A voltage-gated K⁺ current in renal inner medullary collecting duct cells

Laura I. Escobar, Julio C. Martínez-Téllez, Monica Salas, Salvador A. Castilla, Rolando Carrisoza, Dagoberto Tapia, José Vázquez, José Bargas, and Juan J. Bolívar. A voltage-gated K⁺ current in renal inner medullary collecting duct cells. Am J Physiol Cell Physiol 286: C965–C974, 2004. First published December 18, 2003; 10.1152/ajpcell.00074.2003.—We studied the K⁺-selective conductances in primary cultures of rat renal inner medullary collecting duct (IMCD) using perforated-patch and conventional whole cell techniques. Depolarizations above –20 mV induced a time-dependent outward K⁺ current (I_out) similar to a delayed rectifier. I_out showed a half-maximal activation around 5.6 mV with a slope factor of 6.8 mV. Its K⁺/Na⁺ selectivity ratio was 11.7. It was inhibited by tetraethylammonium, quinidine, 4-aminopyridine, and Ba²⁺ and was not Ca²⁺ dependent. The delayed rectifying characteristics of I_out prompted us to screen the expression of Kv1 and Kv3 families by RT-PCR. Analysis of RNA isolated from cell cultures revealed the presence of three Kv α-subunits (Kv1.1, Kv1.3, and Kv1.6). Western blot analysis with Kv α-subunit antibodies for Kv1.1 and Kv1.3 showed labeling of ~70-kDa proteins from inner medulla plasmatic and microsome membranes. Immunocytochemical analysis of cell culture and kidney inner medulla showed that Kv1.3 is colocalized with the Na⁺-K⁺-ATPase at the basolateral membrane, although it is also in the cytoplasm. This is the first evidence of recording, protein expression, and localization of a voltage-gated Kv1 in the kidney IMCD cells. Cation conductances of the IMCD membranes have been studied with microelectrodes and patch-clamp techniques. Microelectrode recordings in isolated and perfused IMCD have demonstrated the presence of a basolateral K⁺ and an apical amiloride-sensitive Na⁺ conductance (20, 41). Patch-clamp studies of the apical membrane have identified two nonselective cation channels: an amiloride-sensitive (in IMCD1) and an amiloride-insensitive cation channel (in IMCD2 and IMCD3), as well as an amiloride-sensitive Na⁺ channel (30, 35, 47). In a murine IMCD3 cell line, an amiloride-sensitive cation channel and two K⁺-selective channels have been observed in the apical membrane (36, 40). Cl⁻ conductances have been registered by whole cell clamp studies of cells from IMCD in primary culture (19, 48). The first functional findings indicating evidence of Kv channel expression in the kidney were reported in a rabbit papillary epithelial cell line and in the medulla (45, 50).

In the present work we have examined the K⁺-selective conductances by the perforated-patch and conventional whole cell clamp in a primary culture of IMCD from rat. Our results demonstrate the presence of a time-dependent voltage-activated outward K⁺ current with a high voltage activation threshold in these cells. We detected mRNAs encoding three members of the Kv1 (Shaker) family: Kv1.1, Kv1.3, and Kv1.6 in cell cultures. Western blot showed Kv1.1 and Kv1.3 protein expression in plasmatic and microsone membranes from inner medulla. Immunocytochemistry analysis confirmed Kv1.3 distribution and localization in the cytoplasm and at the basolateral membrane of collecting duct cells. Kv1.x channel subunits have been reported to assemble into heterotetrameric channels with distinct biophysical and pharmacological properties when expressed in vitro (9). We suggest that in IMCD, the functional expression of an heteromultimer of Kv1.3/Kv1.x gives rise to this outward K⁺ current.

MATERIALS AND METHODS

Cell Culture

Primary cultures of IMCD cells were obtained by using a modified hypotonic lysis method (30). Four male Wistar rats (175–225 g) were intraperitoneally injected with furosemide (Lasix; 1 mg/100 g; Hoechst, Mexico) 20 min before death. Animals were anesthetized with chloroform and killed by decapitation. The inner medulla of the kidneys was aseptically removed and sectioned in its outer and inner halves, which were handled in parallel. The tissue was finely minced and incubated, at 37°C, for 60 min as described (30). After this incubation, 1.75 volumes of distilled water were added, and the mixture was centrifuged at 500 g for 6 min. The pellet was suspended...
as described (30) and centrifuged at 110 g for 2 min. This last
procedure was repeated three times. The resulting pellet was
suspended in DMEM (GIBCO, Grand Island, NY) supplemented
as described (6) and seeded on glass coverslips contained in a 35-mm
plastic petri dish (15 coverslips per dish) and kept incubated, at 37°C,
in a humidified air-5% CO2 atmosphere, with medium changed every
other day. With this procedure two types of IMCD cell cultures were
obtained: one from the IMCD outer half and one from its inner half
(IMCDo and IMCDc, cultures, respectively). Five to six days after
plating, both cultures showed confluent cell monolayers in at least five
of the coverslips. These monolayers remained stable for at least
another week. Only confluent cells were studied.

To evaluate our cultures (42), some monolayers were incubated for 40
min in PBS (GIBCO) with 0.25 mg/ml peroxidase-labeled Dolichos
biflorus lectin (Sigma, St. Louis, MO). Lectin binding was observed with
diaminobenzidine plus hydrogen peroxide and estimated with an inverted
microscope (Diaphot 300; Nikon) equipped with Hoffman modulation
optics (Modulation Optics, Greenvane, NY).

RNA Purification and RT-PCR

Total RNAs from primary cultures of IMCD cells, kidney inner
medulla, and brain from rats were extracted with CsCl (39). RNA (1 μg)
from each sample was converted to cDNA, using the random primer
random priming (Roche Diagnostics) and reverse transcribed with SuperScript II
RT (Life Technologies), according to the manufacturer’s instructions.
Contaminating DNA was removed using DNAse I (Pharmacia).

PCR Amplification and DNA Sequencing

cDNA products of the above-described samples were used directly
as templates for PCR amplification with Taq DNA polymerase (Life
Technologies). Only primers 5'-GAG TAC TTC TTC TGC AGC CG-3'
(antisense) and 5'-CAT GGT CAC CAC AGG CCA CAA GGA-3'
(sense) (Life Technologies) (Table 1) were designed according to the
conserved amino acid sequences at the amino terminus of a Kv1.x
channel. The clones yielded a sequence corresponding to a Kv1.3. Therefore,
cDNA fragments were purified from the agarose gel (Marligen-Biosciences) to further
sequencing (n = 6).

Table 1. Specific primers used to amplify unique DNA fragments corresponding to regions of rat
Kv1.x and Kv3.x by RT-PCR

<table>
<thead>
<tr>
<th>Channel</th>
<th>Accession No.</th>
<th>Sequence</th>
<th>Sense</th>
</tr>
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<tr>
<td>Kv1.1</td>
<td>P16388</td>
<td>5'-GAA GAA GAT TGC TCG CAC TGG CAC ATG CTC 3'</td>
<td>Forward</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5'-TTA AAG ATC GGT CAG CAG CTG GCT 3'</td>
<td>Reverse</td>
</tr>
<tr>
<td>Kv1.2</td>
<td>AAA19867</td>
<td>5'-GAT GAG CGA GAT TCC CAG TCC CCC AGC ATC 3'</td>
<td>Forward</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5'-TCA GAC ATC AGT TAA TAT TTG GGT 3'</td>
<td>Reverse</td>
</tr>
<tr>
<td>Kv1.3</td>
<td>See MATERIALS AND METHODS</td>
<td>5'-GAG TGC TAC TTC TGC CAG CCA CAC 3'</td>
<td>Forward</td>
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<tr>
<td></td>
<td></td>
<td>5'-TCA CAC ATG ATC CAC ACC CTT TCC 3'</td>
<td>Reverse</td>
</tr>
<tr>
<td>Kv1.4</td>
<td>P15385</td>
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<td>Forward</td>
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<td></td>
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<td>5'-TCA CAC ATG CTC CAC ACC CTT TCC 3'</td>
<td>Reverse</td>
</tr>
<tr>
<td>Kv1.5</td>
<td>Q61762</td>
<td>5'-AAT CAG GGG TCG CAA CTT CAC AGT ATG CCG 3'</td>
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<td>5'-TTA GAA ATC TGT TCC CCG GAT GCT 3'</td>
<td>Reverse</td>
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<td>Kv1.6</td>
<td>X176219</td>
<td>5'-GGT GAT TCG CTC TGC CAC ATG CCA CAC 3'</td>
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<td>5'-CTG CTG CCC TCC CAG CAA GGT 3'</td>
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<td>Kv1.7</td>
<td>NM031886</td>
<td>5'-GAT TGG GGC GCG CCG CCT ATG TCG GGC 3'</td>
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<td>5'-TCA CAC CTC ATG CAC GTC TGG CTC 3'</td>
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<tr>
<td>Kv1.10</td>
<td>X227577</td>
<td>5'-CCG AGT GCC CAA CAG AAC ATG TCC CTC GGC 3'</td>
<td>Forward</td>
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<td></td>
<td></td>
<td>5'-TCA TTT CCT AGA GTC CTT CTC GTC GAA AGA 3'</td>
<td>Reverse</td>
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Accession numbers refer to DNA sequences published in the GenBank of the National Center for Biotechnology.
Whole Cell Clamp Recordings

Membrane currents were studied mainly with the perforated-patch whole cell clamp technique (17, 38); only some experiments were performed with the conventional whole cell clamp technique (34). Coverslips containing confluent cell monolayers were placed on a superfusion chamber (0.3 ml) fixed to the stage of an inverted microscope provided with Hoffman modulation optics. Cells were maintained in a bath solution containing (in mM) 157 gluconic acid, 146 NaOH, 5 KOH, 2 Ca(OH)\textsubscript{2}, 1 Mg(OH)\textsubscript{2}, 10 HEPES, and 10 superfusion chamber (0.3 ml) fluid cell monolayers were placed on a Coverslips containing corperformed with the conventional whole cell clamp technique (34).

Whole Cell Clamp Recordings

KOH; and 2 Ca(OH)\textsubscript{2}, with a calculated free Ca\textsuperscript{2+} concentration of <10\textsuperscript{-8} M; 2) a “15 mM K+ bath solution” containing 136 mM NaOH and 15 mM KOH; and 3) a “45 mM K+ bath solution” containing 106 mM NaOH and 45 mM KOH. All experiments were performed at room temperature (20–25°C) with 30 μM amiloride in the bath. Recording of membrane currents was performed through Ag-AgCl electrodes by using an Axopatch-1D amplifier with a CV-4 (500 MΩ) head stage (Axon Instruments, Foster City, CA). Micropipettes from Kimax-51 glass (Kimble, Vineland, NJ) were fabricated in a two-step vertical pipette puller (PB7; Narishige). The tips were fire-polished in a microforge (MP9, Narishige). Micropipettes were filled from the tip, up to a distance of 0.4–0.5 mm, with a pipette solution composed of (in mM) 156 gluconic acid, 141 KOH, 10 NaOH, 1.54 Ca(OH)\textsubscript{2}, 1 Mg(OH)\textsubscript{2}, 2.3 EGTA, and 10 HEPES, ph 7.4, with a calculated free Ca\textsuperscript{2+} concentration of 3 × 10\textsuperscript{-7} M. Pipette filling was completed, from the back, with a pipette solution with or without 200 μg/ml amphotericin B. In some experiments a “Ca\textsuperscript{2+}-free pipette solution” was employed, containing 0.1 mM Ca(OH)\textsubscript{2} and 2 mM EGTA, with a calculated free Ca\textsuperscript{2+} concentration of <10\textsuperscript{-8} M. Once filled, micropipettes had a resistance of 2–3 MΩ. With the use of a hydraulic micromanipulator (WR-60; Narishige), pipettes were applied to the cells to contact the membrane, and a gentle suction was applied and maintained until a seal of at least 1 GΩ was obtained. Perforated-patch whole cell configuration was reached 4–8 min after the membrane contact as monitored when a voltage square pulse (20 mV, 5 ms) evoked a capacitative current transient shorter than 4 ms and a series resistance (R\textsubscript{s}) smaller than 20 MΩ. In conventional whole cell recordings the membrane was ruptured with suction. Membrane potential was clamped at −50 mV. Membrane capacitance and R\textsubscript{s} were compensated (80%) and then measured by using the Axopatch-1D compensation systems. The voltage-clamp protocols were performed with the conventional whole cell clamp technique (17, 38); only some experiments were performed with the conventional whole cell clamp technique (34).

Current Kinetics Analysis

Time course of the capacitative current. Current was evoked by a voltage pulse from −50 to −60 mV, and its time course was fitted with a single exponential function of the form

\[ I_t = I_0 \exp(-t/\tau) \]  

where \( I_0 \) is the current measured at steady state, \( I_t \) is the current at time \( t \), \( I_0 \) is the value of \((I_t - I_0)\) extrapolated at \( t = 0 \), and \( \tau \) is the time constant of the capacitative current decay.

Time course of the voltage-activated outward current. The time course of the voltage-activated outward current was adjusted to a Hodgkin-Huxley-type model with one activation gate and one inactivation gate (15, 16). Leak current was measured [from the recordings obtained in the presence of 10 mM tetraethylammonium (TEA) or 1 mM quinidine in the bath] and subtracted. Currents were evoked by the basic stimulation protocol. The time course of current activation was fitted to a single exponential function of the form

\[ I_t = I_{\text{max}}[1 - \exp(-t/\tau)] \]  

where \( I_{\text{max}} \) is the maximum value of the current and \( \tau \) is the time constant of activation. If inactivation was observed, the current was fitted with a product of exponential functions of the form

\[ I_t = I_{\text{max}}[1 - \exp(-t/\tau_1)\exp(-t/\tau_2)] \]  

where \( \tau_1 \) and \( \tau_2 \) are the time constants of inactivation.

Voltage dependence of the outward current activation. The voltage dependence of the outward current activation was studied with a voltage protocol departing from a holding potential of −50 mV to depolarizing voltage pulses, applied every 4 s for 300 ms, from −40 to 65 mV in 5-mV increments. Leak current was subtracted as before. The peak outward current (\( I \)) recorded after each voltage step (\( V \)) was transformed into conductance (\( g \)) according to the relation \( f = g(V - V_{\text{rev}}) \), where \( V_{\text{rev}} \) is the reversal potential of the current, which was assumed to be −50 mV (see RESULTS). Conductance values were fitted with the following (Boltzmann type) equation

\[ g/g_{\text{max}} = \frac{1}{1 + \exp[(V - V_h)/k]}^{-1} \]  

where \( g_{\text{max}} \) is the maximum value reached by \( g \), \( V_h \) is the voltage at which \( g \) is half maximal, and \( k \) is a constant that gives the steepness of the voltage dependence.

Tail currents. In tail current experiments, the conventional whole cell clamp technique was employed. From a holding potential of −50 mV, the current was activated by a prepulse to 40 mV during 180 ms, and then voltage was returned to various test potentials ranging from −100 to −30 mV in 10-mV increments for 32 ms. When high-K+ concentration bath solutions were used, the test potentials ranged from −100 to 0 mV. Leak current was measured (from the recordings obtained with a similar protocol in which a prepulse to −35 mV was used) and subtracted. Tail currents were fitted with a single exponential of the form

\[ I_t = I_{\text{gisk}} \exp(-t/\tau) \]  

where \( I_{\text{gisk}} \) is the calculated value of \( I \) at \( t = 0 \) (when the voltage change occurs) and \( \tau \) is the time constant of the current deactivation.

The tail currents measured in the first 2 ms were averaged and plotted against voltage. The reversal potential of the tail currents was measured at the point where the best curve that fitted the plotted current points crossed the voltage axis.

RESULTS

Cell Morphology

The monolayers observed in IMCD cultures (IMCD\textsubscript{o} and IMCD\textsubscript{r}) were mainly formed by 20- to 40-μm-diameter cells showing an almost uniform appearance: flat and polygonal, with a large nucleus (Fig. 1). This morphology is similar to that previously described for cells from the IMCD in primary culture (30, 35). Smaller cells (10–20 μm) were present in some monolayers, with oval or rounded appearance. However, >85% of the cells in IMCD\textsubscript{o} cultures and >90% of the cells in IMCD\textsubscript{r} cultures exhibited positive binding for D. biflorus lectin (not shown). All
these cells were flat and polygonal, indicating that these were IMCD cells in the IMCD\(_i\) cultures or principal and IMCD cells in the IMCD\(_o\) cultures (7, 42). Only these cells were used for the electrophysiological recordings. Therefore, electrophysiological recordings were performed in IMCD and principal cells. The monolayers exhibited blisters, providing evidence that cells were polarized and capable of transepithelial transport (29).

Capacitance and Resistance of Cells from IMCD\(_i\) and IMCD\(_o\) Cultures

According to the criterion established in MATERIALS AND METHODS, perforated-patch whole cell configuration was reached in 6.2 ± 0.4 min, the time at which \(R_o\) was 13.9 ± 0.3 MΩ (mean ± SE; \(n = 121\)). \(R_o\) became smaller during the following minutes and reached a minimum value of 9.9 ± 0.4 MΩ. When studied \((n = 15)\), the time course of the capacitative current was well described by Eq. 1. A good fit of the time course of the capacitative current with a single exponential function is indicative of the absence of electrical coupling between cells (6, 19), an indispensable condition for obtaining space clamp.

Membrane capacitance was 24.0 ± 0.9 and 24.8 ± 1.0 pF in IMCD\(_i\) \((n = 77)\) and IMCD\(_o\) cells \((n = 44)\), respectively, values identical to those previously reported (19). Cell input resistance, measured by means of the current change elicited by a voltage change from −60 to −40 mV, was 1.35 ± 0.17 and 1.07 ± 0.15 GΩ in IMCD\(_i\) \((n = 77)\) and IMCD\(_o\) cells \((n = 44)\), respectively. The difference is not statistically significant.

Membrane Currents in Cells

At the voltage range explored (from −160 to 80 mV), cells showed both inwardly and outwardly rectifying currents (Fig. 2). The present work focuses on voltage-dependent outward current.

A time- and voltage-dependent outward current \((I_{\text{vto}})\) was observed in ∼27% of the cells (IMCD\(_i\): 29%, \(n = 77\); IMCD\(_o\): 25%, \(n = 44\)). Figure 2A shows that these currents activate at potentials between −20 and 0 mV, require ∼100 ms to complete their activation at 0 mV, and activate more quickly with larger depolarizations. Figure 2B shows the mean current-voltage \((I-V)\) relationship obtained in cells with \(I_{\text{vto}}\). The outward rectification is due to \(I_{\text{vto}}\). Considering the ionic conditions in our experiments (virtual absence of any potentially permeant anion) and the outward direction of \(I_{\text{vto}}\), it can be expected that this is a K\(^+\) current. Figure 2B also shows that...
these cells exhibited an inward rectification and a leak component that reverses close to 0 mV, which are mainly due to an inward rectifying Cd$^{2+}$-sensitive current (unpublished results).

**Effect of Inhibitors on $I_{vto}$**

To test whether $I_{vto}$ is a K$^+$ current, we explored its sensitivity to four K$^+$ channel blockers. The effect of the inhibitors studied, expressed as the mean percentage block of the total outward currents recorded at membrane potentials between 0 and 80 mV, was TEA (10 mM), 82 ± 4% ($n = 7$); TEA (1 mM), 57 ± 2% ($n = 2$); quinidine (1 mM), 96 ± 1% ($n = 5$); Ba$^{2+}$ (5 mM), 25 ± 4% ($n = 2$); and 4-aminopyridine (4-AP; 10 mM), 54 ± 10% ($n = 2$). TEA, quinidine, 4-AP, and Ba$^{2+}$ inhibition of similar voltage-activated outward K$^+$ currents has been observed in other classes of epithelial cells (6, 18, 21, 27, 45, 49). Therefore, the present experiments suggest that $I_{vto}$ is a voltage-activated K$^+$ current present in IMCD cells. Figure 3 illustrates the reversible effect of TEA on $I_{vto}$. TEA does not affect the Cd$^{2+}$-sensitive cationic current, and its apparent inhibition is mainly due to its rundown (unpublished results).

**Ca$^+$ Independence of $I_{vto}$**

Time- and voltage-dependent outward K$^+$ currents similar to $I_{vto}$ may be Ca$^{2+}$ dependent (6, 21). To test this possibility, we employed the conventional whole cell clamp technique with a Ca$^{2+}$-free intracellular solution (pCa > 8; see MATERIALS AND METHODS). Extracellular Ca$^{2+}$ removal did not reduce the $I_{vto}$ current amplitude after 6 min (not shown), as should happen when the current is Ca$^{2+}$ dependent (21). Also, the presence of 5 mM Cd$^{2+}$ in the bath did not inhibit the current. These results suggest that $I_{vto}$ is not a Ca$^{2+}$-dependent current (1).

**Time-Dependent Kinetics of $I_{vto}$**

Current activation was followed by a slow inactivation. Figure 4A shows that the time course of current activation and inactivation can be well described by Eq. 2 or 3. Current activation shows voltage dependence, with $\tau_a$ becoming smaller as depolarization increases (Fig. 4B). Inactivation was observed in 73% of the currents recorded at potentials between 20 and 80 mV, $\tau_i$ exhibited a weak voltage dependence, from 5.1 ± 1.1 s (at 20 mV) to 3.3 ± 0.8 s (at 80 mV; $n = 9$). $I_{vto}$ activation and inactivation had $\tau_a$ and $\tau_i$ values comparable to those reported by other authors in delayed rectifier-type K$^+$ outward currents observed in other classes of epithelial cells and fitted with similar equations (18, 21, 45, 49, 50). The similarities extend to the voltage dependence of $\tau_a$ and $\tau_i$, which appears to be a common characteristic for this type of current when observed in epithelial cells. Therefore, these experiments suggest that $I_{vto}$ is a K$^+$ current of the delayed rectifier type. This current was the only one with delayed rectifier characteristics recorded in IMCD cultures.

**Voltage Dependence of $I_{vto}$ Activation**

The voltage dependence of $I_{vto}$ activation is well described by Eq. 4 (Fig. 4C). The mean values of the activation parameters were $V_o = 5.6 ± 3.3$ mV and $k = 6.8 ± 1.0$ mV ($n = 4$). The $V_o$ value is similar to (18, 24) or 15–25 mV more positive than (45, 49) the $V_o$ value reported for delayed rectifiers in other epithelial and nonepithelial cells. The value of $k$ compares to those already reported (24, 46). The relative conductance tends to decline at potentials more positive than that at which $g_{max}$ is reached (not shown). This phenomenon also has been observed in delayed rectifiers of other cells (12, 24).

**K$^+$/Na$^+$ Selectivity of $I_{vto}$**

To further characterize the ionic selectivity of $I_{vto}$, a tail current analysis of its reversal potential was performed. The conventional whole cell clamp technique was employed for these experiments. Figure 5A shows the tail currents recorded in a representative cell bathed in the control solution. The $I-V$ relationship of these currents (Fig. 5B) exhibited a reversal potential of about −52 mV, which is much closer to $E_K$ (−85 mV) than to $E_{Na}$ (68 mV). The reversal potential of the tail currents depended on the external K$^+$ concentration. Figure 5C
shows the mean reversal potentials measured in the presence of 5 (n = 9), 15 (n = 2), and 45 mM (n = 3) external K\(^{+}\). The straight line that joins the points is the best fit obtained with the minimum squares method; it has a slope of 30.4 that does not suggest a complete K\(^{+}\) selectivity. However, from this slope, the Goldman equation predicts that \(I_{\text{vto}}\) channels are 11.7 times more selective to K\(^{+}\) than to Na\(^{+}\). This value compares with that obtained for the delayed rectifier of the squid axon (5).

### RT-PCR Analyses of Kv.x mRNA Expression in Rat IMCD Cells

Delayed rectifying K\(^{+}\) channels are found in the Kv1 and Kv3 voltage-gated channel families. Therefore, our first strategy was to design primers to amplify a conserved region from the pore toward the amino-terminal end of both families. A single base band of roughly 900 bp was amplified with the IMCD cell culture and kidney inner medulla cDNA samples (Fig. 6). Sequence analysis of the 900-bp cDNA identified it as a Kv1.3 channel. Because the kinetic properties of the \(I_{\text{vto}}\) did not correspond to a Kv1.3 homomultimer (see above and Ref. 9), we carried out further RT-PCR reactions with specific primers for all of the Kv1.x family members (see Table 1). We observed amplification of the expected cDNA fragments for Kv1.1 and Kv1.6 (Fig. 6). GAPDH cDNA from IMCD cells and GAPDH and Kv1.x cDNAs from brain samples were always amplified as positive controls of the PCR reaction.
**Kv α-Subunit Protein Expression and Localization in Inner Medulla Cells**

To determine whether Kv1.x α-subunits are expressed as protein, we performed Western blot experiments with commercially available antibodies directed against Kv1.1 and Kv1.3 in plasmatic membrane and microsome fractions. A representative blot (Fig. 7) identifies proteins with masses of ~70 kDa that were specifically labeled with antibodies to these Kv α-subunits in both fractions.

Confocal microscopy images of the inner medullary slices and cultures (Fig. 8) were helpful in studying the Kv1.3 distribution. Fluorescence was observed mainly in the intracellular perinuclear zones. Colabeling of Kv1.3 and Na⁺-K⁺-ATPase proteins was observed, supporting the expression of Kv1.3 α-subunits at the basolateral membrane. Interestingly, this colabeling was also observed at the basolateral membrane of the papillary epithelial cells (Fig. 8 F), in agreement with the hypothesis raised by others (50).

**DISCUSSION**

The present work demonstrates for the first time the current recordings of an outward K⁺ current in ~27% of confluent IMCD cells. IMCD cells cultured on glass (untreated) cover slips behave as a single electrical compartment, as inferred from the monoexponential decay of the capacitative current. This result resembles the one obtained with MDCK cells (cell line probably derived from the collecting duct) cultured under the same conditions (6). In contrast, when IMCD cells are cultured on collagen, they become electrically coupled. Therefore, under the latter conditions, the cells have to be decoupled in a Ca²⁺-free medium so that reliable whole cell clamp measurements can be performed (19, 48). The procedure reported in this work offers an alternative way to perform whole cell clamp studies in IMCD uncoupled cells in primary cultures. On the other hand, the total membrane area of cultured IMCD cells, as inferred from whole cell capacitance, is the same with both procedures (19).

The cells of IMCDᵢ and IMCDᵦ cultures exhibited the same type of currents. IMCDᵢ cultures must be formed mainly by IMCD cells from the IMCD2 and IMCD3, whereas IMCDᵦ cultures must be enriched with principal cells from the IMCD1. Both cultures exhibited positive binding for D. biflorus lectin in at least 85% of the cells, indicating that they are formed mainly by these cellular types (7). It is known that cells from different IMCD segments show important functional differences (32, 33). However, with regard to the cationic conductances we observed, all cells behaved similarly. Therefore, functional differences between IMCD segments may involve other conductance types.

Our data suggest that a Kv1.3 channel protein is present at the basolateral membrane. In contrast, patch-clamp studies performed on the apical membrane of IMCD cells in primary cultures have only identified amiloride-sensitive Na⁺ channels (47) and two nonselective cation channels (one amiloride sensitive and the other amiloride insensitive; Refs. 30 and 35). Kv1.2 and Kv1.3 channels have been identified in the rabbit kidney medulla and in GRB-PAP1 cells (45, 50), although their activation occurs at voltages ~10 mV more negative. However, the values of the activation time constants and their voltage dependence are equivalent to those reported here.

Even when the RT-PCR and immunocytochemistry assays identified the presence of Kv1.3 channels in the IMCD cells,

![Fig. 6. RT-PCR amplification of rat Kv α-subunits with RNA isolated from IMCD cell cultures and brain. Molecular weight marker (MWM) was φ X174 RF DNA/HaeIII (2.5 μg). Kv1.1, ~441 bp (400 ng); Kv1.3, ~891 bp (300 ng); Kv1.6, ~387 bp (300 ng), GAPDH, ~459 bp (300 ng), positive control; (−), no cDNA, negative control.](http://ajpcell.physiology.org/)

![Fig. 7. Western blot showing specific antibody labeling to membrane proteins with molecular masses of ~66 kDa for Kv1.1 (A) and 74 kDa for Kv1.3 (B). C, cerebellum; M, inner medulla. HD (plasmatic membrane) and LD (microsomes), high- and low-density fractions, respectively.](http://ajpcell.physiology.org/)
Fig. 8. Localization of Kv1.3 and Na\(^+\)-K\(^+\)-ATPase in IMCD: transverse sections (A–C), sagittal sections (D–F), and cultures (G–I). Localization of Na\(^+\)-K\(^+\)-ATPase at the basolateral membrane (A, D, and G; red) and localization of Kv1.3 (B, E, and H; green) are shown. Colocalization of Kv1.3 and Na\(^+\)-K\(^+\)-ATPase (C, F, and I; yellow) is shown and indicated by arrows. Kv1.3 and Na\(^+\)-K\(^+\)-ATPase colocalization was also observed in the papillary epithelium (arrowheads; F). Bars, 50 \(\mu m\).
the biophysical and pharmacological properties of the \( I_{\text{to}} \) did not correspond to those reported for a Kv1.3 homomultimer. The voltage at which \( I_{\text{to}} \) is half-maximally activated (\( V_{1/2} \)) was 5.6 mV, in contrast to the \( V_{1/2} \) of about −30 mV of a Kv1.3 channel (13). The time constant of \( I_{\text{to}} \) inactivation (5.1 s at 20 mV) was about five to more than seven times slower than that expected for a Kv1.3 (250–600 ms; Ref. 9). Also, the \( I_{\text{to}} \) is about 10 times more sensitive to TEA and about 2 orders of magnitude less sensitive to 4-AP, compared with a Kv1.3 homomultimer (ID_{50} of 10 mM TEA and 195 μM 4-AP; Ref. 13). To compare cloned channels with native channels in IMCD cells, we have to consider that heteromultimer channels might be able to form in these cells with the resulting biophysical and pharmacological differences. The other two Kv channels (Kv1.1 and Kv1.6) identified in the cell culture also correspond to delayed rectifier channels. Although we observed expression of Kv1.1 and Kv1.3 proteins in the inner medulla, further immunoprecipitation experiments are needed to determine the Kv-α-subunits that conform the heteromultimer that probably gives rise to \( I_{\text{to}} \).

The physiological role of Kv1.x channels in IMCD cells would depend on the membrane (apical or basolateral) where the channels are located and the membrane potential in physiological conditions. Colabeling of Kv1.3 and the Na⁺-K⁺-ATPase in our fluorescent assays suggests that Kv1.3 is expressed at the basolateral membranes of IMCD cells. However, there is no information about the membrane potential in IMCD in physiological conditions. Conflicting results are available from in vitro studies performed in extracellular K⁺ concentrations of 4–5 mM (not physiological for IMCD; Refs. 2 and 8). Meanwhile, mean basolateral membrane potentials of −82 and −51 mV have been reported (20, 41), and it was also observed that 60% of the cells exhibited a positive membrane potential (mean: 24 mV), whereas the remaining 40% exhibited a negative membrane potential (mean: −15 mV; Ref. 43). Because the transepithelial potential difference is very small, apical and basolateral membrane potentials must be approximately the same, at least in control conditions (20, 41). Because the IMCD is exposed to two different extracellular media (tubule and inner medullar interstitial fluids) that exhibit large variations in their ionic concentrations and osmolarity (2, 3, 8, 11, 14, 22, 23), it is possible that, depending on the physiological conditions, the IMCD membrane potential (apical and basolateral) undergoes large variations. Accordingly, there will be situations in which the basolateral membrane is sufficiently depolarized to activate these Kv1 channels. This activation, by inducing a K⁺ efflux, would oppose further depolarization. One can speculate that Kv1 channel activation may contribute to the maintenance of the driving force for Na⁺ reabsorption in conditions in which Na⁺ is reabsorbed at a high rate by the IMCD cells. Furthermore, because of the large variations in interstitial osmolarity, Kv1 channels might also play a role in volume regulation (10).

ACKNOWLEDGMENTS

We appreciate the assistance of Lidia Morales and Tomás Cruz for the artwork and Carolina Salvador and Ivonne Mora for technical support with the molecular biology assays. We thank Dr. R. G. Contreras (Centro de Investigación de Estudios Avanzados, Instituto Politécnico Nacional, Mexico) for helpful discussions on the confocal images and Dr. Carol Deutsch for critical reviewing of the manuscript.

GRANTS

This work was partially supported by Dirección General de Asuntos del Personal Académico (DGAPA), Universidad Nacional Autónoma de México (UNAM) Grants IN206393 and IN216396 (to J. J. Bolivar) and DGAPA UNAM Grant IN233802, Consejo Nacional de Ciencia y Tecnología Grants 41365 (to L. I. Escobar).

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