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Properties of a Potassium Channel in the Basolateral Membrane of Renal Proximal Convoluted Tubule and the Effect of Cyclosporine on It

B. YE, Y. LIU[1], Y. ZHANG

Department of Physiology, Xiamen Medical College, Xiamen University, Xiamen City, Fujian Province and [1]Division Nephrology, 153Hospital of People´s Liberation Army, China

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Summary

We studied the potassium channel in the basolateral membrane of the rat proximal convoluted tubule as affected by cyclosporine A. Proximal convoluted tubules were dissected from the rat kidney under a stereoscopic microscope, without a preliminary enzyme treatment. The standard configuration for single-channel tight seal patch-clamp technique was used to record channel currents. A small conductance, stretch-sensitive potassium channel could be observed spontaneously in most of the cell-attached patches as the gigaohm seal was formed. In the inside-out configuration, channel activity was diminished. The K^+ channel appeared to be an inward rectifier. The limiting inward slope conductance was 28.3±1.7 pS (Vp was between 40 mV and 80 mV, n=6) and the outward chord conductance was 5.6±0.3 pS (Vp was between -40 and -60 mV, n=5). The open dwell time constants of the potassium channel were 0.524 ms and 5.087 ms, while the closed dwell time constants were 1.029 ms and 16.500 ms. The opening probability of the channel decreased when the extracellular fluid was acidified. Cyclosporine A had no significant effect on the potassium channel of the proximal tubular cell in the basolateral membrane at concentrations of 10 and 50 μg/ml, while at 100 μg/ml, it decreased the opening probability.

Key words
Proximal convoluted tubule • Basolateral membrane • Potassium channel • Cyclosporine A

Introduction

A large number of microelectrode studies have firmly established that the potassium conductance of the basolateral membrane of proximal convoluted tubule cells is a critical regulator of transport since it is the major determinant of the negative cell membrane potential and is necessary for pump-leak coupling to the Na^+,K^+-ATPase pump (Mauerer *et al.* 1998a). Despite this pivotal physiological role, the properties of this conductance have been incompletely characterized, in part due to difficulty of gaining access to the basolateral membrane of the proximal convoluted tubule. In the initial studies, the kidneys were dissected following treatment with collagenase. It was found that tubules pretreated with collagenase were not satisfactory for functional studies. Due to the polarity of tubule cells, it is difficult to gain access to the basolateral membranes of tubule cells when they are adhering to plastic tissue culture dishes. In this study, we report a potassium

Fax +420 241 062 164
E-mail: physres@biomed.cas.cz http://www.biomed.cas.cz/physiolres

channel in the basolateral membrane of the renal proximal convoluted tubule on a model of fresh tubules suitable for studying the basolateral membrane, with no preliminary enzymatic treatment.

Cyclosporine, a currently employed primary immunosuppressant for the prevention of allograft rejection in solid organ transplantation, may cause both acute and chronic nephrotoxicity (Paul and De Fijter 2004, Grinyo and Cruzado 2004, Vítko and Viklický 2004). However, it has not been reported whether cyclosporine A affects the activity of potassium channels in the kidney directly. In this study we thus tested the effects of cyclosporine A on potassium channels of the basolateral membrane of the renal proximal convoluted tubule.

Methods

Tubule preparation

Wistar rats were killed by decapitation and the kidneys were removed immediately. Thin kidney slices were obtained from a vertical section through the center of the kidney, and placed immediately in a cold preservation fluid. The solution contained (in mM) 30 NaCl, 5 KCl, 1.2 $MgSO_4$, 1.8 $CaCl_2$, 1 NaH_2PO_4, 3 Na_2HPO_4, 4 sodium acetate, 1 trisodium citrate, 25 $NaHCO_3$, 10 glucamine chloride, 128 mannitol, 5.5 glucose and 6 alanine. The solution was stirred constantly and oxygenated by bubbling with a mixture of 95 % O_2 and 5 % CO_2. Using steel needles and fine forceps, the tubules were dissected by hand under a stereo microscope at 15-40 x magnification.

Patch-clamp technique

The standard configuration for single-channel tight seal patch-clamp technique (Hamill *et al.* 1981) was used for recording channel currents from the basolateral membrane of proximal convoluted tubules. The patch pipettes were fabricated from borosilicate glass capillary tubes by means of a two-stage patch pipette puller (PP-83, Narishige Co., Ltd, Tokyo, Japan), and fire-polished. Pipettes were filled with a solution contained (in mM) 140 KCl, 1.5 $MgCl_2$, 10 HEPES, pH 7.4 with KOH. The open tip pipette resistance was 3-8 MΩ when placed in the bath solution. A hydraulic micromanipulator (Narishige) was used to guide the patch microelectrode to the basolateral membrane of the tubular cell. High resistance gigaohm seals (up to 50 GΩ) were obtained on the basolateral membrane of a proximal convoluted tubule by applying gentle suction to the pipette just after it touched the cell membrane. Data were not corrected for liquid-junction potentials since in most solutions they were less than 4 mV when measured as follows: the bath Ag-AgCl ground electrode was connected to the control KCl bath through a 3 % agar bridge made of KCl pipette solution.

Voltage-clamped membrane currents were amplified with a CEZ-2200 patch-clamp amplifier (Nihon Kohden, Japan) controlled by a Tsinghua Tongfang computer (China) with Digidata 1200 A/D board and recorded with pCLAMP software (version 6.0.1, Axon Instruments, USA).

Effect of cyclosporine

In accordance with the report of Carvalho *et al.* (2003), cyclosporine A was prepared in the concentration of 10, 50, 100 μg/ml of the bath solution. The isolated proximal convoluted tubules were perfused with cyclosporine A in different concentrations.

Data analysis

Channel current records were analyzed with pCLAMP software (version 6.0.1 Axon Instruments, USA). Channel activity (nP_o) was calculated over a period of 60 s. The open and closed dwell-time histograms were fitted with exponential components.

Data are given as means ± S.E.M. Statistical analysis was performed with Student's *t*-test where appropriate.

Results

Overview

Under the stereoscopic microscope at 15-40 x magnification, we dissected tubules from a kidney slice, and distinguished the proximal convoluted tubule near to the glomerulus (Fig. 1). Controlled by the experimental set-up, we succeeded in detecting the K^+ channel activity. We obtained the K^+ channel in 76 of 94 patches sealed on the basolateral membrane (81 %) (Fig. 2).

Activity of the channel

The channel activity could be observed spontaneously in most of the cell-attached patches with the gigaohm seal (Fig. 2A). In a minority of cell-attached patches, with no spontaneous channel activity, the channel current could be observed when the microelectrode was pressed into the cell further (Fig. 2B).

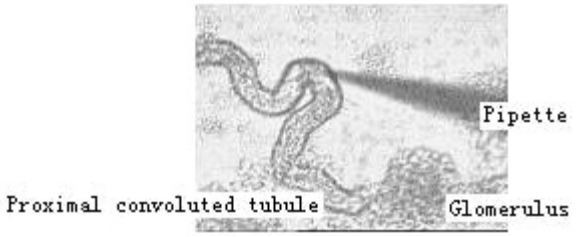

Fig. 1. Proximal convoluted tubule dissected from a slice of the kidney, with no preliminary enzyme treatment

When the cell-attached patch was converted into the excized patch (inside-out configuration), channel activity began to decline and then disappeared. Sucking the pipette with a negative pressure (about 10 cm H_2O), the channel could be transiently activated again in some experiments if the gigaohm seal was not broken (Fig. 2C). The currents were recorded under Vpip = 40 mV in the symmetry solution, i.e. when the membrane potential was –40 mV. The activity of the channel was sensitive to glibenclamide, which suggested it is a kind of potassium channel (see our previous paper, Ye *et al.* 2003).

Single-channel conductance characteristics

Figure 3A shows a series of single recordings obtained at the basolateral membrane of the proximal convoluted tubule at different potentials with the pipette containing 140 mM KCl, with the same solution of KCl in pipette and bath solution. The current-voltage (I-V) characteristic is shown in Figure 3B. The basolateral membrane potassium channel appeared to be an inward rectifier. The channel activity increased with hyperpolarization. The limiting inward slope conductance was 28.3±1.7 pS (Vpip was between 40 mV and 80 mV, n=6) and the outward chord conductance was 5.6±0.3 pS (Vpip was between –40 and –60 mV, n=5).

Kinetics of activated channels

It was observed that there was more than one K^+ channel in most of the patches. To accumulate enough transitions for a meaningful analysis of the long closed state, long recordings were required. The curves of the open dwell time and closed dwell time channel were fitted with an exponential equation. The open dwell time constants of the potassium channel were 0.524 ms and 5.087 ms, while the closed dwell time constants were 1.029 ms and 16.500 ms.

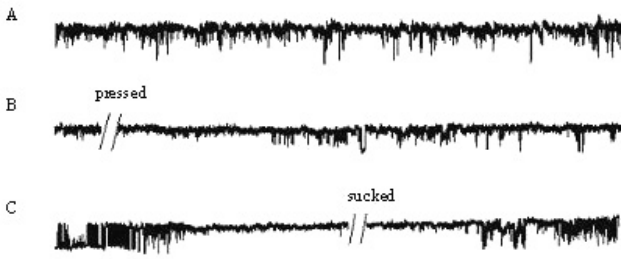

Fig. 2. The channel activity. (**A**) The channel current occurred spontaneously. (**B**) In the minority cell-attached patches, with no spontaneously channel activity, the channel current occurred when the microelectrode was pressed into the cell further. (**C**) When the cell-attached patch was converted into the inside-out configuration, channel activity typically began to decline and then disappeared. Sucking the pipette with negative pressure, the channel current occurred again if the gigaohm seal was not broken. The currents were recorded under Vpip = 40 mV in the symmetry solution.

pH sensitivity

The activity of the K^+ channel was sensitive to pH. Figure 4 shows the effects of different pH on the K^+ channel in the basolateral membrane of proximal tubular cell. Figures 4A, 4B and 4C depict the K^+ channel activities recorded at pH as 7.4, 7.2, 7.0, respectively, in cell-attached patches, under Vpip as 40 mV in the symmetry solution. Figure 4D shows the opening probability of the K^+ channel which was 0.58, 0.31, 0.14 at pH as 7.4, 7.2, 7.0, respectively (n=5).

Effect of cyclosporine

Cyclosporine A had no significant effect on the potassium channel of the proximal tubular cell basolateral membrane at concentrations of 10 and 50 μg/ml. At 100 μg/ml, cyclosporine A decreased the opening probability. Table 1 gives the effects of cyclosporine A on the opening probability of the potassium channel at pH 7.4, Vpip = 40 mV in the symmetry solution (n=5).

Discussion

The proximal convoluted tubule can be considered to function within the general scheme of the epithelial transport model first proposed by Koefoed-Johnsen and Ussing (1958), in which the apical membrane is primarily Na^+ selective and the basolateral membrane is primarily K^+ selective. Since then, a number of studies have shown that K^+-selective ionic channels may contribute significantly to the ionic permeability of the basolateral membrane in many epithelial cells. In the basolateral membrane of the proximal convoluted tubule, potassium channels have been described in the frog,

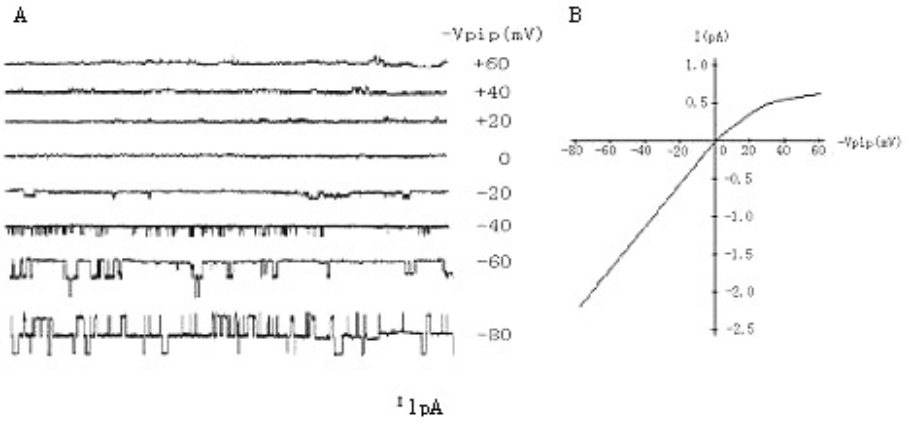

Fig. 3. (**A**) Representative currents recorded at various potentials from a cell-attached basolateral membrane patch in a symmetry solution of KCl. The basolateral membrane potassium channel appeared to be an inward rectifier. The channel activity increased with hyperpolarization. (**B**) Current-voltage relation for the basolateral membrane potassium channel described in A. The limiting inward slope conductance was 28.3±1.7 pS (Vpip was between 40 mV and 80 mV, n=6) and the outward chord conductance was 5.6±0.3 pS (Vpip was between -40 and -60 mV, n=5)

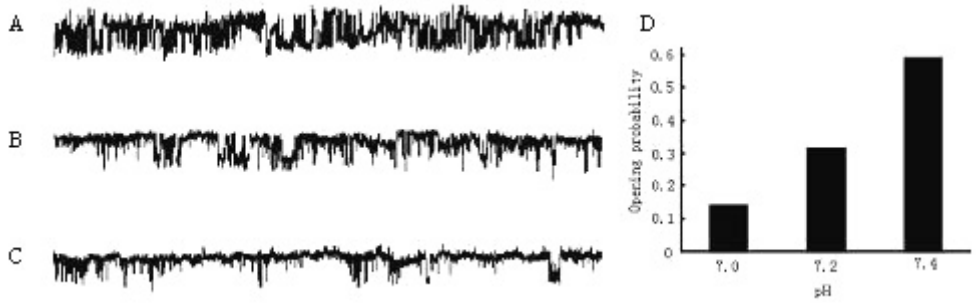

Fig. 4. The effect of pH on the potassium channel in the basolateral membrane of proximal tubular cells. (**A**), (**B**) and (**C**) are currents recorded at pH as 7.4, 7.2, 7.0, respectively, in cell-attached patches. Vpip = 40 mV in the symmetry solution. (**D**) is the opening probability of the channel at different pH. It was 0.58, 0.31, 0.14 at pH as 7.4, 7.2, 7.0, respectively (n=5).

Necturus and rabbit (Beck *et al*. 1993). It has rarely been demonstrated in the rat. In the present study, we are reporting the behavior of the potassium channel at the basolateral membrane of the rat proximal convoluted tubule recorded on a model of fresh tubule, without a preliminary enzyme treatment.

In this experimental system, the channel activity could be observed spontaneously in most of the cell-attached patches after the gigaohm seal had been formed. We tested this in 94 patches, and the K^+ channel activity was detected in 76 patches. It means that 81 % of the patches were positive, which illustrates that this kind of channel is universally present. In a minority of cell-attached patches which show no spontaneous channel activity, but a channel current could be observed when the microelectrode was pressed into the cell further. This implies that the channel is stretch-sensitive.

When the cell-attached patch was converted into the excised patch, the inside-out configuration, channel activity typically began to decline and then disappeared. This indicates that it exhibited the phenomenon of rundown. The mechanism was not explored in this study further. It might be regulated by some factor, for example, when the membrane patch was deprived of the cytosolic components. Further analysis showed that this channel was an inwardly rectifying small conductance potassium channel.

As for the kinetic analysis, the channel exhibited conventional brief openings as in a normal control group. The open-time histogram and the closed-time histogram were both fitted by two exponential components, which conforms with the four-state model of two open states and two closed states. The potassium channel recorded in the basolateral membrane of rat proximal convoluted tubule had two apparent open states and two apparently closed states. Furthermore, the potassium channel was sensitive to pH. This is in agreement with microelectrode studies which showed that the basolateral membrane

Table 1. Effects of cyclosporine A on the opening probability of K^+ channel (n=5)

Cyclosporine A concentration (μg/ml)	Opening probability
0	0.58±0.07
10	0.56±0.05
50	0.61±0.04
100	0.27±0.12*

Data are means ± S.E.M., * Significantly different ($P<0.05$) vs. control group (without cyclosporine)

potassium selectivity is pH sensitive (Kuwahara *et al.* 1989). The results of this study are similar to the reports that studied basolateral membranes in rabbit and in amphibian (Noulin *et al.* 1999, Mauerer *et al.* 1998b).

In this study, we have shown that the potassium channel of proximal convoluted tubule basolateral membrane was stretch-sensitive, which indicates that might depend on the volume or shape of the cell. According to other reports, proximal convoluted tubule potassium channels play a pivotal physiological role in the regulation of membrane voltage, potassium recycling, and ultimately of transepithelial solute and water reabsorption (Giebisch 1999, Hebert *et al*. 2005). The Na^+,K^+-ATPase pump in the basolateral membrane provides the energy that makes ion transport thermodynamically favorable, but a continuous operation of the pump requires the presence of a K^+ exit pathway. The basolateral membrane potassium conductance provides such a pathway and thus a steady-state intracellular K^+ activity can be maintained in the face of large transcellular fluxes of salt and water.

The introduction of cyclosporine into organ transplantation was a landmark achievement leading to a substantial improvement of the early transplant results. On the other hand, cyclosporine may cause both acute and chronic nephrotoxicity (Paul and De Fijter 2004, Grinyo and Cruzado 2004, Vítko and Viklický 2004). In earlier research, it has been demonstrated that cyclosporine is capable of inducing strong vasoconstriction in both pre- and postglomerular vessels, which decrease glomerular filtration and renal blood flow and increase renal vascular resistance. Concomitantly, the sympathetic and renin-angiotensin system may be activated, thereby enhancing impaired intrarenal hemodynamics. In recent reports, cyclosporine was found to affect the renal tubule directly (Aker *et al*. 2001, Petrussa *et al*. 2001, Watanabe *et al*. 2005), causing renal tubular acidosis. This mechanism is complicated. Cyclosporine can prevent Cl^- : HCO_3^- exchange (Watanabe *et al*. 2005), reduce the expression and activity of Na^+-K^+-ATPase and Na^+-K^+-$2Cl^-$ cotransport activity (Aker *et al*. 2001). Furthermore, it has been reported that high cyclosporine concentrations caused Ca^{2+}- and Mg^{2+}-dependent proximal convoluted tubule injury (Carvalho *et al*. 2003). As to the potassium-channel, it was reported that cyclosporine A induces the opening of a potassium selective channel in higher plant mitochondria (Petrussa *et al*. 2001). However, whether cyclosporine affects the renal tubule potassium channel is not clear. In this study, we report that cyclosporine A had no significant effect on the potassium channel of the proximal tubular cell basolateral membrane at concentrations of 10 and 50 μg/ml. At 100 μg/ml, it decreased the opening probability. The consequence of this remains to be elucidated.

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Reprint requests

Benlan Ye, Department of Physiology, Xiamen Medical College, Xiamen University, Xiamen City, Fujian Province, People's Republic of China. E-mail: yebl@sina.com