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A calcium-permeable non-selective cation channel in the thick ascending limb apical membrane of the mouse kidney

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ABSTRACT

Non-selective cation channels have been described in the basolateral membrane of the renal tubule, but little is known about functional channels on the apical side. Apical membranes of microdissected fragments of mouse cortical thick ascending limbs were searched for ion channels using the cell-free configuration of the patch-clamp technique. A cation channel with a linear current–voltage relationship (19 pS) that was permeable both to monovalent cations [$P_{NH4}(1.7) > P_{Na}$ $(1.0) = P_K$ (1.0)] and to Ca^{2+} ($P_{Ca}/P_{Na} \approx 0.3$) was detected. Unlike the basolateral TRPM4 Ca^{2+}-impermeable non-selective cation channel, this non-selective cation channel was insensitive to internal Ca^{2+}, pH and ATP. The channel was already active after patch excision, and its activity increased after reduced pressure was applied via the pipette. External gadolinium (10^{-5} M) decreased the channel-open probability by 70% in outside-out patches, whereas external amiloride (10^{-4} M) had no effect. Internal flufenamic acid (10^{-4} M) inhibited the channel in inside-out patches. Its properties suggest that the current might be supported by the TRPM7 protein that is expressed in the loop of Henle. The conduction properties of the channel suggest that it could be involved in Ca^{2+} signaling.

1. Introduction

Cation channels are a heterogeneous molecular family including ligand-gated channels [1], cAMP- and cGMP-dependent channels [2], mechanosensitive channels and TRPs [3], many of which are permeable at least to some extent to calcium. Many cation channels have been identified on native tissues using the patch-clamp technique. In the renal tubule, patch-clamp studies have revealed the functional expression of the Ca^{2+}-activated non-selective cation channel TRPM4 [4–6], of a cGMP-inhibited channel[7], of a pressure activated cation channel, and of a volume sensitive channel [8–10]. There is also non-electrophysiological evidence for the presence of P2X receptor-channels [11].

One specific domain of research concerns the channels that mediate the entry of calcium into the cell, and are involved in hormonal/paracrine transduction cascades [12,13]. Store-operated channels and agonist-activated channels with high selectivity for calcium have been reported in several tissues, and although their precise identity is still a matter of debate, this has made it possible to some extent to disentangle the modes of hormonal activation in these tissues. Electrophysiological data on Ca^{2+} permeable channels are especially sparse for the renal tubule, where research has so far focused on channels involved in the transepithelial absorption of calcium and magnesium: TRPV5, TRPV6 and TRPM6 [14].

Here we report the properties of a non-selective cation (NSC) channel in the cortical thick ascending limb (CTAL), which has hitherto unprecedented properties in the renal tubule: this channel is located at the apical membrane, and it has a conductance of 20 pS that is similar to that of a previously described Ca^{2+}-dependent, non-selective, cation channel located at the basolateral membrane of the mouse CTAL, but which is not permeable to Ca^{+} [4–6], in contrast to this new channel. Its electrophysiological properties are also clearly different from those of TRPV5, TRPV6 and TRPM6, which are highly selective for calcium and magnesium. The physiological role of the channel requires further investigation, but its location near the key ion transport systems (Na^{+}–K^{+}–Cl^{-} and K^{+} channels) suggests a possible role in calcium signaling and ion transport regulation.

Abbreviations: CTAL, cortical thick ascending limb; E_r, reversal potential; FA, flufenamic acid; IMCD, inner medullary collecting duct; NSC channel, non-selective cation channel; NSC_{Ca} channel, calcium-activated non-selective cation channel; P_o, open probability; TRP, transient receptor potential

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2. Methods

2.1. Tubule isolation

All animal experiments were performed according to the European Commission directive 2010/63/EU. Male mice (15–20 g) were euthanized by cervical dislocation, and renal tubules were obtained as previously described [6]. One kidney was perfused *via* the renal vein with Leibowitz medium (Eurobio, Les Ulis, France) containing 300 U/ml Worthington collagenase (CLS II). Small kidney slices of varying mass were incubated in the same solution at 37 °C for 45–75 min. Fragments of CTAL were dissected out from tissue immersed in ice-cold Leibowitz medium under a stereomicroscope. The fragments were placed on a plastic dish coated with poly-L-lysine in a recording chamber, and cut open along their long axis with a micropipette to expose the apical membrane.

2.2. Solutions and chemicals

The standard solution (140 mM NaCl solution) used in the bath and in the pipette contained (in mM): 140 NaCl, 4.8 KCl, 1.2 $MgCl_2$, 1 $CaCl_2$, 10 glucose, and 10 HEPES. Low NaCl solutions had the same composition, except that the NaCl concentration was reduced to 42 or 14 mM NaCl (200 or 256 mM sucrose was added to maintained osmolarity and, no KCl was included).

For tests of the selectivity between monovalent cations, 140 mM NaCl was replaced by the same concentration of KCl or NH_4Cl. The Ca^{2+} permeability was determined using a solution containing (in mM) 100 $CaCl_2$, 10 glucose, and 10 HEPES. All solutions were adjusted to pH 7.2. Internal Ca^{2+} concentrations below 10^{-5} M were produced with $CaCl_2$ and Ca-EGTA buffers [6]. Flufenamic acid, nucleotides, EGTA, gadolinium tetrachloride and ammonium chloride were obtained from Sigma-Aldrich-Fluka (L'Isle d'Abeau Chesnes, France), other chloride salts were from Merck (Nogent-sur-Marne, France), and amiloride was from LC laboratories (MA, USA).

2.3. Measurements

Single-channel currents were recorded with List LM-EPC7 (List Electronics, Darmstadt, Germany) or RK 400 (Bio-logic, Claix, France) patch-clamp amplifiers from patches of apical membranes using the inside-out and outside-out variants of the patch-clamp technique. Patch pipettes with a tip resistance of 7–12 MΩ (in 140 mM NaCl solution) were made from microhematocrit borosilicate glass tubes, and were coated with Sylgard (Dow Corning, Seneffe, Belgium). The signal was displayed on an oscilloscope (Tektronix, Beaserton, CA, USA) or a computer-generated scope (Axoscope, Axon, CA) and stored on a DAT recorder (Sony PCM 701 ES). The bath reference was an Ag/AgCl pellet connected to an agar bridge filled with a 0.5-M KCl solution. Liquid junction potentials were determined by placing a patch pipette filled with 2.7 M KCl successively in standard and test solutions, and measuring the voltage deflections in zero current clamp. The applied potentials ($Vm = V_{bath} - V_{pipette}$) were corrected accordingly. The pipette-filling solution differed from the initial bath solution (140 mM NaCl) for one experimental protocol in which a Ca^{2+}-rich solution was used instead of a solution containing 140 mM NaCl solution was used. In this case, we took into account the fact that the liquid junction potential disappeared after sealing, unmasking the compensating voltage used to offset the junction potential before sealing. Currents due to the migration of cations from the inner to the outer surface of the membrane were positive, and were registered as upward deflections in single-channel current tracings. Experiments were conducted at room temperature (20–24 °C).

2.4. Data analysis

Signals for analysis were filtered at 300 Hz with an 8-pole Bessel filter (Frequency Devices, Haverhill, MA, USA), and digitized at 1 kHz using a Digidata 1200A analog–digital interface and Axoscope software (Axon Instruments, Foster City, CA, USA). The channel-open probability (P_o) was calculated from the equation $I = N\ P_o\ i$, where i is the amplitude of the unit current, and N the apparent number of channels of the patch. The mean current (I) passing through the N channels present in the patch was estimated from current amplitude histograms. Relative permeabilities and associated reversal potentials for current flow (E_r) were estimated by fitting experimental measurements to the Goldman–Hodgkin–Katz equation:

$$i = gV \sum_s (P_s/P_{Na}) z_s{}^2 \left([S]_i e^{\frac{zFV}{RT}} - [S]_o \right) / [Na]_o \left(e^{\frac{zFV}{RT}} - 1 \right)$$

with $g = P_{Na}[Na]_o F^2/RT$, where V, P_{Na}, F, R and T have their usual meaning. P_s represents the permeability of the channel to the S ion; subscripts i and o refer to the inside and outside, respectively. The relative permeabilities of the non-selective cation channel to monovalent cations were deduced from E_r values after linear fitting of the data points since no current rectification was apparent when K^+ or NH_4^+ was substituted for Na^+ in the bath. The activity coefficients used to correct the ion concentrations were taken from Robinson and Stokes [15], and had the following values: 0.75 (140 mM monovalent chloride salts), 0.83 (40 mM NaCl), 0.89 (20 mM NaCl) and 0.52 (100 mM divalent chloride salts). Experimental values are given as means ± SEM, and n denotes the number of results. The groups were compared using Student's *t*-test or Mann–Whitney Rank sum test (SigmaStat; SpSS GmbH, Erkrath, Germany).

3. Results

A calcium-activated non-selective cationic channel has been characterized in basolateral membrane of mouse CTAL [6], but nothing is known about the presence of non-selective cation channels in the apical membrane. To investigate this, we recorded single channel currents in apical membranes from microdissected mouse CTAL.

Channel activity was recorded using the inside-out patch-clamp configuration with 140 mM NaCl (1 mM $CaCl_2$) on both sides of the membrane. Channel activity was detected in most excised patches (27 out of 36 patches, 75%), with $P_o = 0.66 \pm 0.04$ ($n = 24$) and 4.2 ± 0.6 channels per patch ($n = 24$) (Fig. 1).

3.1. Conductance and permeabilities

The current recordings in Fig. 1A show the activity of this channel at various voltages. No voltage dependence was apparent (Fig. 1A, B). The channel displayed a linear current–voltage relationship (Fig. 1C), with a unit conductance of 19 ± 0.9 pS ($n = 10$) and an E_r (0.8 ± 0.9 mV) not significantly different from 0 mV.

Ion selectivity was assessed by ion-substitution experiments. When a membrane patch was exposed to 140 mM NaCl in the pipette (extracellular side) and 42 or 14 mM NaCl in the bath (intracellular side), the current/voltage relationship was shifted toward the positive voltage range (Fig. 1B, C). The reversal potentials were 22.1 ± 1.1 mV ($n = 4$) and 40.2 ± 1.8 mV ($n = 7$) for 42 and 14 NaCl, respectively, without any change in single channel conductance (see Table 1). When mean reversal potentials were plotted as a function of log ($[cation]_e/[cation]_i$), the points fell on a line with a slope of 39.91 ± 0.02 mV per decade (Fig. 1D), indicating that the channel had substantial cation selectivity. The permeability ratio P_{Na}/P_{Cl} calculated from the reversal potentials using the Goldman–Hodkin–Katz equation was similar under both conditions (*i.e.*, 0.08 ± 0.02 and