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Absence of KCNQ1-dependent K^+ fluxes in proximal tubular cells of frog kidney

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Abstract

The present study was designed to investigate the functional significance of KCNQ1-mediated K^+ secretory fluxes in proximal tubular cells of the frog kidney. To this end, we investigated the effects on rapid depolarization and slow repolarization of the peritubular membrane potential after luminal addition of L-phenylalanine or L-alanine plus/minus KCNQ1 channel blockers. Perfusing the lumen with 10 mmol/L L-phenylalanine plus/minus luminal 293B, a specific blocker of KCNQ1, did not modify the rapid depolarization and the rate of slow repolarization. Perfusing the lumen with 10 mmol/L L-alanine plus/minus luminal HMR-1556, a more potent KCNQ1 channel blocker, did not also alter the rapid depolarization and the rate of slow repolarization. Pretreatment (1 h) of the lumen with HMR-1556 also failed to modify rapid depolarization and rate of slow repolarization upon luminal 10 mmol/L L-alanine. Perfusing the lumen with 1 mmol/L L-alanine plus/minus luminal HMR-1556 did not change the rapid depolarization and the rate of slow repolarization. The pretreatment (1 h) with luminal HMR-1556 did not modify the rapid depolarization and the rate of slow repolarization upon luminal 1 mmol/L L-alanine. The pretreatment (1 h) of the lumen with HMR-1556 did not change transference number for K^+ of peritubular cell membrane. Finally, luminal barium blunted the rapid depolarization upon application of luminal 1 mmol/L L-alanine. RT-PCR showed that KCNQ1 mRNA was not expressed in frog kidney. In conclusion, the KCNQ1-dependent K^+ secretory fluxes are absent in proximal tubule of frog kidney.

Keywords: Barium; Cell membrane potential; Chromanol 293B; Frog kidney; HMR1556; KCNQ1; KCNQ1 mRNA expression; Sodium coupled transport; Proximal tubule

1. Introduction

The cotransport of an electroneutral substrate like phenylalanine or alanine with Na^+ across the luminal membrane of epithelia is often associated with an increase of the K^+ conductance at the basolateral cell membrane (Gunter-Smith et al., 1982; Messner et al., 1985). The increase in luminal substrate concentration has been shown to increase Na^+ entry across the luminal membrane, thereby stimulating the basolateral Na^+–K^+–ATPase to extrude more Na^+ in exchange for K^+. Without a commensurate increase in K^+ conductance, the cell would suffer an accumulation of K^+ and excessive osmotic swelling. However, the basolateral membrane of renal proximal tubule exhibits a high K^+ conductance that is largely mediated by K^+-selective ion channels. Although the conductance and voltage dependence of these K^+ channels differ among species, amphibian proximal tubule and small intestine have stretch-activated (Sackin, 1987; Hunter, 1990; Kawahara, 1990; Dubinsky et al., 1999) and ATP-sensitive (Mayorga-Wark et al., 1995; Mauerer et al., 1998a; Dubinsky et al., 2000) K^+ channels. Thus, direct evidence is available for the activation of basolateral stretch-activated K^+ channels of frog proximal tubule in response to isotonic swelling accompanying Na^+-coupled solute absorption (Cemerikic and Sackin, 1993). There is also a good evidence for an ATP-regulated K^+ conductance that allows recycling of K^+ taken up by Na^+–K^+–ATPase (Mauerer et al., 1998b).

Two different K^+ channels have been identified in the luminal membrane of an amphibian proximal tubule. One is a potassium-selective, large conductance K^+ channel which opens probability

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that increases with depolarization and activated by raising intracellular calcium (Kawahara et al., 1987; Filipovic and Sackin, 1991). The other channel is poorly selective for K^+ and activated by a membrane stretch (Filipovic and Sackin, 1992). The activation of these K^+ channels minimizes the depolarization of the luminal membrane upon activation of electrogenic sodium reabsorption (sodium-dependent glucose and amino acid transport): hyperpolarization of the apical membrane maintains the driving force for apical entry of sodium with organic solutes (Giebisch and Wang, 2000; Hebert et al., 2005). Direct evidence for this hypothesis has been presented in the KCNQ1-deficient mice (KCNQ1−/−) with reduced ability of proximal renal tubule cells to maintain the cell membrane potential during Na^+-coupled transport (Vallon et al., 2005). This suggests an important role of the luminal KCNQ1 K^+ channel as the driving force for electrogenic Na^+ and substrate reabsorption.

A K^+ channel α subunit KCNQ1, formerly known as KvLQT1 (Bleich and Warth, 2000; Jespersen et al., 2005) associates with KCNE1 (minK channel, IsK) a K^+ channel β subunit expressed in the apical cell membrane of mammalian proximal renal tubules (Sugimoto et al., 1990; Vallon et al., 2001), the heart and a number of epithelial cells (Demolombe et al., 2001), to produce a current that resembles the slow delayed rectifier current (I_{Ks}) in cardiac myocytes (Barhanin et al., 1996; Sanguinetti et al., 1996). The time course of this slow delayed rectifier current (I_{Ks})—expressed in oocytes (Takumi et al., 1988), is identical to the slowly activating K^+ conductance in the amphibian kidney observed after an enhanced load of substrates for Na^+ coupled transport (Lang and Rehwald, 1992).

The aim of our study is to investigate the functional significance of KCNQ1 K^+ secretory fluxes in proximal tubular cells of the frog kidney. Using conventional electrophysiology, we tested whether the slowly activating K^+ conductance in the amphibian kidney after an enhanced load of substrate for Na^+-coupled transport is pharmacologically identical with $I_{Ks.}$ We tested this hypothesis by using inhibitors of KCNQ1 K^+ channel, such as chromanol 293B and a compound with chromanol structure HMR-1556 (Bleich et al., 1997; Loussouarn et al., 1997; Gerlach, 2001; Gerlach et al., 2001). We monitored the effects of luminal addition of L-phenylalanine or L-alanine, plus/minus KCNQ1 channel blockers, on rapid depolarization and the slow repolarization of the peritubular cell membrane potential difference. The ion selectivity of the peritubular cell membrane for K^+ and the effects of luminally added K^+ channel blocker barium were also evaluated. RT-PCR analysis was used to detect KCNQ1 mRNA expression in frog kidney. Preliminary results were previously reported (Nesovic and Cemerikic, 2005).

2. Materials and methods

2.1. Experimental animals, solutions and chemicals

The experiments were performed at room temperature on isolated, doubly perfused kidneys of *Rana esculenta* of both sexes, prepared according to Wang et al. (1983). The dorsal surface of the kidney, which consists almost entirely of proximal tubule segments (Deeds et al., 1977; Stoner, 1977), was exposed for micropuncture. Aortic and portal vein perfusions were maintained using hydrostatic pressure (50 cm H_2O for aortic perfusion, 30 cm H_2O for portal vein perfusion), and checked by infusion of 0.05 mg/ml fast green dye (Sigma, St. Louis, MO, USA). Amphibian Ringer solution of the following composition (in mmol/L) was used: NaCl 90, KCl 2.5. $NaHCO_3$ 10, NaH_2PO_4 0.5, $CaCl_2$ 1.8, $MgCl_2$ 1, glucose 2.2, dextran (mol/wt. 80 000) 15 g/L and heparin 2000 U/L. The Ringer solution was equilibrated with 1% CO_2–99% O_2 to pH 7.6.

The electrogenic response of the proximal tubular cells to L-phenylalanine (Sigma) was evaluated by switching the aortic perfusate from the control Ringer solution to Ringer solution with addition of 10 mmol/L of L-phenylalanine for iso-osmotic replacement of NaCl with mannitol. Similarly, we tested the electrogenic response of the proximal tubular cells to L-alanine (Sigma) by evaluating the effects of switching the aortic perfusate from control Ringer solution to a solution containing 1, 2, or 10 mmol/L of L-alanine. Barium chloride (Sigma) at a concentration of 5 mmol/L, was also added in aortic perfusate for iso-osmotic replacement of NaCl. K^+ Ringer solution (25 mmol/L) was prepared by iso-osmolar replacement of Na^+ with K^+. Chromanol 293B and its derivative, compound HMR-1556, were generous gifts from Aventis Pharma (Frankfurt, Germany). Both compounds were dissolved in 0.01 ml dimethyl sulfoxide (Sigma) as 100 mmol/L stock solutions and stored in the refrigerator. They were added to the aortic perfusate separately, chromanol 293B at a final concentrations of 30 μmol/L or 90 μmol/L, the compound HMR-1556 at a final concentration of 1.2 μmol/L.

2.2. Electrical measurements

Peritubular cell membrane potentials (PD) were measured with conventional 3 mol/L KCl microelectrodes, made from thick wall capillary tubings having an internal fiber (1.2 mm outside diameter, 0.6 mm inside diameter, Frederick Haer Co., Brunswick, ME, USA). Microelectrodes were fabricated on a horizontal puller (PD-5, Narishige Instrument Lab., Tokyo, Japan). The tip diameter was <1 μm, tip potentials were <5 mV, and input resistance was 40–60 MΩ in standard Ringer solution. Impalement of a proximal tubule cell by the microelectrode was accepted if resulted in an abrupt change in the potential, which was stable for several minutes, and upon withdrawal of the microelectrode from the cell the measured potential returned rapidly to the baseline.

The microelectrodes were connected to the probe of a very high impedance dual electrometer (Analog Devices AD 515 L, Norwood, MA, USA) via an Ag–AgCl wire, and voltage signals were recorded by a two-channel recorder (Linseis, Selb, Germany) and digitally displayed on LED panel. The ground electrode was an Ag–AgCl wire connected to the kidney by a 3 mol/L KCl 3% agar bridge.

2.3. Calculation of the transference number for potassium (t_K)

The apparent transference number for potassium (t_K, apparent slope potassium conductance over slope membrane conductance) was calculated from the peak rapid voltage deflection (ΔPD) of the peritubular cell membrane observed upon rapid change (by changing the aortic for portal vein