KCl 140, $MgCl_2$ 1.8, HEPES 10, egtazic acid 1, pH adjusted to 7.4 with KOH; erysimin G was dissolved in ethanol and freshly prepared with distilled water; cell-Tak were purchased from American Collaborative Research and HEPES and egtazic acid were purchased from Sigma Co.

Diuretic study Rabbits were divided into three groups in random. The animals were anesthetized and fixed on the anatomic table, 5 % glucose 10 mL·kg^{-1} was administered by an intravenous injection. Urine in bladder was emptied through urethal intubation and the normal quantity of urine were collected and measured every 30 min twice. Then erysimin G 50 $\mu g \cdot kg^{-1}$ was administered to group A by an intravenous injection; to group B erysimin d_1 50 $\mu g \cdot kg^{-1}$ was administered; and the same volume physiologic saline was administered in group C. Urine was collected once every half an hour three times after administration of the drug and the change in the volume of the urine was measured.

Preparation of TAL of rat kidney The TAL

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was prepared according to the method as previously reported[3]. Rat was sacrificed by decapitation, and the kidneys were removed immediately. After the capsule was removed, the middle section of the kidney was cut with a razor blade into several 1.0 mm-thick slices. The TAL tubules were dissected in HEPES-buffered NaCl Ringer solution under an anatomic microscope at room temperature and transferred onto a 5 mm × 5 mm cover glass coated with cell-Tak to immobilize the tubules. The glass was placed in a chamber mounted on an inverted microscope, and the tubule was superfused with HEPES-buffered NaCl solutions. The tubule was cut open with a sharpened pipette to expose the apical memberane. The temperature of the chamber was maintained at (37 ± 1) ℃.

Patch-clamp technique Patch electrodes were pulled from a vertical pipette puller model PP-830 (Narishige Scientific Instrument Laboratory, Narishige, Tokyo, Japan) in two stages with borosilicate glass capillaries. The pipettes were fire-polished and had resistances of 4 – 6 MΩ when filled with KCl 140 mmol/L. After a tight pipette-membrane seal had been obtained, single channel current was recorded in cell-attached mode[4]. Recordings were made with an Axopatch 200B patch-clamp amplifier and were filtered at 1 kHz. For analysis, the data were collected to an IBM computer and analyzed with the use of pCLAMP software system 6.04 (Axon Instrument California, USA). We define NP_o as an index for determining channel activity in these patches with multiple channels. N means channel number and P_o means open probability. Results are presented as $\bar{x} \pm s$ and compared with paired t test.

RESULTS

Diuretic effect of erysimin G To examine the diuretic effect of erysimin G, we used dose of 50 $\mu g \cdot kg^{-1}$. Data is summarized in Tab 1. Erysimin G induced an increase in urine volume 60 min after administration. Whereas the urine volume increased by erysimin d_1 was less than erysimin G. Futhermore, the time course of the changes in urine volume after administration of erysimin G was about 120 min.

Effect of erysimin G on activity of 70-pS potassium channel Addition of erysimin G 1 $\mu mol \cdot L^{-1}$ decreases the initial control channel activity (NP_o = 1.67 ± 0.10) within 5 min by 94 % ± 4 % (n = 4). Fig 1 is a representative recording showing the effect of erysimin G on the activity of apical 70-pS channel in a cell-attached patch.

Tab 1. Effect of erysimin G on urine volume (UV) every half an hour. n = 6. $\bar{x} \pm s$. $^cP < 0.01$ *vs* control. $^eP < 0.05$ *vs* erysimin G.

Group	UV before/mL	UV after/mL		
		60 min	90 min	120 min
Control	2.6 ± 0.8	4.0 ± 1.6	4.6 ± 1.8	4.1 ± 1.4
Erysimin G	2.4 ± 0.7	9.0 ± 2.1^c	9.8 ± 2.4^c	4.6 ± 1.4
Erysimin d_1	2.8 ± 1.0	7.2 ± 1.2^e	6.0 ± 0.9^e	4.7 ± 1.8

DISCUSSION

Active NaCl reabsorption in thick ascending limb (TAL) takes place by Na^+ transport across the apical membrane by a Na^+-K^+-$2Cl^-$ cotransporter and subsequently by active Na^+ movement via the Na-K-ATPase. The apical K^+ channel plays a key role in K^+ recycling across the apical membrane[5], a process which is essential for maintaining the function of Na^+-K^+-$2Cl^-$ cotransporter and thus for the Na^+ transport in TAL. First, K^+ recycling across the apical membrane maintains the transepithelial current flow, since K^+ exit hyperpolarizes the cell membrane and provides the driving force for Cl^- diffusion across the basolateral membrane. Secondly, K^+ recycling across the apical membrane potentiates the lumen-positive potential, the driving force for paracellular NaCl re-absorption. Thirdly, K^+ recycling provides an adequate K^+ supply for the Na^+-K^+-$2Cl^-$ cotransporter in the cortical TAL, where the K^+ concentration in the lumen is at least one order of magnitude lower than those of Cl^- and Na^+. Although three types of K^+ channel have been found in the apical membrane of TAL, the 30-and 70-pS K^+ channel are mainly responsible for the apical K^+ conductance. A previous study has further shown that 70-pS K^+ is predominant in the TAL[6].

Our studies demonstrated that erysimin G, a main component of erysimum cheiranthoides, increased urine volume. We deduced that the diuretic effect was mediated by inhibition of recycling of potassium across the apical membrane of the cells of TAL which inhibits reabsorption of sodium and chloride. A large body of evidence supports the view that electroneutral Na^+-K^+-$2Cl^-$ cotransporter mediates the entry of these ions from the lumen into the cell[7,8]. Apical potassium channel provides a segment of current loop that includes passive

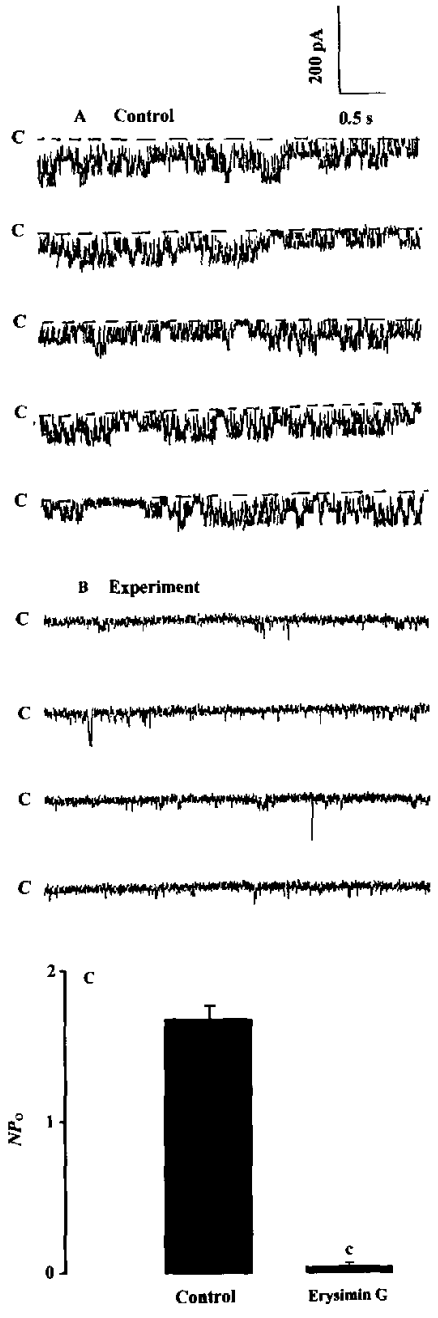

Fig 1. Effect of erysimin G on 70-pS K^+ channel. Pipette holding potential was 0 mV, and "C" indicates channel closed level. A: Control; B: Erysimin G 1 μmol/L in the pipette and KCl 5 mmol/L and NaCl 140 mmol/L in the bath solution. C: The effect of erysimin G on channel activity. Control and erysimin G NP_o was 1.67 ± 0.10 and 0.05 ± 0.02, respectively. $n = 4$. $\bar{x} \pm s$.

sodium movement to blood through the intracellular transport pathway between cells, and a return root that consists of chloride channels in the basolateral membrane and potassium channel in the apical membrane. Interference with such current flow may inhibit the exit of chloride ions from cells and cause a rise in cell chloride concentration. As a consequence, activity of apical Na^+-K^+-$2Cl^-$ cotransporter declines and sodium absorption falls.

An important finding of the present study is that the apical 70-pS potassium channel in TAL is inhibited by erysimin G, which exhibits a diuretic effect. It results in an effective sodium diuresis without potassium loss. And it can be used in treatment of hypertension and some kinds of edema without the side effects of traditional diuretics such as potassium loss.

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桂竹糖芥苷 G 对肾小管功能及髓袢升支粗段 70-pS 钾通道活性的影响[1]

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关键词 桂竹香糖苷；肾；膜片箝技术；钾通道

目的：研究桂竹糖苷 G(erysimine G)的利尿作用及其作用机制. **方法**：应用尿道插管观察给药前后尿量变化；并应用膜片箝技术记录大鼠肾脏髓袢升支粗段 70-pS 钾通道的活性改变. **结果**：给予桂竹糖苷 G 后，实验动物尿量显著增加. 单通道实验记录表明：应用桂竹糖苷 G 1 $\mu mol \cdot L^{-1}$抑制 TAL 70-pS 钾通道的活性，NP_o 由正常对照的 1.67±0.10 降低至 0.05±0.02 ($P<0.01$, $n=4$). **结论**：桂竹糖苷 G 有明显的利尿作用，其利尿机制可能与抑制 TAL 单细胞 70-pS 钾通道的活性有关.

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IMPORTANT ANNOUNCEMENT: WORLD CONGRESS OF PHARMACOLOGY 2010

Subject to approval by the General Assembly at the XIV World Congress of Pharmacology in San Francisco in 2002, the World Conference on Clinical Pharmacology and Therapeutics and the World Congress of Pharmacology will be merged from 2010 to from a joint meeting. The proposed title of the combined meetings will be the "IUPHAR World Congress of Pharmacology", which will take place every two years. Pre-clinical and clinical pharmacology will feature in the complementary programmes for each congress.

Bids from member societies to host the first joint Congress in 2010 are now invited and should be addressed to the IUPHAR Secretary-General:

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