Regulation of apical K channels in rat cortical collecting tubule during changes in dietary K intake

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Palmer, Lawrence G., and Gustavo Frindt. Regulation of apical K channels in rat cortical collecting tubule during changes in dietary K intake. Am. J. Physiol. 277 (Renal Physiol. 46): F805–F812, 1999.—Long-term adaptation to a high-K diet is known to increase the density of conducting secretory K (SK) channels in the luminal membrane of the rat cortical collecting tubule (CCT). To examine whether these channels are involved in the short-term, day-to-day regulation of K secretion, we examined the density of K channels in animals fed a high-K diet for 6 or 48 h. CCTs were isolated and split open to provide access to the luminal membrane. Cell-attached patches were formed on principal cells with 140 mM KCl in the patch-clamp pipette. SK channels were recognized from their characteristic single-channel conductance, inwardly rectifying channel with a high, voltage-independent open probability (19). These channels are most likely the product of the ROMK gene (10).

Previous studies showed that the density of SK channels in the apical membrane of the rat CCT increased when the animal was fed a high-potassium diet for 1 wk or more (9, 20). This finding is consistent with a role for regulation of SK channels in K homeostasis. Under these conditions, however, there are complex morphological as well as physiological changes in the cells of the CCT. Activities of apical Na channels and the basolateral Na/K pump increase, and the surface area of the basolateral membrane is enhanced through basal infoldings (9, 18). However, Rabinowitz et al. (13) showed that the rat kidney adapts much more rapidly to increased K intake. Renal K excretion rises to the matched K intake within 1 to 2 days.

In this study, we asked whether changes in SK channel density could occur over a more rapid time course and contribute to the day-to-day maintenance of plasma K. We also addressed the question of whether animals on a low-K diet, which need to avoid loss of K in the urine, have a reduced density of conducting SK channels.

METHODS

Biological preparations. Sprague-Dawley rats of either sex (100–150 g) raised free of viral infections (Charles River Laboratories, Kingston, NY) were fed with one of the following matched and nutritionally adequate diets (Harlan-Teklad, Madison, WI): high-K (10% KCl), control K (1.2% KCl), or K-deficient diet (no added KCl). All diets contained 0.39% Na. In a few experiments, the rats were given a low-Na diet (Na content 3.8 mg/kg; K content 8.6 g/kg; ICN, Cleveland, OH). The food intake per rat ranged between 6 and 15 g/day. In the experiments designed to measure the effect of high K intake for 6 h, the control K diet was withdrawn overnight, and the next morning the rats were given high-K food or control diet for 6 h. During this interval, the rats ate 2–4.5 g of food each. In this group, the ratio of Na/K concentrations in the urine was 1.30 ± 0.18 in controls and 0.20 ± 0.05 in the high-K rats. To increase levels of circulating aldosterone, rats were implanted subcutaneously with osmotic mini-pumps (model 2002 or 1007D; Alza, Palo Alto, CA). Aldosterone was dissolved in polyethylene glycol 300 at concentrations calculated to give the desired infusion rate according to the pumping rate specified by the manufacturer.

Animals were killed by cervical dislocation, the kidneys were removed, and CCTs were dissected free and opened manually to expose the luminal surface. Under these conditions the tissues retain their epithelial structure, and the cells are presumed to remain polarized. The split tubules were attached to a small plastic rectangle coated with Cell-Tak (Collaborative Research, Bedford, MA) and placed in a perfusion chamber mounted on an inverted microscope. The...
chamber was continuously perfused with solution which was prewarmed to 37°C by a miniature water-jacketed glass coil (Radnotti Glass Technology, Monrovia, CA). Principal cells of the tubule were identified visually.

Solutions. For cell-attached patch recordings, tubules were superfused with a physiological saline solution consisting of (in mM) 140 NaCl, 2 CaCl2, 1 MgCl2, 2 glucose, and 10 HEPES adjusted to pH 7.4 with NaOH. The patch-clamp pipettes were filled with solutions containing (in mM) 140 KCl and 10 HEPES adjusted to pH 7.4 with KOH to measure K channel currents, or one in which KCl was replaced by LiCl to measure Na channel currents.

Electrical measurements. Basic patch-clamp methods were as described previously (2, 3, 17). Pipettes were fabricated from hematocrit glass capillaries using a three-stage pulling process and were coated with Sylgard. Pipette resistances were 2-5 MΩ. Seals were made on the apical surface of the cells and were normally between 2 and 10 GΩ. Recording of currents and analysis of data were carried out with an Atari model 1040 ST computer equipped with interface and data acquisition software (Instrutech, Mineola, NY). Current records were stored on videotape using a pulse-code modulator (Instrutech). For analysis, records were filtered at 1 kHz. Single-channel currents were measured from 10–20 clear transitions in the current records. Computation of mean open and closed times was carried out using the TAC program (Instrutech).

Patch membrane area. We wished to measure the area of the cell-attached patches from which channel activities were measured. We could not detect the movement of membrane into the patch pipette using the Hoffman modulation contrast optics on our microscope. We also tried to visualize the membrane interface by including the water-soluble dye Lissamine green in the pipette. As shown in Fig. 1, the dye could be discerned. We concluded that the area of membrane comprised within the resolution of the optical system.

To estimate the tip diameter, we used the formula (14)

\[ R = R_s + \frac{\rho cot(\phi/2)}{\pi[(1/r_t - 1/r_s)]} \]

where \( R \) is the resistance of the entire pipette, \( R_s \) is the resistance of the shank, \( \rho \) is the resistivity of the solution assumed to be 51 Ω·cm, \( \phi \) is the angle of the cone at the tip, and \( r_t \) and \( r_s \) are the radii of the tip and the shank, respectively. We measured the following parameters on typical pipettes:

- \( R = 4.1 \) MΩ
- \( R_s = 0.9 \) MΩ
- \( \phi = 9.8^\circ \)
- \( r_t >> r_s \)

We then calculated that \( r_t = 0.58 \) µm. This gives an estimate of tip area of 1.06 µm². This number was used to normalize channel densities to channels per square micrometer as reported in the text and Table 1.

Analysis. In the animals in which plasma K level was measured, the rats were anesthetized with methoxyflurane, and blood was obtained from the abdominal aorta. K was measured by flame photometry (model 943; Instrumentation Laboratory, Lexington, MA).

Radioimmunoassay for plasma aldosterone was carried out with the ImmuChem double antibody kit from ICN (ICN Pharmaceuticals, Costa Mesa, CA) according to the manufacturer’s instruction and including the following modification. To avoid cross-reactivity with the high endogenous level of corticosterone found in the rat, the aldosterone was separated from corticosterone using Celite column chromatography. Columns contained ~1 g Celite mixed with 0.33 ml of 40% ethylene glycol in water. The dried ethyl acetate:hexane extract corresponding to 0.4 ml of plasma was resolubilized in 0.1 ml of ethyl acetate and subsequently mixed with 0.4 ml of isooctane. This entire volume was transferred onto the Celite column. The column was washed with 3.5 ml of 15% ethyl acetate in isooctane, then with 3.5 ml of 40% ethyl acetate in isooctane, and these washes were discarded. Aldosterone was eluted with 3.5 ml of 60% ethyl acetate in isooctane into a test tube, and this eluate was evaporated to dryness. The sample residue was then reconstituted with 2 ml of the steroid diluent provided with the kit, and the assay was completed as described.

RESULTS

Representative traces for several patch-clamp recordings of SK channel activity are illustrated in Fig. 2A. These traces also illustrate a difficulty in counting the number of conducting channels in each patch. This is commonly done by counting the number of discrete current levels observed and subtracting one for the state in which all channels are closed. SK channels, however, have a very high open probability (Pₒ). Thus when there are several channels in the patch, the probability that all are closed at the same time can be vanishing small. If this minimum current level is not observed over the time period of the recording, then the number of channels in the patch will be underestimated using this approach.

To circumvent this problem, we made use of an advantage of the high Pₒ: that the state in which all channels are open is frequently observed and well defined, as shown in Fig. 2. We also took advantage of

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Fig. 1. A: photomicrograph of a pipette filled with solution containing Lissamine green (50 mg/ml). The dye extends to the tip of the pipette within the resolution of the optical system. B: photomicrograph of a similar pipette in which the tip was prefilled with mineral oil. The dye-free tip can easily be resolved. The length of the oil-filled portion of the tip was 15 µm, as measured with an eyepiece micrometer.
Fig. 2. Secretory K (SK) channel activity in cell-attached patches. A: traces from four different patches containing different numbers of SK channels. In all cases, the pipette voltage was zero relative to the bath. Horizontal lines to the right of each trace indicate the current level at which all channels are open. Histograms beside each trace indicate the distribution of dwell time in the state with all channels open. Solid lines indicate best fits with single exponentials with time constants of (from top to bottom) 24.6, 12.0, 8.1, and 4.0 ms. Number of channels ($N$) was determined independently in the top three traces and from Eq. 2 in the bottom trace. B: rates of closure computed as the inverse of the time constants of the open-time histogram are plotted vs. $N$ for patches in which $N$ could be independently determined. Data are given as means ± SE. Line is drawn according to the average value of rate $k_{N} = N^{-1}$ for each measurement.
the findings that the kinetics of these channels are quite reproducible from patch to patch, and that in all single-channel recordings analyzed so far, the dwell-time histograms are consistent with the presence of a single open state of the channel. If the rate at which any one channel leaves the open state is $k_{\text{close}}$, then for a patch with $N$ channels the rate for transitions from the state in which all $N$ channels are open to the state in which one channel is closed ($k_{N-N-1}$) is given by

$$k_{N-N-1} = Nk_{\text{close}}$$

(2)

In the top three traces in Fig. 2A the number of channels in the patch was measured independently from the number of current levels. In general, $N$ could be determined with certainty for all patches with either one or two channels, and for some patches with three channels. In these cases, a minimum current level was found in which no rapid closing events were observed. Open time histograms for these patches were constructed from the dwell times in the state of maximum current in which all channels were open, and the rates of leaving the all-open state were estimated. As shown in Fig. 2B, these rates are linear functions of $N$ as expected from Eq. 2.

For a patch with three or more channels in which the fully closed state could not be determined, $N$ was estimated from the ratio of $k_{N-N-1}/k_{\text{close}}$. For example, in the bottom trace of Fig. 2A, the fully closed state was not observed, but the rate $k_{N-N-1}$ could be estimated readily. From Eq. 2, we estimated $N$ to be 6.

In a few cases, $N$ was so large (>10) that open-time histograms were difficult to construct. In these instances, $N$ was estimated from the absolute inward current when the pipette voltage was held at 0 mV. In these circumstances most of this current will go through the channels, whose aggregate conductance is much larger than that through leak pathways. The total maximum inward current was divided by the single-channel current measured in the same patch to obtain an estimate of $N$ from the relationship $I = iN$ which is valid when all the channel are open. This method had to be used in less than 2% of the patches analyzed.

The distribution of channels in patches from rats fed the control diet is shown in Fig. 3A. The majority of patches had no detectable activity, so these were assigned a value of $N = 0$. Other patches had from 1 to 4 channels. The histogram can be approximated by a Poisson distribution with a mean density of 0.43 channels/µm$^2$, consistent with a random distribution of active channels in the apical membrane.

The distributions of channels in patches from rats fed the high-K diet for either 6 or 48 h are shown in the Fig. 3B and C. In both cases, it is clear that a much larger percentage of patches contained channel activity and that many more patches had a large number (>5) of conducting SK channels. These histograms could not be described by Poisson distributions. First, there were many more patches with no channels than would be predicted from a single Poisson distribution. These findings are consistent with the idea that channels are clustered. Since we generally made only a single seal on a given cell, we cannot say whether this clustering represents a heterogeneity from cell to cell or from one area to another on the same cell.

To more easily make statistical comparisons of the effects of the diet, we computed the average channel density for each animal studied. These averages included values from at least 12 different patches on at least three different tubules for each rat. These data are shown in Fig. 4. Values for control animals ranged from 0.21 to 0.85 with a mean value of 0.40 (Table 1). Values for rats fed the high-K diet were from 0.6 to 3.6 channels/µm$^2$. The mean values both for the 6-h and the 48-h K loading protocols were significantly larger.

![Histograms of N (channels/patch) for rats on control diet (A), on a high-K diet for 6 h (B), and on a high-K diet for 48 h (C). Each determination of N represents a single cell-attached patch.](http://ajprenal.physiology.org/DownloadedFrom/10.220.33.4/201710142017)
than controls using the Student's t-test. Since it is not obvious that the values for individual animals follow a normal distribution, we also compared the data using the nonparametric Mann-Whitney ranking test. This comparison indicated differences between controls and both experimental groups at an even higher level of significance (P < 0.001) in both cases. The values at 6 h of high K were not significantly different from those at 48 h.

To determine whether SK channels were downregulated when animals were deprived of K, rats were fed a low-K diet for 5–10 days before measurement of channel activity. In these animals plasma K fell from control levels of 3.68 ± 0.07 mM to 2.44 ± 0.04 mM. In this circumstance, channel densities were similar to those of the control group (Fig. 4). In four animals, the average density was 0.53 ± 0.13 channels/µm². This was not significantly different from the group of all controls (0.40 ± 0.05) or from a smaller group of paired controls run over the same period (0.65 ± 0.17, n = 4).

In separate experiments, the activity of Na channels was measured using Li in the pipette as the major conducted ion. A typical patch containing Na channels is illustrated in Fig. 5A. As reported previously, these channels had slow kinetics (Fig. 5A) and a slope conductance of 12–14 pS for inward Li current at 37°C (Fig. 5B). As reported previously, the density of conducting Na channels under control conditions was very low. In this series, the mean value was 0.08 channels/µm², which was accounted for by two patches from the same animal with one and two channels, respectively. All other patches were devoid of channel activity. Na channels were much more prevalent in patches from K-loaded animals. The average density was 1.00 and 1.08 after 6 h and 48 h on a high-K diet, respectively. The time course of the changes in SK and Na channel density is plotted in Fig. 6. As with the SK channels, the Na channels observed in K-loaded rats appeared to be clustered in the membrane. Four of 33 patches made in animals on the high-K diet for 48 h had five or more conducting Na channels. This probability (0.12) is more than 10 times greater than that expected from a Poisson distribution with mean density of 1.08 channels/patch (P < 0.01). The significance of the appearance of Na channels for K secretion is discussed below. The effects of long-term (>1 wk) K adaptation on SK channels appeared to be independent of changes in plasma aldosterone concentration (9). We reevaluated this relationship with respect to the short-term increase in SK channel activity. First, plasma aldosterone levels were measured in animals fed high K for 6 and 48 h. There was approximately a twofold increase in aldosterone after 6 h, and a larger, although variable increase after 48 h (Table 1). This suggests the possibil-

Fig. 4. Average values of N for individual animals. Each point represents the mean of 12 or more patches from 3 or more tubules. *Distribution of values for animals on a high-K diet for either 6 h or 48 h was significantly different from controls.

Fig. 5. Na channel activity in a cell-attached patch from an animal on a high-K diet for 48 h. A: traces of a typical channel-containing patch. Horizontal lines to left of each trace indicate the current level at which all channels were closed. Numbers to the right indicate the voltage of the pipette relative to the bath. B: current-voltage relationship for the channels shown in A. Line through the data points indicates a slope conductance of 13.0 pS. Vpipette, pipette voltage in mV.
that mineralocorticoids could mediate the response to a high-K diet. However, much larger increases in aldosterone are observed when the animals are fed a low-Na diet (8). Under these conditions there was no increase in SK channel density.

Because other physiological changes may occur when dietary Na is restricted, we also investigated the effects of infusing the animals with known amounts of aldosterone through osmotic minipumps. The pumps were filled with aldosterone at a concentration designed to increase plasma concentrations to levels greater than those with a high-K diet. There was no significant increase in SK channel density in these animals after 2 days of infusion (Table 1). However, as expected, plasma K was reduced under these conditions. We next studied animals infused with the same concentration of aldosterone but fed a high-K diet (48 h). Here plasma K was close to normal, whereas aldosterone was strongly elevated. Again, there was no measurable increase in SK channel density (Table 1). This suggests that increases in plasma aldosterone per se are not sufficient to upregulate the SK channels.

**DISCUSSION**

**Density of SK channels.** The mean number of conducting SK channels in cell-attached patches in the apical membrane of the rat CCT varied from 0.5 to 2 channels/patch depending on the physiological circumstance. To convert these values to a true channel density, we estimated the surface area of a patch from the geometry of the patch pipettes. This value was about 1 µm², which may be an underestimate if the membrane is drawn slightly into the pipette tip.

The single-channel conductance (for inward current) of these channels is about 40 ps with 140 mM K in the pipette. We do not have direct measurements of single-channel conductance under physiological conditions, but with an extracellular K of 5 mM, the conductance of ROMK2 channels expressed in Xenopus oocytes is about one-third of that measured with isotonic K. If we assume a single-channel conductance of 13 pS and a P₀ of 0.9, then the overall K conductance through SK channels in the principal cells of the CCT would vary from 0.6 to 2.4 mS/cm². There are no direct measurements of the K conductance of the apical membrane of principal cells in the rat CCT. An indirect estimate, based on net flux measurements, suggested a value of 2.6 mS/cm² (16). This is higher than the most comparable values that we estimate from control or aldosterone-treated animals but is close to that we obtained for K-loaded rats. We think that it is likely that SK channels contribute a major fraction of the overall apical K conductance, particularly since these are by far the most abundant K-conducting channels that we and others have observed using patch-clamp techniques. However, we cannot rule out the possibility that other channel types may contribute to apical K conductance and/or K secretion in this nephron segment.

As indicated in the histograms in Fig. 3, SK channels appeared to be distributed in clusters rather than randomly in the apical membrane of the CCT. This was particularly evident with the animals on the high-K diet. In this case there were more patches with no channels than would be predicted from a Poisson distribution. In principle, the heterogeneity of the channel distribution could be from patch to patch within the same cell, from cell to cell within the same tubule, from tubule to tubule within the same animal, or from animal to animal. The latter two possibilities are less likely, since SK channel density was higher than the mean control value in every K-loaded animal tested (Fig. 4) and in nearly every tubule examined (data not shown). It is impossible to distinguish the former two possibilities on the basis of our data, since we generally studied only a single patch from each cell. The clustering could reflect insertion of channels into the membrane at specific sites, or heterogeneous biochemical events leading to activation of channels within certain parts of the cell. However, we cannot entirely rule out the idea that the apparent clustering is an artifact, resulting from slight damage to some of the cells during the isolation and opening procedures.

Effects of dietary K. Previous studies had shown that chronic (>10 days) elevation of dietary K intake increased the density of SK channels in the apical membrane of the rat CCT (9, 20). However, renal K excretion in the rat can increase to match the augmented uptake within 1 or 2 days (13). It was not clear

<table>
<thead>
<tr>
<th>Condition</th>
<th>K Channel Density, channels/µm²</th>
<th>Plasma Aldo, ng/dl</th>
<th>Plasma K, mM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.41 ± 0.06</td>
<td>15 ± 4</td>
<td>3.68 ± 0.07</td>
</tr>
<tr>
<td>High-K diet (6 h)</td>
<td>1.51 ± 0.15</td>
<td>36 ± 8</td>
<td>NM</td>
</tr>
<tr>
<td>High-K diet (48 h)</td>
<td>2.13 ± 0.37*</td>
<td>98 ± 23*</td>
<td>4.37 ± 0.19*</td>
</tr>
<tr>
<td>Low-Na diet (7 days)</td>
<td>0.48 ± 0.20</td>
<td>1,260 ± 130*</td>
<td>NM</td>
</tr>
<tr>
<td>Aldo infusion (48 h)</td>
<td>0.44 ± 0.08</td>
<td>550 ± 46*</td>
<td>2.44 ± 0.04*</td>
</tr>
<tr>
<td>Aldo infusion + high-K diet</td>
<td>0.32 ± 0.07</td>
<td>521 ± 65*</td>
<td>3.80 ± 0.17</td>
</tr>
<tr>
<td>Low-K diet</td>
<td>0.53 ± 0.13*</td>
<td>22 ± 3</td>
<td>2.30 ± 0.10*</td>
</tr>
</tbody>
</table>

Values are means ± SE. Aldo, aldosterone; NM, not measured. *P < 0.05, significantly different from control values (Student's t-test).
from the earlier results whether upregulation of these channels was relevant to this day-to-day regulation of K excretion.

To address this question we gave a high-K diet to rats for 48 h and for 6 h. The first time period was selected to allow the rate of renal excretion to reach a steady state (13). The second time period represents essentially a single high-K meal. Under both of these circumstances, there was a significant increase in channel density. In fact, the densities reached levels comparable to those observed with chronic K loading (9). This indicates that the regulation of the number of conducting K channels could be an important component in the balancing of K intake and excretion over the course of a day, as well as in long-term adjustments to high intake levels.

In addition to the increased number of SK channels, the density of conducting Na channels was also increased by short-term administration of a high-K diet. The presence of these channels will enhance K secretion by the CCT in two ways. First, influx of Na into the cell will depolarize the apical membrane, increasing the driving force for K efflux into the urine. Second, the ensuing increase in intracellular Na concentration stimulates the basolateral Na/K pump, bringing K into the cell at a higher rate.

In contrast, lowering the intake of K did not affect SK channel density, despite a marked decrease in plasma K concentrations. It is possible that changes in principal cell surface area could affect the number of channels without a change in channel density. However, we did not notice any marked changes in cell morphology. We suspect that the reduction in K excretion seen did not notice any marked changes in cell morphology. We suspect that the reduction in K excretion seen under these conditions may result more from a stimulation of K reabsorption (21), particularly in the outer medullary collecting ducts. However, we did not notice any marked changes in cell morphology. We suspect that the reduction in K excretion seen under these conditions may result more from a stimulation of K reabsorption (21), particularly in the outer medullary collecting ducts.

Role of aldosterone. The importance of mineralocorticoids in the regulation of apical K channels is an issue that remains unsettled. Measurements of apical membrane K conductance in the rabbit CCT indicated a delayed activation in response to deoxycorticosterone acetate (DOCA) administration (15). Furthermore, in primary cultures of rabbit CCT cells the density of SK channels was dependent on the presence of aldosterone in the growth medium (6). In the rat CCT, Schafer et al. (16) argued that the increased K secretion in response to DOCA treatment of the animal could be accounted for by an increased driving force for K movement across the apical membrane. Consistent with this idea, we found that SK channel density was not increased either with infusion of aldosterone or when the animals were fed a low-Na diet to stimulate endogenous aldosterone secretion (Table 1, see also Ref. 9). It therefore appears, at least in the rat CCT, that increased aldosterone levels are not sufficient for upregulation of the SK channels.

We found previously that the increase in SK channel density with long-term high-K diet was abolished by adrenalectomy (9). This raises the possibility that slightly elevated levels of plasma aldosterone might be a necessary cofactor in this process. Again, data from rabbit CCT are somewhat different. Muto et al. (7) found that apical K conductance increased in adrenalectomized animals on a high-K diet. In addition, Wingo et al. (22) found increased net K secretion in tubules isolated from K-loaded, adrenalectomized rabbits.

A number of studies have indicated a synergistic interaction between elevated plasma aldosterone levels and increased plasma K concentrations in promoting K excretion in rats (5), dogs (23), and sheep (12). This interaction may reflect the separate control of apical Na and K channels in the distal nephron segments. High plasma K due to increased K intake would increase apical K conductance. High levels of aldosterone would activate Na channels, depolarizing the apical membrane and increasing the driving force for K secretion. In addition, Na entering the cells would stimulate the basolateral Na/K pump, bringing more K into the cell.

This picture is undoubtedly an oversimplification. First, a high K intake itself can to a small extent activate Na channels through a mechanism that is apparently independent of aldosterone (Table 1; Ref. 9). Second, at least in the rabbit CCT as discussed above, aldosterone may itself increase K conductance. Third, Beesley et al. (1) recently showed that aldosterone infusion increased the amount of mRNA coding for several ROMK isoforms in the kidney, although the specific nephron segments involved were not identified (1). Nevertheless, this basic idea of distinct pathways for controlling Na and K channels could explain many of the experimental observations and provide a mechanism for the separate regulation of Na and K transport in these segments.

Regulators of K channels. The above discussion implies the existence of factors other than aldosterone which by themselves or in conjunction with the mineralocorticoid control SK channel activity. Rabinowitz (11) has suggested that a high K intake could release kaliuretic factors from the central nervous system through a reflex involving receptors in the gut or portal vein. This would provide for control of K excretion that would be independent of both aldosterone and of elevation of systemic plasma K. In our studies, high plasma K appears to be essential for upregulation of SK channels. In aldosterone-infused animals, plasma K is maintained near normal levels in the face of a high K intake (Table 1). Since SK channel density is not increased under these circumstances, increased K absorption by the intestines does not appear to be a sufficient signal. High plasma K could have a direct effect on the renal tubular cells to stimulate SK channel expression or activation. Such an effect would have to be retained by the tubules through the process of isolation and superfusion with defined media. Alternatively, high plasma K could release a kaliuretic hormone that would act on the CCT to activate the channels. Both the putative hormone and its site of secretion remain unidentified.

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