Apical membrane of native OMCD\textsubscript{i} cells has nonselective cation channels

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Received 26 June 2000; accepted in final form 18 December 2000

The apical membrane of native OMCD\textsubscript{i} cells has nonselective cation channels. Am J Physiol Renal Physiol 280: F48–F55, 2001.—The purpose of this study was to examine cation channel activity in the apical membrane of the outer medullary collecting duct of the inner stripe (OMCD\textsubscript{i}) using the patch-clamp technique. In freshly isolated and lumen-opened rabbit OMCD\textsubscript{i}, we have observed a single channel conductance of 23.3 ± 0.6 pS (n = 17) in cell-attached (c/a) patches with high KCl in the bath and in the pipette at room temperature. Channel open probability varied among patches from 0.06 ± 0.01 at −60 mV (n = 5) to 0.31 ± 0.04 at 60 mV (n = 6) and consistently increased upon membrane depolarization. In inside-out (i/o) patches with symmetrical KCl solutions, the channel conductance (22.8 ± 0.8 pS; n = 10) was similar as in the c/a configuration. Substitution of the majority of Cl− with gluconate from KCl solution in the pipette and bath did not significantly alter reversal potential (E\textsubscript{rev}) or the channel conductance (19.7 ± 1.1 pS in asymmetrical potassium gluconate, n = 4; 21.4 ± 0.5 pS in symmetrical potassium gluconate, n = 3).

Xia, Shen-Ling, Seung-Hyun Noh, Jill W. Verlander, Craig H. Gelband, and Charles S. Wingo. Apical membrane of native OMCD\textsubscript{i} cells has nonselective cation channels. Am J Physiol Renal Physiol 280: F48–F55, 2001.—The purpose of this study was to examine cation channel activity in the apical membrane of the outer medullary collecting duct of the inner stripe (OMCD\textsubscript{i}) using the patch-clamp technique. In freshly isolated and lumen-opened rabbit OMCD\textsubscript{i}, we have observed a single channel conductance of 23.3 ± 0.6 pS (n = 17) in cell-attached (c/a) patches with high KCl in the bath and in the pipette at room temperature. Channel open probability varied among patches from 0.06 ± 0.01 at −60 mV (n = 5) to 0.31 ± 0.04 at 60 mV (n = 6) and consistently increased upon membrane depolarization. In inside-out (i/o) patches with symmetrical KCl solutions, the channel conductance (22.8 ± 0.8 pS; n = 10) was similar as in the c/a configuration. Substitution of the majority of Cl− with gluconate from KCl solution in the pipette and bath did not significantly alter reversal potential (E\textsubscript{rev}) or the channel conductance (19.7 ± 1.1 pS in asymmetrical potassium gluconate, n = 4; 21.4 ± 0.5 pS in symmetrical potassium gluconate, n = 3).

Experiments with 10-fold lower KCl concentration in bath solution in i/o patches shifted E\textsubscript{rev} to near the E\textsubscript{rev} of K+. The estimated permeability of K+ vs. Cl− was over 10, and the conductance was 13.4 ± 0.1 pS (n = 3). The channel did not discriminate between K+ and Na+, as evidenced by a lack of a shift in the E\textsubscript{rev} with different K+ and Na+ concentration solutions in i/o patches (n = 3). The current studies demonstrate the presence of cation channels in the apical membrane of native OMCD\textsubscript{i} cells that could participate in K+ secretion or Na+ absorption.

THE OUTER MEDULLARY COLLECTING DUCT of the inner stripe (OMCD\textsubscript{i}) is a major renal segment for luminal fluid acidification (15, 29) and exhibits large rates of proton secretion due to H\textsuperscript{+}-ATPase and H\textsuperscript{+}-K\textsuperscript{+}-ATPase (34). Although this segment actively reabsorbed K\textsuperscript{+} when the animal was fed a K\textsuperscript{+}-restricted diet (K\textsuperscript{+} restriction; see Refs. 30 and 31), the OMCD\textsubscript{i} had little net K\textsuperscript{+} transport when the animal was fed a normal K\textsuperscript{+}-containing diet (K\textsuperscript{+} replete; see Refs. 4, 25, and 30). In addition, either luminal K\textsuperscript{+} removal or luminal application of Ba\textsuperscript{2+} inhibited acidification in the OMCD\textsubscript{i} of K\textsuperscript{+}-replete rabbits (2). These observations suggest that luminal K\textsuperscript{+}, which is reabsorbed via H\textsuperscript{+}-K\textsuperscript{+}-ATPase, recycles back to the lumen through K\textsuperscript{+} exit pathways under K\textsuperscript{+}-replete circumstances.

However, cation channel conductances have never been directly demonstrated in the apical membrane of the native OMCD\textsubscript{i}. Although a whole cell K\textsuperscript{+} conductance was observed in primary culture of rabbit OMCD\textsubscript{i} cells (23) and a cation channel mRNA was detected in the mouse outer medullary collecting duct (OMCD; see Ref. 6), the contribution of the channel conductance from the apical or basolateral membrane was not detailed.

The purpose of the present study was to examine directly whether K\textsuperscript{+}-permeable ion channels are present at the apical membrane of the native OMCD\textsubscript{i}. Using cell-attached patch and inside-out patch configurations, we detected single channel conductances on the apical membrane of the native OMCD\textsubscript{i} under K\textsuperscript{+}-replete circumstances. The primary channel we observed had an increased channel open probability (P\textsubscript{o}) when the patched membrane was depolarized, a high permeability of K\textsuperscript{+} over Cl−, and approximately the same permeability to Na\textsuperscript{+} as to K\textsuperscript{+}. Determination and characterization of this cation channel in the apical membrane of the native OMCD\textsubscript{i} cells extend our previous observations and help us to better understand ion regulation in the acidification process.

METHODS

Cell preparations. The dissection of OMCD was similar as described previously (2, 30, 31). In brief, New Zealand White female rabbits (1.0–1.8 kg) were fed a normal-K\textsuperscript{+} diet and were allowed free access to tap water. The rabbits were decapitated, and one kidney was quickly removed, sliced into 1- to 2-mm slices, and placed in chilled Ringer solution (<10°C) that contained (in mM) 135 NaCl, 5 KCl, 1.5 CaCl\textsubscript{2}, 1 MgCl\textsubscript{2}, 5 glucose, and 10 HEPES (pH 7.4 with NaOH). Isolation of single OMCD tubules from those slices was...
carried out in the same solution. The inner border of the OMCD was defined as the transition of the white medulla into the pink medulla, and the segment of the OMCD was chosen from those directly adjacent to thin descending limbs and thick ascending limbs of Henle's loop. In the case of the outer stripe of the OMCD (OMCDo), the outer border of the segment was defined as the transition of the brown cortex into the pink medulla.

The microdissection of the OMCD was much harder than that of the cortical collecting duct (CCD), and most of the dissection procedures were completed under a dissection microscope with up to \( \times 40 \) amplification (M32; Wild, Heerbrugg, Switzerland). The native tubules were manually slit open by a fine needle or a dissection probe (500135; World Precision Instruments, Sarasota, FL), and then the opened area was extended by a pair of fine forceps. The lumen-opened tubule was placed on a small glass coverslip coated with tissue adhesive (Cell-Tak; Collaborative Biomedical Products, Bedford, MA), which was then transferred to a perfusion chamber (area \( 0.75 \text{ cm}^2 \), volume \( 0.5 \text{ ml} \)) for patch-clamp recording. The tubule was continuously perfused at room temperature (\( \sim 20^\circ \text{C} \)). Figure 1 shows a typical OMCD preparation with the apical membrane facing up.

The experiment was conducted under an inverted microscope (TE300; Nikon, Melville, NY) with a \( \times 40 \) air objective (numerical aperture is 0.65 for Phase Contrast optics and 0.55 for Hoffman Modulation Contrast optics). Photomicrography was done on a Nikon F-3 camera mounted on the microscope front port (intermediate magnification, \( \times 2 \)).

**Single channel recording and solutions.** An Axopatch 200B amplifier with a DigiData 1200 interface (Axon Instruments, Foster City, CA) connected to a 166-MHz Pentium computer (Optiplex GXi; Dell Computer, Round Rock, TX) was used for all recordings. Patchex software (pClamp 6.0.4; Axon Instruments) was used for data collection. Single channel currents were sampled at 10 kHz, filtered at 2 kHz, and stored on the computer hard disk and on a digital videotape recorder (500C videotape recorder with 3000A PCM recording adaptor; Vetter Digital, Rebersburg, PA) for further analysis.

Patch pipettes were pulled from borosilicate capillary glass using a micropipette puller (PP-83; Narishige International, East Meadow, NY) and fire polished using a microforge (MF-83; Narishige International); the pipettes typically had tip resistance between 5 and 10 M\( \Omega \) when filled with 140 mM KCl solution. The reference electrode was an Ag/AgCl wire connected to the bath through a salt bridge made of a glass pipette filled with 2% agar (Difco Laboratories, Detroit, MI) in 3 M KCl.

Single channel recordings were carried out as described by Hamill et al. (7). The pipette solution contained (in mM) 140 KCl (or 135 KCl, 5 NaCl), 1 MgCl\(_2\), and 10 HEPES. For cell-attached patch experiments, KCl bath solutions contained (in mM) 135 KCl, 5 NaCl, 1.5 CaCl\(_2\), 1 MgCl\(_2\), 5 glucose, and 10 HEPES. For inside-out patch experiments, KCl bath solution contained (in mM) 135 KCl and 5 NaCl (or 140 KCl), 1 MgCl\(_2\), 1–2 EGTA, and 10 HEPES. Potassium gluconate solution used for both bath and pipette contained (in mM) 130 potassium gluconate, 10 KCl, 1 MgCl\(_2\), 10 HEPES, and 1 EGTA. Tetraethylammonium chloride (TEA-Cl) bath solution contained (in mM) 130 TEA-Cl, 5 CsCl, 5 NaCl, 1 MgCl\(_2\), 10 HEPES, and 1 EGTA. Low-KCl solution used for bath and pipette contained (in mM) 14 KCl, 1 MgCl\(_2\), 10 HEPES, and mannitol (osmolarity of 290–296 mmol/kg\( \text{H}_2\text{O} \)). NaCl solution for inside-out patches contained (in mM) 135 NaCl, 5 KCl, 1 MgCl\(_2\), 10 HEPES, and 1 EGTA. All of the solutions were adjusted to pH 7.4 with KOH or NaOH according to the major cation in the solutions. Chemicals used in this study were purchased from Sigma Chemical (St. Louis, MO).

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**Fig. 1.** Appearance of native rabbit outer medullary collecting duct inner stripe (OMCD\(_i\)) cells. Lumen-opened OMCD\(_i\) in phase-contrast optics (A) and in Hoffman modulation contrast optics (B) are shown. Images were made under a Nikon Eclipse TE300 inverted microscope with a \( \times 40 \) air objective in phase-contrast optics \([\text{numeric aperture (NA) } = 0.65]\) or Hoffman modulation contrast optics \([\text{NA} = 0.55]\). Final magnification is \( \times 683 \), and the size of each panel is \( 300 \times 92 \text{ \mu m} \).
Data analysis. Single channel analysis was performed on records obtained from patches with seal resistance >1 MΩ. Fetchan and pSTAT software (pClamp 6.0.4; Axon Instruments) were used for calculating the mean channel current amplitude and for estimating channel $P_o$. The threshold of current amplitude between the two current levels was manually set. Because most patches contained more than one active channel and the total number of channels within a patch could not be determined, $P_o$ was used to describe the channel open activity (or to estimate the open activity from total number of channels in the patch, if $NP_o$ is used)

$$P_o = \frac{[\Sigma (n\cdot t_n)/T]/N}{P}$$

where $N$ is the number of active channels observed in the patch recording, $T$ is the total recording time at a given holding potential (20- to 40-s data sampling periods), and $t_n$ is the total channel opening time at each current level ($n = 1$ to $N$). The number of active channels was estimated by observing the number of peaks on current amplitude histograms. Because the active channels in the patch may open at different times without overlapping each other, $N$ might be underestimated (therefore $P_o$ may be overestimated).

In the present studies, all of the voltages ($V$) are reported as transmembrane potentials of the patch from inside the cell with respect to the inside of the recording pipette, and single channel unit currents ($i$) are called outward currents as the flow of cations is from the inside of the cell moving out of the membrane into the recording pipette (plotted as upward transitions). The Gaussian digital filter with cutoff frequency of 200 Hz was applied to all current traces plotted in Figs. 2 and 5. The mean current amplitude was graphed against the membrane potential. The unitary conductance and reversal potentials ($E_{rev}$) were derived from the slope by fitting a linear regression (with confidence of 95%) to the data points in the $i$-$V$ curve. The liquid junction potentials were estimated by using JPCalc, a program developed by Barry (5), and the correction was made only to $i$-$V$ curves when asymmetrical solutions were presented in the bath and pipette (18). Origin 4.0 (Microcal Software, Northampton, MA) was used for statistical analysis and graphics. The data are reported as means ± SE. Statistical significance was examined using the two-tailed paired or unpaired Student’s $t$-test. A value of $P < 0.05$ was considered significant.

RESULTS

Cell-attached patch studies. To examine whether single ion channel conductances exist in the apical membrane of the OMCD, we first used the cell-attached patch-clamp technique. A high-KCl bath solution was used in these experiments to depolarize the cell membrane potential. With the use of this approach, the voltage across the patch membrane could be determined by the command potential in the patch pipette. In 268 patches when KCl was in the bath and pipette solutions, we observed 50 patches with single channel activity. Figure 2 presents current traces of spontaneous channel activity recorded from an OMCD cell at different membrane potentials. The channel activity was also detected in the apical membrane of the OMCD (8 of 35 patches) and the OMCD (the gray area between the OMCDm and the OMCD; 7 of 38 patches). Because the single channel data among the segments did not show a distinguishable difference, we have grouped the cells together for data analysis.

The channel opening was variable in each experiment as $P_o$ varied between 0.03 and 0.1 at −60 mV and between 0.21 and 0.47 at 60 mV. However, $P_o$ was consistently increased upon membrane depolarization in the cells recorded, and $P_o$ averaged from 0.06 ± 0.01 at −60 mV ($n = 5$) to 0.31 ± 0.04 at 60 mV ($n = 6$), Figure 3B shows the relation between $P_o$ and membrane potentials. $P_o$ at −20 mV (0.12 ± 0.02; $n = 4$; $P = 0.05$) and at 20 mV (0.21 ± 0.06; $n = 4$; $P = 0.02$) are higher than that at −60 mV (0.06 ± 0.01; $n = 5$). The single channel $i$-$V$ curve had an $E_{rev}$ near 0 mV when KCl was in the bath and pipette solutions (Fig. 4A). The single channel conductance obtained from the $i$-$V$ curve was 23.3 ± 0.6 pS ($n = 17$).

Inside-out patch studies. When inside-out patches were formed from these cells, >60% of the cells (23 of 37) could maintain the channel activity for a number of minutes. Figure 4B shows the $i$-$V$ curve from excised patches when KCl was present in the pipette and bath solutions. The slope conductance was 22.8 ± 0.8 pS ($n = 10$), which is similar to that in the cell-attached configuration.

Ion selectivity studies. We carried out experiments in the inside-out patch configuration to clarify the ion...
selectivity of the channel observed in the OMCD cells.

First, to distinguish cationic current from an anionic one, we replaced all but 10 mM KCl in the pipette solution with potassium gluconate [i.e., Cl\(^{-}\) concentration ([Cl\(^{-}\)]) was altered from \(\approx 140\) to \(\approx 10\) mM]. When KCl solution was present in the bath, similar channel activity was observed in cell-attached patches (data not shown) and in inside-out patches (Fig. 5A). When the bath solution was changed from KCl to potassium gluconate, the channel activity was unaffected. Because K\(^{+}\) concentration ([K\(^{+}\)]) was much greater than [Cl\(^{-}\)], both in the pipette solution and in the bath solution, these data suggest that the current conducted through the recording channels was mainly carried by K\(^{+}\).

In the excised patch with KCl in the pipette solution, however, when the bath KCl solution was replaced with TEA-Cl, the current was abolished (Fig. 5B). Because the [Cl\(^{-}\)] on both sides did not change, this implies that the channel current was carried by K\(^{+}\).

The \(i-V\) curve in the asymmetrical potassium gluconate/KCl condition shows that the \(E_{\text{rev}}\) did not shift notably, which suggests that anionic conductance (Cl\(^{-}\) in this case) did not play a major role in this channel activity (Fig. 6A). The slope conductance at this recording condition was \(19.7 \pm 1.1\) pS (\(n = 4\)). When K\(^{+}\) became the only major permeable ion in the symmetrical potassium gluconate/potassium gluconate recording condition (Fig. 6B), the single channel conductance was \(21.4 \pm 0.5\) pS (\(n = 3\)). These data further support the idea that the major conductance was contributed by K\(^{+}\).

To evaluate the selectivity between K\(^{+}\) and Cl\(^{-}\), we reduced KCl in the pipette solution from 140 to 14 mM (i.e., reducing KCl by substitution with the nonelectrolyte mannitol). Figure 7A shows that, with 140 mM KCl in the bath, average \(E_{\text{rev}}\) was shifted in a negative direction to about \(-53\) mV (\(-52.8 \pm 1.6\) mV; \(n = 3\)) in the cell-attached configuration. Because intracellular [K\(^{+}\)] usually is high, this would be expected if the conductance was contributed from K\(^{+}\). Data from inside-out patches had similar \(E_{\text{rev}}\) (approximately \(-51\) mV; \(n = 2\)). With the use of the Goldman-Hodgkin-
Katz (GHK) voltage equation, the relative permeability of Cl⁻ over K⁺ ($P_{Cl}/P_{K}$) is estimated as 0.034 or 1:30. The channel conductance at this recording conduction was 19.3 ± 0.9 pS ($n = 3$) in cell-attached patches and 20 pS ($n = 2$) in inside-out patches.

We did another series of experiments in the inside-out configuration with 140 mM KCl in the pipette (Fig. 7B). When the bath solution was changed from 135 mM KCl to 14 mM KCl, $E_{rev}$ was shifted from near 0 mV to a positive membrane potential (i.e., depolarization direction, K⁺ reversal potential ~60 mV). This indicates that the change was due to the decrease of cationic conductance ($E_{rev} = 42.3 ± 1.5$ mV; $n = 3$). If the conductance change was due to Cl⁻, we would expect the $E_{rev}$ to shift from zero to a negative membrane potential (i.e., hyperpolarization direction, Cl⁻ reversal potential approximately ~60 mV). With the use of the GHK equation, estimated $P_{Cl}/P_{K}$ is ~0.09 or 1:11. The slope conductance was changed from 23.7 ± 0.5 pS in high-KCl bath solution to 13.4 ± 0.1 pS in low-KCl bath solution ($n = 3$). Thus this series of studies further supports the conclusion that a cationic channel conductance is present in the apical membrane of the rabbit OMCD₁ cells.

**Cation selectivity studies.** The next set of experiments tested the cation selectivity of the single channel conductance in this segment. In inside-out configuration with KCl solution in the pipette, when the bath solution was switched from KCl solution to NaCl solution, only a very small shift in $E_{rev}$ to positive potential occurred (~2.7 mV; $n = 3$; Fig. 8). The relative permeability of this channel to Na⁺ over K⁺ is ~0.9 or 1:1.1. The channel conductance was 21.3 ± 1.5 and 21.6 ± 2.3 pS in KCl/KCl solution and in KCl/NaCl solution, respectively (paired, no significant difference; $n = 3$). These data suggest that this channel could conduct Na⁺ as well as K⁺ under the same recording conditions. A similar i-V curve was obtained with a slope conductance of 22.7 ± 0.9 pS ($n = 5$) in the inside-out configuration with KCl solution in the pipette. Traces show the outward current carried by K⁺ either in the KCl bath or K-glu bath in the same cell. B: in another inside-out patch recording with KCl solution in the pipette, replacing KCl by tetraethylammonium chloride (TEA-Cl) in the bath (i.e., the Cl⁻ concentration remained the same) abolished the outward current. Transmembrane potential was at 40 mV.

Fig. 5. Permeable K⁺ channel. A: representative current traces from an inside-out patch recording with potassium gluconate (K-glu) solution in the pipette. Transmembrane potential was at 55 mV. Traces show the outward current carried by K⁺ either in the KCl bath or K-glu bath in the same cell. B: in another inside-out patch recording with KCl solution in the pipette, replacing KCl by tetraethylammonium chloride (TEA-Cl) in the bath (i.e., the Cl⁻ concentration remained the same) abolished the outward current. Transmembrane potential was at 40 mV.

Fig. 6. Channel conductance was not affected significantly in the asymmetrical concentration or low concentration of Cl⁻. A: i-V curve averaged from 4 cells in the inside-out patches when 130 mM potassium gluconate + 10 mM KCl was in the pipette and 140 mM KCl in the bath. The reversal potential was not shifted and was still near 0 (which is near K⁺ reversal potential; Cl⁻ reversal potential would be near 70 mV at this condition). The channel conductance (19.7 ± 1.1 pS) was not changed significantly when comparing with those recorded at symmetrical KCl solution. B: when excised patches were in the symmetrical 140 mM K⁺ and 10 mM Cl⁻ solution, the i-V curve averaged from 3 cells showed that the conductance was carried mainly by K⁺.
configuration with NaCl in the pipette solution and KCl in the bath solution (data not shown).

Other observations. Besides this most frequently observed 23-pS channel current, other ionic unitary current levels were also observed. Among them, one exhibited a relative small channel conductance of 10 pS ($n = 7$) and had a relative linear $i$-$V$ relationship. The other two were outward rectified with chord conductance (inward/outward) of 16/50 pS ($n = 11$) and of 41/67 pS ($n = 3$). All of the conductances were measured in the cell-attached configuration with KCl solution in the pipette and in the bath. Our observations indicate that the 16/50 pS channel was consistent with a cation channel (35). Because these channels were infrequently observed, their characteristics were unable to be explored in detail. It is not clear if their low occurrences were due to either the low density of the channel or the inactive state of the channel.

**DISCUSSION**

The present study has demonstrated for the first time that the apical membrane of the native OMCDi cells contains a 23-pS nonselective cation channel (see Fig. 2). The spontaneous activity of this cation channel was also detected in the apical membrane of the OMCDo. The channel had a wide range of $P_o$, and membrane depolarization increased $P_o$ (see Fig. 3B). The renal medulla is the only tissue in which extracellular osmolarity exceeds that of systemic plasma in mammals. The lumen $[K^+]$ in the outer medulla varies with dietary $K^+$ content, and the degree of antidiuresis and may exceed 50 mM. Although the role for voltage-dependent cation channels in the OMCDi is unknown, given the condition of such high lumen $K^+$, the membrane potential of OMCDi cells should be depolarized to a certain degree, which may facilitate the channel opening.

The $i$-$V$ curve of the 23-pS channel is almost linear in both cell-attached and in inside-out patch configurations (see Fig. 4). The rectification of the inward-rectifier $K^+$ channel is caused by both the block of the outward current by cytoplasmic $Mg^{2+}$ and by intrinsic channel gating (19). In the present study, the pipette and bath solutions always had 1 mM $Mg^{2+}$ present, and cytoplasmic $Mg^{2+}$ did not appear to block the outward current.

The channel is selective for $K^+$ over $Cl^-$. Paired experiments in inside-out patches showed that, when most of the $Cl^-$ in the bath (and in the pipette) was replaced by gluconate, channel current and conductance were not significantly changed (see Figs. 5A and 6), but, when most of the $K^+$ in the bath was replaced by TEA, it failed to show outward current (see Fig. 5B). These data indicate that $K^+$ was the contributive ion for the channel current. The estimated selectivity of

![Fig. 7. Channel is highly selective for $K^+$ over $Cl^-$.](image)

![Fig. 8. Channel is nonselective for $K^+$ and $Na^+$.](image)
the 23-pS channel for K\(^+\) over Cl\(^-\) was ~11 to 30 times in inside-out patches (see Fig. 7). This estimation of selectivity using the GHK equation was mainly based on measured \(E_{rev}\) under the condition that solutions contained both K\(^+\) and Cl\(^-\). The possible effect of a small portion of Cl\(^-\) permeability cannot be excluded.

This study also shows that the channel could pass both of the monovalent cations K\(^+\) and Na\(^+\) (see Fig. 8). A selective Na\(^+\) conductance has not been detected in the OMCD\(_1\) segment by microperfusion and intracellular recording studies in rabbits (9, 17). It is also reported that, in whole cell patch-clamp studies of cultured OMCD\(_1\) cells, a 10-fold change in the bathing solution [K\(^+\)] caused a membrane potential shift, but a 10-fold change in Na\(^+\) had no effect (23), which indicates only the presence of a K\(^+\) conductance. The differences could be due to the recording configuration (i.e., single channel current vs. whole cell macroscopic current) and the cell preparation (i.e., native cells vs. cultured cells).

In our previous microperfusion study, 2 mM Ba\(^{2+}\) inhibited bicarbonate absorption and decreased transepithelial voltage in perfused OMCD\(_1\). In the whole cell patch-clamp study of cultured OMCD\(_1\), 1 mM Ba\(^{2+}\) did not alter the cell membrane conductance, and an inhibitory effect was seen only when the concentration was 10-fold greater (23). Ciampolillo et al. (6) demonstrated the presence of mRNA for a 28-pS cation channel in the mouse OMCD. As shown by patch-clamp studies in mouse inner medullary collecting duct (IMCD) cells in culture, this 28-pS channel does not discriminate between Na\(^+\) and K\(^+\) and does not alter channel activity by voltages, Ba\(^{2+}\), TEA, and bath pH changes (13). Studies from the IMCD cell line, mIMCD-3 cells, also showed a 24-pS nonselective cation channel that was activated by a negative suction in the pipette (21). This channel was modulated by bath Ca\(^{2+}\) in excised patches but did not appear to be sensitive to Ba\(^{2+}\) because the inhibitory effect of Ba\(^{2+}\) on channel activity was observed only in very high concentrations. Although the channel described in the present study may not share all of the properties with channels described in the apical membrane of mouse IMCD (13, 21) and mouse OMCD (6), the similarity of both K\(^+\) and Na\(^+\) permeability in these channels would suggest that, in the medullary collecting duct, such channels constitute a general mechanism for K\(^+\) and Na\(^+\) regulation.

The OMCD\(_1\) has been shown to reabsorb Na\(^+\) and to secrete K\(^+\) but at rates that are less than that in the CCD (24, 26, 32). Although it is not clear whether the OMCD\(_1\) has the same ability to secrete K\(^+\) and to reabsorb Na\(^+\) under certain conditions, the presence of cation channels in this segment suggests that this possibility exists.

Nonselective cation channels (with similar permeability for monovalent cations Na\(^-\) and K\(^+\)) have also been found in various CCD cells (1, 10, 12, 14, 16). Most of these channels have a linear \(i-V\) relationship with slope conductance of 20–30 pS and have an increased channel activity at depolarized voltages. Such channels have similarities with the channels found in the native OMCD\(_1\) cells.

It is clear from immunohistochemistry and electron microscopic studies that principal cells and intercalated cells exist in the rabbit OMCD\(_1\) (27, 33). However, our current experiments under light microscopic observations, including the use of Hoffman modulation contrast optics, could not identify the cell type being patched. In contrast to the CCD, in which “hexagonal” cells and “circular” cells are clearly observed (20, 22, 35), most cells of the OMCD\(_1\) observed using Hoffman modulation contrast optics exhibit irregular shapes (Fig. 1B). Nevertheless, we observed detectable ionic channel activity in ~20% of the patches that were investigated, a percentage that is similar to the percentage of intercalated cells in this segment (8, 11, 33).

Further studies are required to specifically identify the cell types that have cation channel activity.

It is known that intercalated cells have a higher rate of H\(^+\) secretion by H\(^+\)-K\(^+\)-ATPase than principal cells (28, 29) and thus may require a pathway to recycle K\(^+\) back from these cells to the lumen. For example, if the apical membrane H\(^+\)-K\(^+\)-ATPase absorbs K\(^+\) in exchange for H\(^+\) (or H\(_2\)O\(^+\)), then K\(^+\) must either exit basolaterally (in which case K\(^+\) reabsorption would occur) or exit apically (in which case there would be no net K\(^+\) reabsorption). Previous studies have shown that the OMCD\(_1\) has little net K\(^+\) reabsorption in K\(^+\)-replete rabbits (4, 25, 30). Our current data suggest that the renal H\(^+\)-K\(^+\)-ATPase may function in parallel with an apical K\(^+\)-permeable channel in intercalated cells of the OMCD\(_1\).

In summary, we have successfully prepared the apical membrane of the native OMCD\(_1\), to directly examine single channel activity. The current study demonstrates the presence and biophysical properties of cation channels in a subpopulation of cells in this segment. We speculate that these channels could provide K\(^+\) recycling during proton secretion by H\(^+\)-K\(^+\)-ATPases in the K\(^+\)-replete condition. However, the exact physiological role of these channels in Na\(^+\) absorption and K\(^+\) secretion needs to be explored in future studies.

We thank Jeanette Lynch, Lance Parker, and Robin Moudy for technical support, Dr. Jeff Martin for interaction in the early experiments, and Dr. Kirsten Madsen for use of key equipment during this study and the staff of the Medical Media at the Veterans Affairs Medical Center for photographic processing.

Part of this work was presented in abstract form at the American Society of Nephrology 31st and 32nd annual meetings held in Philadelphia, PA, October 25–28, 1998, and Miami Beach, FL, November 5–8, 1999, respectively.

This work was supported by National Institute of Diabetes and Digestive and Kidney Diseases Grants DK-49750 and by the Medical Research Service of the Department of Veterans Affairs.

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