High-conductance K channels in intercalated cells of the rat distal nephron

Lawrence G. Palmer and Gustavo Frindt
Department of Physiology and Biophysics, Weill Medical College of Cornell University, New York, New York

Submitted 30 May 2006; accepted in final form 14 October 2006

Palmer LG, Frindt G. High-conductance K channels in intercalated cells of the rat distal nephron. Am J Physiol Renal Physiol 292: F966–F973, 2007. First published October 24, 2006; doi:10.1152/ajprenal.00191.2006.—High-conductance (BK or maxi) K⁺ channels were observed in cell-attached patches of the apical membrane of the isolated split-open rat connecting tubule (CNT). These channels were quite rare in cells identified visually as principal cells (PCs; 5/162 patches) but common in intercalated cells (ICs; 24/26 patches). The BK-expressing intercalated cells in the CNT and cortical collecting duct (CCD) were characterized by a low membrane potential (~36 mV) under short-circuit conditions, measured from the reversal potential of the channel currents with similar K⁺ concentrations on both sides of the membrane. Under whole-cell clamp conditions with low intracellular Ca²⁺, ICs had a very low K⁺ conductance. When cell Ca²⁺ was increased to 200 nM, a voltage-dependent, tetraethylammonium (TEA)-sensitive outward conductance was activated with a limiting value of 90 and 140 nS/cell in the CNT and CCD, respectively. Feeding animals a high-K⁺ diet for 1 wk did not increase these currents. TEA-sensitive currents were much smaller in PCs and usually below detection limits. To examine the possibility that the ICs participate in transepithelial K⁺ secretion, we measured Na/K pump activity as a ouabain-sensitive current. Although these currents were easily observed in PCs, averaging 79 ± 14 and 250 ± 50 pA/cell in the CNT and CCD, respectively, they were below the level of detection in the ICs. We conclude that ICs have BK channel densities that are sufficient to support renal secretion of K⁺ if cell Ca²⁺ is elevated. However, a pathway for K⁺ entry into these cells has not been identified.

maxi-K channels; principal cells; intercalated cells; K⁺ secretion; cortical collecting duct; connecting tubule

RENAL K⁺ SECRETION TAKES PLACE in the distal nephron, primarily in the late distal convoluted tubule, the connecting tubule (CNT), and probably to a lesser extent in the cortical collecting duct (CCD) (31). The classic secretory mechanism involves entry of K⁺ into the cells via the Na/K pump on the basolateral membrane and exit through K-selective channels on the apical membrane (4). Although ROMK (Kir1.1) channels are thought to comprise the major secretory channel type under most conditions (9, 16, 27), high-conductance BK (maxi-K) channels are also observed in the apical membrane of the CCD (3, 11, 12, 15, 26).

The involvement of BK channels in K⁺ secretion has been controversial. Earlier experiments from our laboratory suggested a minor role. (3). More recent work, however, has suggested that these channels could be important, at least under some conditions. K⁺ secretion by isolated perfused rabbit CDs was reduced by the BK channel blockers tetraethylammonium (TEA) and charybdotoxin at high flow rates (29).

Furthermore, mice in which the β1-subunit of the BK channels was deleted excreted less K⁺ than did controls in response to an acute volume expansion, also consistent with a role of these channels in mediating flow-dependent K⁺ secretion (22). Finally, in mice in which ROMK channels were deleted net secretion of K⁺ by in vivo perfused distal tubules was inhibited by iberiotoxin, a specific inhibitor of BK channels (1).

We previously reported that in the CCD, the density of BK channels was higher in the intercalated cells (ICs) than in the principal cells (PCs) (15). Here, we show a similar distribution in the cells of the CNT, an important site of K⁺ secretion. We have carried out a further electrophysiological analysis of ICs to assess their possible role in K⁺ secretion.

METHODS

Animals

Sprague-Dawley rats (Charles River Laboratories, Kingston, NY), weighing 100–150 g, were fed either control rat chow (0.6% K, 0.4% Na, 0.9% Cl, 0.2% Mg, 0.9% Ca) or a matched high-K⁺ (5.2% K, 5.1% Cl⁻) diet (Harlan Teklad, Madison, WI) for 1–2 wk before the experiments as indicated. After the animals were killed, kidneys were excised and thin sections were cut with a razor blade. CNTs and CDs were isolated with forceps under a dissecting microscope and split open with a fine needle. They were then attached, apical side up, to a small coverslip using Cell-Tak (Collaborative Biomedical Products, Bedford, MA), placed in a glass-bottom chamber on an inverted microscope, and superfused with bath solution at 37°C. All experiments with animals were carried out according to the guidelines of, and with the approval of, the Institutional Animal Care and Use Committee of Weill Medical College.

Patch Clamp

PCs in split-open CNTs and CDs were identified by their flat appearance and polygonal shape, while ICs were either rounded or irregular in shape and appeared to be raised above the plane of the membrane (4). Single-channel and whole-cell currents were recorded with an EPC-7 patch-clamp amplifier (Heka Elektronik, Lambrecht, Germany) and digitized with a Digidata 1332A interface (Axon Instruments, Union City, CA). Data were filtered at 1 kHz and analyzed with pCLAMP8 software (Axon Instruments). Calculations of whole-cell conductances were corrected a posteriori for the series resistance of the pipette.

Solutions

For assessing single-channel currents, the pipette solutions contained (in mM) 140 KCl and 10 HEPES, with pH adjusted to 7.4 with KOH. Final K⁺ concentrations, after pH titration, were determined...
using a flame photometer (model 943, Instrumentation Laboratories, Lexington, MA). Bath solutions contained (in mM) 140 NaCl, 5 KCl, 10 HEPES, 2 glucose, 2 CaCl₂, and 1 Mg(OH)₂, with pH adjusted to 7.4 with NaOH.

For whole-cell clamp measurements, the following combinations of solutions were used.

**K⁺ currents.** The bath solutions contained (in mM) 145 methanesulfonic acid, 5 KI, 2 glucose, 10 HEPES, 2 CaCO₃, and 1 Mg(OH)₂, with pH adjusted to 7.4 with NaOH. TEA or Ba acetate was added to a final concentration of 5 mM. Pipette solutions contained (in mM) 126 aspartic acid, 5 KI, 5 EGTA or BAPTA, 10 HEPES, and 3 MgATP, with the pH adjusted to 7.4 with KOH. CaCO₃ was added to a final concentration of 5 mM. Pipette solutions were used.

**Pump currents.** The bath solution contained (in mM) 130 methanesulfonic acid, 5 KOH, 20 NaCl, 2 CaCO₃, 1 Mg(OH)₂, 5 Ba(OH)₂, 2 glucose, and 10 HEPES, with pH adjusted to 7.4 with NaOH. Ouabain was added to the bath at a final concentration of 1 mM. The pipette solution contained (in mM) 100 aspartic acid, 50 NaOH, 20 KI, 5 EGTA, 10 HEPES, and 3 MgATP, with the pH adjusted to 7.4 with KOH.

**RESULTS**

Figure 1 illustrates typical BK channel activity in a cell-attached patch on an IC identified visually in the CNT. With high K⁺ in the pipette, currents were inward at the cell resting potential and showed a single-channel conductance of 240 pS. The openings were markedly voltage dependent, with open probability increasing sharply with depolarization. These features are common characteristics of BK channels in the kidney and in other cells (9). Nearly every cell-attached patch made on the apical membrane of ICs of the CNT contained active BK channels (Table 1), in contrast to the paucity of channels observed in PCs. The number of BK channels per patch in ICs was roughly estimated after excision of the patches in the inside-out mode, such that the intracellular surface was exposed to the bath fluid containing 2 mM Ca²⁺. The average value was about four channels/patch (Table 1). Assuming a patch area of 1 μm² and an apical surface area of 180 μm² (6, 20), this corresponds to 700 channels/cell.

The currents in Fig. 1 reversed directions at a voltage of +34 mV, i.e., when the pipette potential was −34 mV with respect to the bath (Fig. 1B). If we assume that the concentration of K⁺ in the cell was about the same as that in the pipette (140 mM) and that the channels are highly selective for K⁺, this pipette potential should equal the resting potential of the apical membrane. Since the cells in the split-open tubule are effectively short-circuited, the potentials across the apical and basolateral membranes will be the same. Some patches had no resolvable channel openings with inward currents and could not be used to assess reversal potentials. In those patches in which the reversal potential could be determined, the average value was −36 ± 3 mV. This value is significantly less negative than that of PCs (−81 mV), measured with the same technique but using currents through small-conductance K⁺ (SK) channels (Table 1).

The density of BK channels in the apical membrane of PCs was much lower. As indicated in Table 1, currents characteristic of these channels were observed in only 5 of 162 cells identified visually as PCs in the CNT. BK and the 50-pS SK (ROMK) channels were completely segregated in this series of measurements, never appearing in the same patch. We did not observe any SK channels in ICs. A few BK channels were seen in presumed PCs, but since these patches did not have SK channels we cannot rule out the possibility of a misidentification of cells in these cases. In some of these recordings, it is possible that BK channels, which are activated by membrane depolarization and cell Ca²⁺, were present in the membrane but in an inactive state. In 15 patches that had SK channel activity, the membrane patch could be depolarized by 120 mV or more, reversing the direction of current through these channels (Fig. 2A). Despite this depolarization, no BK currents were observed. In 28 patches that also contained SK channels, cell-attached patches were excised into the bath containing 2 mM Ca²⁺. This maneuver also failed to elicit BK channel activity (Fig. 2C). We conclude that, at least under our recording conditions, BK channel density in the apical membrane of PCs is very low.

### Table 1. **BK channel densities in the rat connecting tubule**

<table>
<thead>
<tr>
<th></th>
<th>PCs</th>
<th>ICs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cells with BK channels</td>
<td>5/162</td>
<td>24/26</td>
</tr>
<tr>
<td>BK channels/patch</td>
<td>&lt;0.1</td>
<td>4.0±0.6</td>
</tr>
<tr>
<td>Reversal potential, mV</td>
<td>−81±1</td>
<td>−36±3</td>
</tr>
</tbody>
</table>

Values are means ± SE. BK, large-conductance K⁺ channel; PCs, principal cells; ICs, intercalated cells.
The presence of BK channels in ICs was confirmed using whole-cell recordings (Fig. 3). To accentuate K⁺/Ca⁺ current, the basolateral Cl⁻ conductance that normally dominates the electrical properties of ICs (19) was suppressed by replacement of Cl⁻ by aspartate and gluconate in the pipette and bath, respectively, and adding 5 mM I⁻ to block this conductance and to facilitate contact with the Ag/AgCl pellets. When the pipette solution contained EGTA and no added Ca²⁺, making the

![Fig. 2. Small-conductance K⁺ (SK) channels, but not BK channels, in cell-attached and excised patches from principal cells (PCs) of rat CNT. A: cell-attached recording with a single SK channel in the patch with depolarization of up to 160 mV. Numbers to the left of the traces indicate the negative of the pipette voltage relative to the bath. The arrows to the right indicate the closed state of the channels. B: I-V relationship for the patch in A. C: excision of a cell-attached patch with an estimated 7 SK channels into the bath solution containing 2 mM Ca²⁺. The pipette potential was 0 mV. The arrow on the left indicates the 0 current level. After excision, SK channel activity persisted but no BK channels were observed.](http://ajprenal.physiology.org/)

Fig. 3. Whole-cell K⁺ currents in an intercalated cell (IC) from rat CNT. Top left: current traces with 5 mM EGTA and no added Ca²⁺ in the pipette solution. Bottom: current traces from a different cell with 200 nM Ca²⁺ in the pipette solution without (left) and with (right) 5 mM tetraethylammonium (TEA) in the bath. Top right: steady-state I-V relationship for the cell in bottom traces.
cytoplasmic-side Ca\(^{2+}\) concentration very low, whole-cell currents were very small, although at the most depolarized potentials (+100 mV) increased noise appeared that could be attributable to the opening of BK channels.

To measure BK currents, we used a 5 mM BAPTA/Ca buffer system with a calculated Ca\(^{2+}\) of 200 nM. A typical result is shown in Fig. 3B. Inward currents were still small, but outward currents increased dramatically as the membrane potential was made positive. The increase was not instantaneous but increased with time in a manner typical of voltage-gated channels. The large fluctuations in these currents probably reflect the high unitary conductance of the voltage-activated channels. In the presence of 5 mM TEA, which blocks BK channels, the time-dependent currents disappeared and the outward conductance and noise decreased. Thus Ca\(^{2+}\) channels. In the presence of 5 mM TEA, which blocks BK channels.

Reflecting the high unitary conductance of the voltage-activated channels. The large fluctuations in these currents probably reflect the high unitary conductance of the voltage-activated channels. In the presence of 5 mM TEA, which blocks BK channels, the time-dependent currents disappeared and the outward conductance and noise decreased. Thus Ca\(^{2+}\) dependence, time and voltage dependence, noise levels, and TEA sensitivity are all consistent with these currents’ being mediated by BK channels. The limiting conductance at large positive voltages was ~90 nS/cell (Table 2). If we assume a single-channel conductance of 200 pS and an open probability near one, this corresponds to a channel density of ~450/cell, in reasonably good agreement with that estimated from cell-attached patches (see above).

Similar results were obtained from ICs in the CCD (Table 2). These segments contain two types of ICs, termed \(\alpha\) and \(\beta\) (8), that we could not differentiate either optically or electrically. To determine which of these types expresses the BK currents, we made similar observations using outer medullary collecting ducts, which have mainly \(\alpha\)-ICs (8). The cells in this segment were more difficult to identify, but four cells were determined to be ICs by both visual (raised appearance) and electrical (high Cl\(^{-}\) conductance, low inward K\(^{+}\) conductance) criteria. All these cells had large BK currents, as illustrated in Fig. 4. Thus it is likely that BK channels are expressed in \(\alpha\)-ICs, but their presence in \(\beta\)-ICs as well cannot be excluded.

These currents were not detected in PCs (Fig. 5). Here, the large conductance is dominated by the K\(^{+}\) conductance of the basolateral membrane (7). In contrast to those of the ICs, the PC currents were inwardly rectifying, and outward currents decreased rather than increased with time. However, it is possible that the large basolateral conductance masks outwardly rectifying BK currents. TEA had no obvious effect on the PC currents (data not shown), but a small effect would have been difficult to detect as under these conditions basolateral conductance runs down rapidly. One strategy to deal with this problem was to allow the large initial currents to diminish to a steady state, which took ~5 min. At this point, both inward and outward K\(^{+}\) currents were very small, and there were no effects of TEA. In 10 experiments similar to that shown in Fig. 5, 7 PCs had no detectable BK-mediated currents. There were three cells with a measurable TEA-sensitive conductance, the largest of which was 7 nS, an order of magnitude smaller than the average value in the ICs. Mean values are shown in Table 2. It is possible that the BK channels also run down under these conditions, although in ICs they are stable over this time period. In four ICs from the same tubules in which the seals could be maintained for 5 min or more, the final TEA-sensitive current was 101 ± 37% of the initial value. In other experiments, we used a pipette solution with pH 6.0 to selectively inhibit the basolateral K channels (7). Under these conditions TEA-sensitive currents were still readily observed in ICs but not in PCs (data not shown). These results are consistent with the idea that BK currents in PCs are much lower than those in ICs, at least under our conditions of study.

Based on these results, we decided to reevaluate the possible role of ICs in the secretory process. We addressed three major questions: 1) is BK channel expression affected by dietary K\(^{+}\)?; 2) how high would intracellular Ca\(^{2+}\) have to be to account for K\(^{+}\) efflux across the apical membrane?; and 3) is the Na/K pump activity of ICs adequate to support K\(^{+}\) influx across the basolateral membrane? These data were obtained mainly in ICs of the CCD, rather than the CNT. This was in large part for technical reasons, since the CCD is much easier to dissect and prepare for patch clamp and the ICs in this segment are generally easier to identify. We feel that the similarities in the basic properties of the cells in the CCD and the CNT, and the fact that BK-mediated K\(^{+}\) secretion has been reported in the isolated, perfused CCD, justify this strategy.

To assess the effect of K\(^{+}\) intake on BK channels, we compared whole-cell currents in rats fed with chow containing either 10 or 1% KCl for 6–8 days. Under these conditions, the abundance of SK (ROMK) channels in the apical membrane of PCs increased three- to fourfold (16). No difference in the TEA-sensitive outward currents in ICs could be documented with this K-loading protocol (Table 2). However, this does not exclude a role of these channels in the regulated secretion of K\(^{+}\). Under both conditions, the density of channels is very high, and the activity can be varied over a wide range by controlling the intracellular Ca\(^{2+}\) activity (see below).

Figure 6 shows the average currents under conditions of 100, 200, and 500 nM Ca\(^{2+}\). Increasing Ca\(^{2+}\) shifted the threshold to a

---

**Table 2. TEA-sensitive conductance in cells of the distal nephron**

<table>
<thead>
<tr>
<th></th>
<th>BK Conductance, nS/cell</th>
<th>No. of Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>CNT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ICs</td>
<td>87 ± 17</td>
<td>15</td>
</tr>
<tr>
<td>PCs</td>
<td>0.9 ± 0.8</td>
<td>10</td>
</tr>
<tr>
<td>CCD ICs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control K diet</td>
<td>145 ± 19</td>
<td>27</td>
</tr>
<tr>
<td>High-K diet</td>
<td>137 ± 18</td>
<td>30</td>
</tr>
<tr>
<td>OMCD ICs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>High-K diet</td>
<td>157 ± 44</td>
<td>4</td>
</tr>
</tbody>
</table>

Values are means ± SE. CNT, connecting tubule; CCD, cortical collecting duct; OMCD, outer medullary collecting duct.
for voltage activation toward more negative values. At 200 and 500 nM, but not 100 nM, significant TEA-sensitive currents could be observed at membrane potentials near 0 mV. Since this represents a reasonable estimate for the potential in the intact, K⁺-secreting tubule (see the appendix), the results suggest that elevation of Ca²⁺ to 200 nM or more may be adequate to stimulate K⁺ secretion across the apical membrane of these cells.

For the ICs to secrete K⁺, the cells would require a pathway for K⁺ influx across the basolateral membrane at rates equal to those of efflux through the apical channels. Since the Na-K-ATPase presumably constitutes the major pathway for K⁺ entry, we measured the pump activity in ICs as a ouabain-inhibited current (17, 18). In these experiments, K⁺ conductances were kept low using internal and external blockers, Cl⁻ currents were minimized by removal of Cl⁻ from the media, and pump activity was stimulated by including 50 mM Na⁺ and 3 mM ATP in the pipette solution. As reported previously (18), these currents are readily seen in PCs of both the CNT and the CCD (Fig. 7A). In ICs, however, ouabain had no measurable effect, and pump currents were below our level of detection (Fig. 7B). Mean values for pump currents are shown in Fig. 7C.
Role of BK Channels in K⁺ Secretion

Earlier work in our laboratory suggested a minimal role for BK channel in K⁺ secretion (3). In isolated, perfused rabbit CCDs, addition of the universal K⁺ channel blocker Ba²⁺ to the luminal perfusate resulted in hyperpolarization of the transepithelial potential, while 5 mM TEA, which is more specific the luminal perfusate resulted in hyperpolarization of the trans-

epithelial potential, while 5 mM TEA, which is more specific to Rb⁺ for BK channels, did not. Furthermore, transepithelial conduc-

tance to Rb⁺ was one-third to one-half that of K⁺, while Rb⁺ conduc-
tance through the channels was quite low. The tubules were taken from DOCA-treated rabbits with high rates of Na⁺

transport and were studied under conditions of high luminal flow rates (15–25 nl/min). These studies were limited, however, in the sense that transepithelial electrical properties were measured, rather than net ion fluxes.

More recently, several studies have produced evidence for a contribution of BK channels to K⁺ transport, at least under certain conditions. Woda et al. (29) used TEA and charybdotoxin as specific blockers of the BK channels. They found that net K⁺ secretion by perfused rabbit CCDs was unaffected by these agents at low luminal flow rates (0–1.5 nl min⁻¹ mm⁻¹) but reduced secretion by 50% or more at high flow rates (5–6 nl min⁻¹ mm⁻¹). Their main conclusion was that K⁺ was transported by ROMK channels in a flow-independent manner and by BK channels through a flow-dependent mechanism.

Pluznik et al. (22) studied mice in which the B1-subunit of the BK channel had been genetically deleted. They reported that these mice excreted K⁺ normally under basal conditions but did not increase K⁺ excretion as much as control mice after an acute volume expansion. This was partially attributable to a smaller increase in glomerular filtration, but the knockout mice also failed to exhibit an increase in fractional K⁺ excretion. This suggested that tubular K⁺ transport was also defective in these animals.

In recent studies of in vivo perfused distal tubules in mice, Bailey et al. (1) found that iberiotoxin, a specific blocker of BK channels, inhibited a significant fraction of K⁺ secretion in ROMK knockout animals and in normal mice on a high-K diet.

Taken together, these observations strongly suggest that BK channels mediate a portion of K⁺ secretion by the distal nephron, at least when flow rates are high. This idea is discussed in more detail in a recent review (21).

Distribution of BK Channels

The results of this paper confirm those reported earlier suggesting that most BK channels in the CCD are in ICs (15) and extend these observations to the CNT, a segment where much of K⁺ secretion in vivo is believed to occur (28). We have also searched for BK channel activity in principal cells using whole-cell recording techniques and found that it is very low, even in the presence of elevated intracellular Ca²⁺. This, however, does not rule out a role of BK channels in the PCs, as it is possible that the channels in these cells might be internalized under our experimental conditions and therefore unable to contribute to membrane conductance.

Immunocytochemical localization of BK channel protein has led to conflicting results. A recent study of the α-subunit of the channel, which contains the conducting pore, showed expression in a minority cell type in the rabbit CCD (14). These cells were identified as ICs based on coexpression of H-ATPase, believed to be IC specific, and the absence of colabeling with the lectin DBA, a PC marker. In contrast, the β1-subunit, an accessory protein that can modulate channel function, has a very different distribution. In the mouse kidney BK channel, β protein was found exclusively in the CNT, and colocalization with aquaporin-3 (AQP3) suggested that it was expressed in PCs (23). Pluznik and Sansom (21) also found no costaining of BK channel β1 with IC markers, supporting a PC location of this subunit. Our electrophysiological data are much more consistent with the reported distribution of the BK α-subunit.
However, the discrepancy regarding BK channel β1 distribution will need to be resolved.

A high-K diet increased the expression of mRNA encoding BK channels as well as a redistribution of channel protein from intracellular to apical membrane locations in ICs of the rabbit CCD (14). We did not detect any change in BK-mediated K⁺ conductance when rats were fed a high-K diet. Thus regulation of these channels during K⁺ adaptation may be species specific.

**Driving Forces for K⁺ Secretion**

The reversal potentials for K⁺ conduction through BK channels in ICs implied that, at least under our experimental conditions, these cells have a much less negative membrane potential than do PCs. It is likely that this reflects a large difference in the basolateral membrane properties of the two cell types, with ICs having a larger Cl⁻ conductance and a much smaller K⁺ conductance than the PCs. This is in good agreement with a previous microelectrode study of the rabbit CCD, in which a minority cell type, presumably ICs, had a basolateral membrane potential of −36 mV compared with −82 mV in the majority PCs (13). In that report, the less negative membrane potential was also attributed to a predominant conductance of the basolateral membrane to Cl⁻. These measured cell potentials should reflect the basolateral membrane potentials under an open circuit, since the generally much larger basolateral conductances will dominate those of the apical membrane under the short-circuit conditions that pertain to the split-open tubule. Also, since the apical membrane resistance is presumably higher than that of the basolateral membrane in the ICs, any transepithelial voltage resulting from Na⁺ reabsorption through PCs will affect mainly the apical membrane potential of the IC (see the APPENDIX). Thus in the intact tubule the apical membrane voltage of the IC will be considerably depolarized relative to that of the PC.

This would have several implications for K⁺ transport through the BK channels of these cells. First, the driving force for K⁺ efflux across the apical membrane would be large, as the concentration difference for K⁺ would not be offset by a negative apical membrane voltage. Second, the depolarized apical membrane would increase the open probability of BK channels. This would, in turn, reduce the requirement for elevation of intracellular Ca²⁺ to open the channels. The depolarized basolateral membrane potential would therefore facilitate K⁺ secretion through BK channels in the apical membrane.

**Role of ICs in K⁺ Secretion**

If ICs are involved in K⁺ secretion, then they must have a mechanism for the uptake of K⁺ across the basolateral membrane. In the classic model of K⁺ secretion, this uptake takes place via the Na/K pump (4). However, we were unable to measure appreciable pump currents in ICs. This finding is consistent with previous immunocytochemical studies in which the antibodies against the Na-K-ATPase stained the basolateral membranes of PCs but not ICs in the rat kidney (10). It is also in agreement with measurements using electron probe analysis (2) that showed that Rb⁺ uptake into ICs, reflecting, at least in part, the activity of the Na/K pump, was much lower than that of PCs. Thus although intracellular K⁺ concentrations are high in these cells, only a very small pump activity is sufficient to maintain these concentrations, presumably because Na⁺ influx rates are also very small.

Model calculations suggest that SK/ROMK channels in the CCD can account for rates of K⁺ secretion as high as 48 pmol·min⁻¹·mm tubule⁻¹ or ~160 pA/cell (6). If ICs secreted K⁺ at roughly the same rate as PCs, then an effective stoichiometry of 2K⁺/1ATP would imply a maximal pump current of at least 80 pA/cell. Our measured currents were <10 pA/cell, suggesting that pump activity is not adequate to sustain a substantial K⁺ secretion through the ICs.

Immunocytochemical studies revealed the expression of Na-K-2Cl cotransporters on the basolateral membrane of ICs, at least in the outer medullary collecting duct (OMCD) (5). These cotransporters could provide a mechanism for K⁺ uptake. However, the Na⁺ that enters the cells would still need to be removed, presumably by the Na/K pump, so that the presence of these transporters would reduce but not eliminate the need for the pump to sustain K⁺ secretion.

A third route for K⁺ influx is through the H-K-ATPase. This pump has been postulated to carry out net H⁺ secretion and K⁺ reabsorption, and both “gastric” and “colonic” isoforms appear to be expressed by ICs (25). In most cases, the enzyme has been localized to the apical membrane, implying that the role of the BK channels would be to recycle rather than to secrete K⁺. Of course, the overall result of a reduction in K⁺ reabsorption in net flux measurements would be the same as the stimulation of secretion.

In summary, we have confirmed a high density of BK channels in the apical membranes of cells of both the CNT and CCD, an essential feature of these segments if these channels are to contribute to K⁺ secretion. However, most of these channels are confined to ICs, cells not normally associated with transepithelial K⁺ transport. In addition to their high channel density, these cells also have an apical membrane voltage that is considerably depolarized relative to that of the PCs. This has the advantage of both increasing the driving force for K⁺ secretion across the apical membrane and activating the voltage-dependent BK channels. The notion of transepithelial K⁺ transport across the ICs raise two questions, neither of which is fully answered in the present study. First, under what conditions is cell Ca²⁺ in the ICs high enough to keep the BK channels open? Woda et al. (30) found that high flow rates could increase Ca²⁺ to ~200 nM in both ICs and PCs of rabbit CCDs. It is also possible that hormones that signal through Ca²⁺ pathways could contribute to elevation of these levels. The second, more difficult, problem is how K⁺ could enter these cells across the basolateral membrane to support a steady-state apical membrane flux. Na/K pump densities do not appear to be adequate for this purpose.

**APPENDIX**

The intercalated cells that we have studied have two major conductances: an apical K⁺ conductance (Gₚₖ) mediated by BK channels and a presumably basolateral Cl⁻ conductance (Gₐ₅) of much larger magnitude. The electrical circuit for these cells is given by

```
  Gₚₖ  Eₚₖ  Gₐ₅  Eₐ₅
lumen |   | cell | | interstitium
```

AJP-Renal Physiol • VOL. 292 • MARCH 2007 • www.ajprenal.org
Under conditions of 500 nM Ca\(^{2+}\) in the cells, K\(^{+}\) conductance is at most \(\sim 10\) nS/cell, corresponding to a resistance of \(10^8\) \(\Omega\). With 5 mM K\(^{+}\) outside the cell, we presume the equilibrium potential of K\(^{+}\) (\(E_k\)) is about \(-80\) mV. The Cl\(^{-}\) conductance is \(\sim 150\) nS/cell (19). Electron microprobe measurements indicated two populations of ICs, one of which had a high Cl\(^{-}\) concentration in the cytoplasm of 35 mM (2), corresponding to \(E_{CI} = -37\) mV. This predicts an intracellular voltage of \(-38\) mV, close to the values (\(-36\) mV) observed in cell-attached patches.

If we now assume a transepithelial voltage of \(-40\) mV, due to active transport of Na\(^{+}\) through the PCs, the transcellular current would be 380 pA. From a simple voltage-divider calculation, this current would hyperpolarize the basolateral membrane by 2 mV and depolarize the apical membrane by 38 mV compared with the short-circuited case. Thus the apical membrane voltage would be \(-30\) mV.

**ACKNOWLEDGMENTS**

This work was supported by National Institute of Diabetes and Digestive and Kidney Diseases Grant DK-27847.

**REFERENCES**


