J. Membrane Biol. 162, 107–116 (1998)

The Journal of
Membrane
Biology

Environmental KCl Causes an Upregulation of Apical Membrane Maxi K and ENaC Channels in Everted *Ambystoma* Collecting Tubule

L.C. Stoner, S.C. Viggiano

Department of Physiology, State University of New York, Health Science Center at Syracuse, Syracuse, New York 13210, USA

Received: 25 August 1997/Revised: 13 November 1997

Abstract. Patch clamp methods were used to characterize the channels on the apical membrane of initial collecting ducts from *Ambystoma tigrinum.* Apical membranes were exposed by everting and perfusing fragments of the renal tubule *in vitro*. Tubules were dissected from two groups of animals; one maintained in tap water, and the other kept in a solution of 50 mM KCl from seven to nineteen days. Patches of apical membranes on tubules taken from animals exposed to tap water expressed low-conductance amiloride sensitive sodium channels (ENaC) in 22 of 49 patches. Only three maxi K channels were observed in this group. In animals exposed to KCl, low-conductance amiloride sensitive sodium channels, 3.7 ± 0.2 pS (36 of 45 patches) and high-conductance 98.3 ± 5.0 pS (19 of 45 patches) potassium channels were observed.

The estimated density of apical maxi K channels increased dramatically from 0.08 to 0.76 channels/μ^2 in tubules taken from animals exposed to KCl. All but four of nineteen patches which contained maxi K channels also expressed the low conductance sodium channels. Therefore, at least 85% of the maxi K channels studied were in principal cells. We speculate that the increase in maxi K channel activity may represent a mechanism for enhancing the potassium secretory capacity of the initial collecting duct.

As expected, exposure of the animals to 50 mM KCl prior to dissection of the initial collecting ducts also increased the estimated density of ENaC from 0.99 to 3.89 channels/μ^2. This upregulation of sodium channel activity is presumably related to the widely recognized effect of potassium loading to increase the plasma aldosterone level.

Correspondence to: L.C. Stoner

Key words: *Ambystoma*—Apical membrane—Collecting tubule—Maxi K channels

Introduction

Increasing the dietary intake of potassium in mammals [6, 20, 26, 29, 36] or exposure of amphibia [26, 35] to environmental potassium ion has long been accepted as a means of elevating plasma aldosterone. Aldosterone stimulates both renal sodium reabsorption and potassium secretion [20, 25, 27]. The relationship between potassium adaptation and aldosterone-induced increases in basolateral membrane surface area [26] and transport capacity [20, 29] have also been established. Dietary manipulation of plasma aldosterone levels has been shown to elevate the number of active apical membrane sodium channels in the rat collecting tubule [7, 23].

The effects of potassium adaptation and aldosterone on maxi K channels identified in the apical membrane of the cortical collecting tubule [1, 5, 11, 13, 17, 21] are less clear. This large-conductance Ca^{2+}-activated potassium channel found in many cell types does not appear to have a single physiological significance. Indeed, the observation that a number of alternatively spliced variants of the Drosophila large-conductance calcium-activated potassium channel can be expressed and that these variants have different gating characteristics is consistent with the notion that the channels have more than one function [15].

In nerve and muscle cells, it has been suggested these channels are used to stabilize the cell resting membrane potential [15, 16]. Numerous investigators have located this channel on the apical membrane of native distal nephron cells [4, 5, 11, 13, 21, 31, 34] and cultured cell lines derived from the distal nephron [1, 8, 17, 18, 34]. Such observations have tempted authors to suggest

that this channel could be involved in renal potassium secretion [8, 13, 18, 19, 24].

Hunter and his coworkers [13] were the first to study this channel on the apical membrane of the rabbit cortical collecting tubule, a nephron segment known to secrete potassium. Based on its sensitivity to barium ion, they speculated that the presence of these apical maxi K channels could be related to potassium secretion by the distal nephron [13]. Another study shows activation with epinephrine or serotonin on MDCK cells [14] and leaves open the possibility that the channels may be involved in secretion. Little other evidence exists to support this view.

Frindt and Palmer [5] found only slight effects of known inhibitors of the maxi K channel on the potassium conductance of the apical membrane of the principal cells of rat collecting tubule. This group was able to show that these channels were primarily identified on the apical membrane of intercalated cells, a collecting tubule cell type not thought to be involved in potassium secretion [21]. These observations have led to the general acceptance that the presence of the maxi K channel may not be related to potassium secretion.

In renal epithelial cells, the maxi K channel is generally found to be in a quiescent state in cell-attached patches [5, 11, 13, 21, 31]. Depolarization of the apical membrane [5, 8, 11, 21, 31], perturbations which elevate cell calcium [5, 8, 11, 13, 21], and cell swelling or membrane stretch [3, 18, 31, 33, 34] have all been implicated in activation of the channel. These characteristics have been used to suggest that a physiological role of the maxi K channel could be cell volume regulation and not potassium secretion. Presumably, activation of the maxi K channel in concert with anion channels results in the loss of KCl and water from the cell causing a volume regulatory decrease. In mammalian renal tubule fragments, membrane stretch or cell volume increase have been shown to activate such channels in the proximal tubule [3], the thick ascending limb [33] and the cortical collecting tubule [11, 31]. In addition, application of antidiuretic hormone to rat collecting tubule cells [31] or cultured collecting tubule cells [18] when a hyposmotic solution is present on the apical surface has been shown to cause modest activation of the maxi K channels. Taken together, these results support the view that the function of the maxi K channel is cell volume regulation.

Little is known about the effects of potassium adaptation on the presence of apical maxi K channels in the distal nephron. Exposure of frogs to potassium has been shown to increase the frequency of potassium channels believed to be involved in secretion in the early distal tubule [35]. On the other hand, exposure of *Amphiuma* to 50 mM KCl for 4 days does not induce any measurable potassium selective conductance of the apical membrane of the initial collecting tubule [12].

We set out to test the possibility that exposure of a larval *Ambystoma* to environmental KCl for longer periods of time might increase the density of active maxi K channels in the apical membrane of the initial collecting tubule, a distal nephron segment known to be involved in potassium secretion [12, 28]. Larval *Ambystoma* were chosen to study because the presence of gills may allow for larger increases in the plasma potassium and therefore an increased need to secrete potassium. Exposure of the animals to potassium containing bathing media for a period of fourteen or more days results in a dramatic increase in the incidence of apical maxi K and ENaC channels.

Materials and Methods

Biological Preparations

Western neonatal tiger salamanders, *Ambystoma tigrinum*, were obtained from Charles Sullivan (Nashville, TN). Animals were kept in special aquaria (Aquaratron, Westminster Scientific, Westminster, MD) containing 1.5 in of circulating tap water at 50°F. Salamanders were fed crickets daily. Under these conditions, neonatal animals can be maintained for many months without undergoing metamorphosis into land-phase animals. To expose animals to an ionic environment of either tap water or 50 mM KCl, animals were transferred to plastic cages in two inches of aqueous media for seven to nineteen days prior to the experiment.

In preliminary experiments, we studied eleven patches from four animals exposed to 50 mM KCl water for four to six days. Only two (18%) of these patches expressed maxi K channels. Since the frequency of observed maxi K channels was higher in animals exposed to the KCl for seven days, we chose to study only animals exposed to KCl for seven days or more.

Patch-Clamp Methods

The methods for dissecting and perfusing amphibian renal tubule fragments have been published previously [2, 4, 28, 32]. All dissections and experiments were carried out at room temperature. In brief, *Ambystoma* were doubly pithed immediately prior to removal of the kidneys via an abdominal incision. Slices of kidney several mm thick were cut and immediately placed in room temperature saline for the dissection of initial collecting tubules. The saline contained in mM: NaCl, 105; KCl, 3.0; $CaCl_2$, 2.0; $MgSO_4$, 1.25; KH_2PO_4, 1.25; HEPES (N-[Hydroxyethylpiperazine-N-]2-ethanesulfonic acid), 5.0; and dextrose, 5.5. The osmolarity of this solution averaged 218 mOsm/Kg H_2O. The nominal sodium content was 109 mM. The pH was titrated to 7.6. For dissection, one gm % of Fraction V bovine serum albumin (Sigma, St. Louis, MO) was added to this saline to reduce the tendency of dissected tubules to stick to glass and the dissection instruments. Unless stated otherwise, the saline solution minus the albumin was used to bathe both surfaces of the everted tubule and to fill the patch pipette. In some cases, the saline in the patch pipette was altered by replacing 95 mM of the NaCl with Na gluconate.

The general features of everting amphibian renal tubule fragments have been described in previous publications [4, 32]. A brief description of the technique is presented here. Dissected tubules were transferred to a setup used to perfuse kidney tubule fragments in vitro [2].

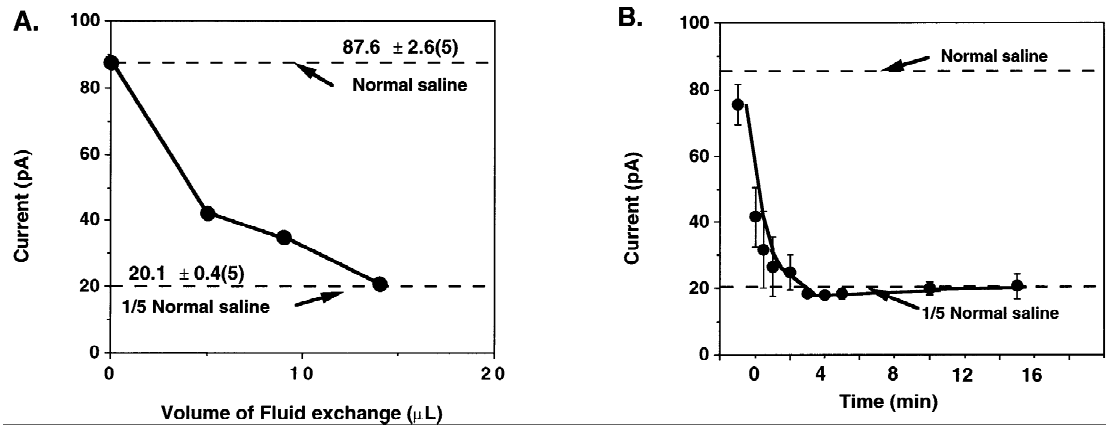

Fig. 1. Efficacy of the fluid exchange method in patch-clamp pipettes. Panel *A* illustrates the current passage through the pipette tip in response to a 0.2 mV pulse after varying the volume of the fluid exchange with 1/5 normal saline. The dashed lines represent the mean current values for groups of 5 other pipettes filled with normal saline (upper) or 1/5 normal saline (lower), diluted with distilled water. Panel *B* illustrates the time course of the current change after a 14 μL exchange of 1/5 normal saline. Five pipettes were studied and the bars represent one SEM. The 1/5 normal saline diffuses into the tip in two minutes and stable currents are recorded for at least 15 min after completion of the fluid exchange.

Pipettes used to perfuse and evert *Ambystoma* collecting tubules were modified from those normally used to perfuse renal tubule fragments [2, 4, 31, 32]. The inner perfusion pipette was pulled with a very long (3.0 to 4.0 mm) narrow parallel section. The outer diameter of this pipette averaged 10 to 15 μm. To prevent the basement membrane from sticking to the inner pipette it was pretreated with the albumin-containing dissection solution for twenty or more minutes prior to mounting the tubule on the pipettes. The outer holding pipette was fabricated with an inner diameter of 85 to 100 μm to allow ample space to evert the tubule.

Eversion was initiated by first retracting the inner perfusion pipette to a point where a small patch of the basement membrane could be snagged and tucked into the lumen of the tubule. The perfusion reservoir was turned off during eversion. After recentering the inner pipette, the fragment could be everted by slowly advancing the inner pipette while applying gentle suction to the outer pipette. Once the tubule was everted onto the inner perfusion pipette, the perfusion was restarted and a suction pipette mounted to the collection end v-track of the perfusion apparatus was used to gently pull the tubule off the inner perfusion pipette. About 200 μm of the inner pipette remained in the lumen of the everted tubule through which saline could be supplied to perfuse the basolateral surface of the everted tubule.

Methods for fabricating patch-clamp pipettes and making seals were modified from those of Hamill et al. [9]. Pipettes were pulled from 150 μL Microcaps (Drummond Scientific, Broomall, PA) on a Brown-Flaming P-80/PC puller (Sutter Instrument, San Rafael, CA) immediately before use. The tips of pipettes were firepolished on a Narishige Microforge (Narishige, Tokyo, Japan) to reduce the chances of damaging the plasma membrane and minimize the capacitative properties of the electrode. Pipette tip diameters were measured and only those pipettes whose tip inner diameter was 1.2 to 1.5 μm were used, since these pipettes routinely formed seals with the apical membrane. These pipettes form seals with the apical membrane (more than 50% of the time).

When patch-clamping cells on a tubule suspended between perfusion and collection pipettes, the preparation is viewed via a high-resolution video monitor (Javelin Electronics, Torrance, CA). The perfused tubule was lowered to touch the surface of the coverslip which was pretreated with CellTac® (Collaborative Research, Boston, MA). To form a seal, the pipette was typically positioned directly above the center of the everted tubule with a mechanical micromanipulator. The final approach to the tissue was made with a three-way Narishige Hydraulic Manipulator.

The data presented are from cell-attached patches. Typically, the seal resistance of patches averaged 29.2 ± 6.1 gΩ (20 patches). To form a seal, pipettes were routinely positioned above areas of the everted collecting tubule which appeared to have a smooth, flat surface. Cells that appeared to bulge out from the surface were avoided. Patches were discarded if the seal resistance was less than 1.0 gΩ.

In some experiments, we applied amiloride to the outside surface of the patch while recording from channels in a cell-attached patch. This necessitated the use of a low noise system which would allow us to change fluid inside the tip of the patch pipette. The methods we use were published previously [32]. In brief, we used 0.01 in. I.D. × 0.03 in. O.D. Microbore tubing (Thomas Scientific, Swedesboro, NJ) fitted with a fine quartz tip which was placed within 2.0 to 0.5 mm of the tip of the patch pipette.

To minimize the introduction of electrical noise, we filled the system initially with stained mineral oil, then aspirated a column of saline containing 2×10^{-6} M amiloride. The volume aspirated was sufficient to exchange 14 μl of fluid inside the tip of the patch clamp pipette.

In preliminary experiments, we observed that when we placed the amiloride-containing fluid exchange pipette less than 2 mm away from the tip of the patch pipette, amiloride would sometimes diffuse to the patch and inhibit the sodium channels even though a fluid exchange had not been attempted. To effect more control over the time at which inhibition occurred, after filling the exchange system with amiloride, we aspirated two centimeters of normal saline into the fluid exchange pipette to serve as a diffusion barrier between the amiloride solution and the membrane patch. Thus, amiloride reached the patch only after gentle pressure was applied to the system to force the amiloride-containing solution into the vicinity of the tip of the patch pipette.

To test the effectiveness of this fluid exchange system we monitored the change in resistance of the pipette tip after exchanging the normal saline with a dilute saline. Panel *A* of Fig. 1 illustrates the completeness of exchange with different volumes of dilute saline being used in the exchange pipette. Panel *B* shows the time course of the exchange for the first 15 min after an exchange of 14 μl of low-conductance saline.

The patch-clamp signal was monitored via an Axopatch 1-B amplifier (Axon Instruments, Burlingame, CA) equipped with a TMA-1 interface. A permanent record of experimental data was digitized (Model VR-10, Instrutech, Mineola, NY) and recorded on videotape for offline analysis. The signal was filtered to tape at 10 kHz. For analysis of sodium channel records, data was fed into the computer at a sampling rate of 200–500 μsec/point and filtered at 50–100 Hz. Events shorter than 25 msec were discarded. In the case of maxi K channel records, the sampling rate was 50 μsec/point and filtered at 2000 Hz. P-CLAMP software (Axon Instruments) was used to analyze the data on a Dell Optiplex PC (Dell Computer).

The chord conductance of channels was determined from the slope of the *I-V* relationship. We routinely applied voltage ramps between a pipette positive 120 mV and pipette negative 120 mV. The signal of an active patch was monitored for 10 to 60 sec at each voltage.

Eighty percent of the seals obtained expressed channels. Many of the patches clearly possessed more than one type of channel. One quarter of the seals yielded patches that appeared to express a single channel type whose conductance and reversal potential were consistent with that reported for sodium channels. When more than one channel was evident in the patch, the open probability was computed as the fraction of time the individual channels were in the open state divided by the maximal number of levels observed. Mean open times were computed from the time a single channel spends in the level 1 state.

Unless stated otherwise, results are presented as the mean ± SE of the mean (number of data points). The *t*-test for the significance between two independent means was used to evaluate the difference between means. A nonparametric test for the difference between two proportions was used to evaluate significant differences between the fractions of patches containing channels.

Results

Exposure of the animals to potassium increased the frequency of observed channels in the apical membrane patches. Among these is a large-conductance (nearly 100 pS) channel expressing an outward current, that while often quiescent under control conditions, is activated by depolarizing voltages. Of the nineteen patches containing maxi K channels reported in Table 1, nine of them were with patch pipettes which contained saline in which most of the chloride was replaced with gluconate. The conductance of these averaged 94.6 ± 9.8 pS and the reversal potential was −8.5 ± 9.1 mV. Thus, the channel activity cannot be explained as an inward movement of chloride. These results, along with the biophysical characteristics of the channel indicate that current carried by this channel is a potassium current. The characteristics of this channel are presented in Table 1 and are consistent with the channel being the so-called maxi K channel.

When clamped to 0 mV only a few of the patches expressed active maxi K channels. In nearly all cases, depolarizing voltage clamps activated the channels. Thus, the open probability numbers presented in Table 1 are zero.

We observe this channel only rarely in animals kept in tap water (3/49 patches). However, 16/45 or 36% of the patches on tissues taken from animals exposed to the potassium chloride solution expressed the channel. As shown in Table 1, the increase in channel density is nearly 10-fold. We are the first to observe such a dramatic increase in maxi K channel density in response to potassium adaptation.

Table 1. Summary of maxi-K channels in the apical membrane of *Ambystoma* initial collecting duct

Parameter	Environment	
	Tap water	50 mM KCl
Conductance (pS)	103.6 ± 3.2	97.2 ± 5.99
Pipette reversal potential (mV)	−34.7 ± 11.4	−13.8 ± 7.4
Open probability	0.00	0.01 ± 0.01
Number of levels	2.0 ± 0.6	2.5^{+} ± 0.3
Fraction of patches with maxi-K	3/49	16/45*
Density (N/μm^2)	0.08	0.76

Density was calculated assuming the surface area under each patch to be 1.40 μm^2. Unitary current, mean open time, and open probability were all measured with the clamp voltage at zero. Three patches were studied in the tap water group and 16 in the KCl exposed group.
* *P* less than 0.001 *vs.* tap water.
$^{+}$ *P* greater than 0.25 *vs.* tap water.

In the nineteen patches used to generate the data in Table 1, we observed the coexistence of the 4 pS sodium channels with the maxi K channels in all three of the patches on membranes from animals in tap water and thirteen of the patches exposed to 50 mM KCl. Sample traces of the coexistence of both channel types in the same patch are presented in Fig. 2. The upper trace (*A*) illustrates maxi K activity with the patch pipette clamped at −80 mV. The lower trace (*B*) shows the presence of numerous 4 pS sodium channels in the same patch.

It is also of interest that the mean pipette reversal potential of the nineteen channels in Table 1 is pipette negative, −16 mV. This particular parameter was found to be highly variable and some channels expressed the more traditional pipette positive reversal potential. Figure 3 shows two representative plots expressing either a positive (panel *A*) or a negative pipette reversal potential (panel *B*). The patch from which the data in panel *A* was taken expressed only maxi K channels, low-conductance sodium channels were not observed. The pipette reversal potential of the maxi K channel in panel A was +15.8 mV. On the other hand, the patch from which the data in panel *B* were taken expressed six of the low-conductance sodium channels. The maxi K channel in this patch has a negative pipette reversal potential of −20.3 mV. Figure 4 plots the observed pipette reversal potential of the maxi K channels in a given patch against the number of sodium channels observed in that patch. A significant correlation exists between these parameters. This may indicate that the electrochemical gradient for potassium across the apical membrane is linked to the presence of sodium channels in the membrane.

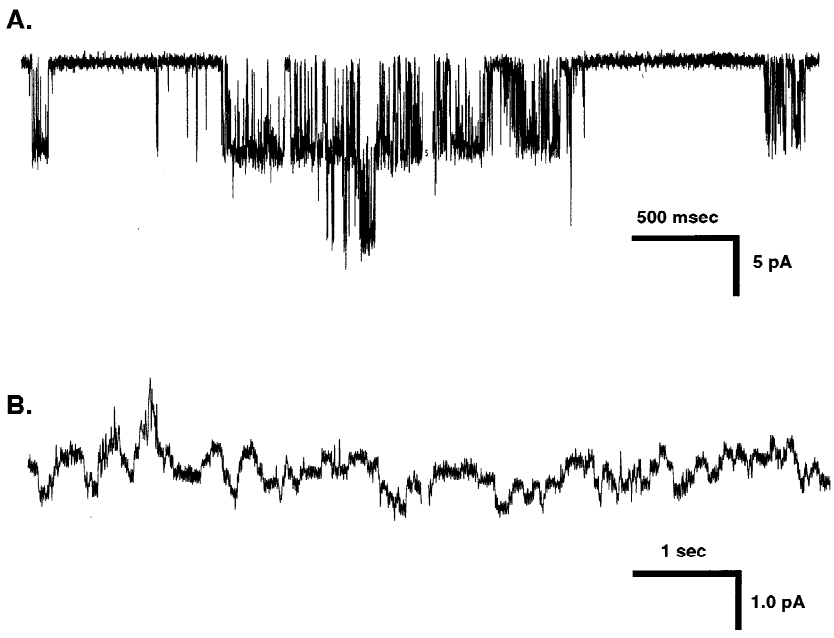

Fig. 2. Traces illustrating the coexistence of low-conductance sodium channels and maxi K channels in the same apical membrane patch. Trace A illustrates the activity of maxi K channels in a patch. The patch was clamped at a potassium voltage of −80 mV and the signal filtered at 1000 Hz. Trace B is from the same patch. The clamp voltage was 0 mV and the signal filtered at 100 Hz.

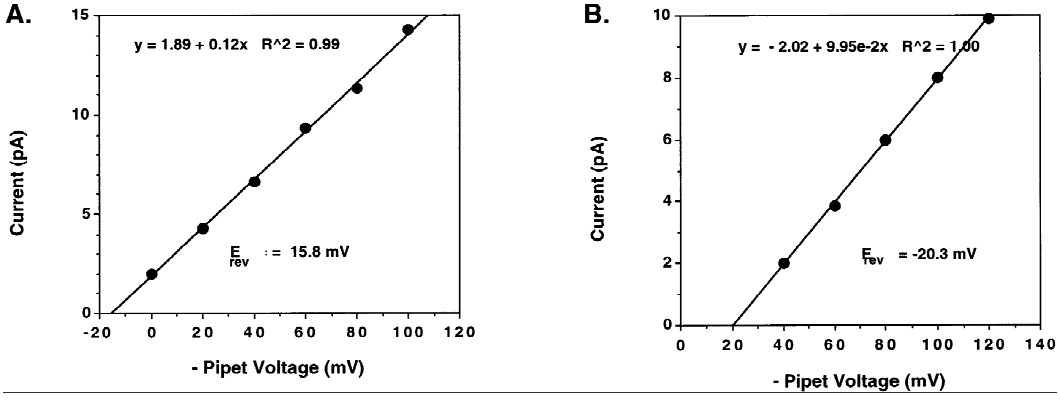

Fig. 3. Current-voltage relationships of representative maxi K channels. Panel *A* presents the traditional relationship in which the reversal potential is a positive pipette voltage so that at a voltage clamp of 0 mV potassium would move into the lumen of the tubule when the channel is open (secretion). There were no sodium channels present in this patch. Panel *B* shows a negative pipette reversal potential so that at 0 mV voltage clamp current moves into the cell when the channel is open (reabsorption). Six sodium channels were present in this patch.

We have seen four patches which expressed maxi K channels and not sodium channels. Table 2 shows that the potassium channels observed in these patches all expressed positive voltages where the current would reverse. In addition, all of the channels express activity at a voltage clamp of 0 mV. The fraction of time the channel spends in the open state is modest. Presumably, these channels are secreting potassium.

The most commonly observed channel type in this study was that of the low-conductance (4 pS) amiloride-inhibitable sodium channel. As illustrated in Table 3, nearly 78% of patches on the apical membrane of collecting tubules dissected from animals exposed to a solution containing 50 mM KCl and 45% of the patches on collecting tubules dissected from animals maintained in tap water expressed the 4 pS sodium channels. The number of channels found per patch also increased from 3.1 to 7.0 in patches on tubules taken from animals exposed to the KCl. The significant increase in frequency of patches containing the channel and the significant increase in the number of channels in a patch expressing the channel together indicate that the density of 4 pS channels is nearly four times higher in the apical membrane of potassium-treated animals (Table 3). The channel conductance, unitary current, open probability and the pipette potential where the current direction would reverse are all consistent with the identity of this channel being a highly-selective sodium channel.

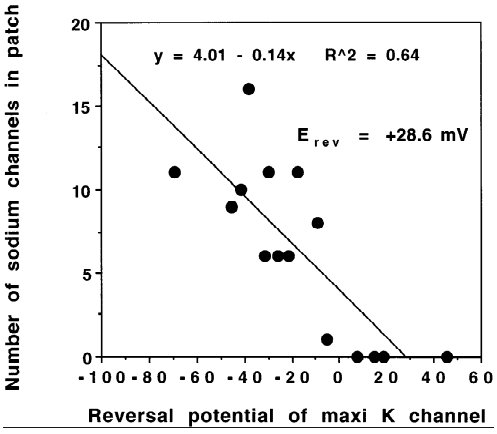

Fig. 4. Plot of the observed reversal potential *vs.* the number of sodium channels observed in the same patch. The reversal potential in patches without maxi K channels averages 28.6 mV. The equation presents the linear regression analysis of the data.

Table 2. Characteristics of maxi K channels in patches which lack 4 pS sodium channels

Parameter	Value
Conductance (pS)	75.5 ± 15.4
Unitary Current (pA)	2.04 ± 0.52
Mean open time (msec)	5.7 ± 1.3
Open probability	0.026 ± 0.008
Pipette reversal potential (mV)	21.8 ± 8.2

Four patches, each from a different animal, were studied. Unitary current, mean open time, and open probability were all measured with the clamp voltage at zero. Since multilevel activity was not seen at 0 mV, the mean open time is the average of all the single level events.

To confirm the identity of these channels we tested whether or not amiloride inhibited this channel. In six patches containing 4 pS channels the normal saline in the patch pipette was exchanged with one containing normal saline and 2×10^{-6} M amiloride. The technique of fluid exchange and its effectiveness are discussed in Materials and Methods. The results are presented in Table 4. The chord conductance, reversal potential and unitary current of the amiloride-treated channels were not different than the values presented in Table 3 for untreated patches. The channels studied exhibited flickering activity as previously reported by others. Mean open time and open probability were nearly 31 msec and 0.008, respectively. These values are significantly lower than those of uninhibited channels (Table 4). Thus, amiloride inhibits the channel activity. Together with the biophysical characteristics of untreated channels shown in Table 3 the sensitivity of these channels to amiloride serves to positively identify these as sodium channels.

Channels of intermediate conductance were also observed in patches on apical membrane of initial collecting ducts dissected from animals exposed to 50 mM KCl. These channels were never observed in animals exposed to tap water. The frequency of patches expressing these channels was 22/45. The identity of these channels are, as yet, unknown. More experiments will be required before we can be certain of their significance.

Discussion

Sodium reabsorption and potassium secretion by the vertebrate distal nephron are regarded as two-step processes [20, 23, 26, 30]. At the basolateral surface, the Na,K-ATPase actively transports sodium into the interstitial fluid and potassium into the cytosol. Ion channels at the apical membrane allow sodium to move from the luminal fluid to the cell (reabsorption) and potassium to move from the cell in to the lumen of the renal tubule (secretion). The hormonal control processes involved and the biophysical and molecular biological identities of the channels involved have been studied extensively. However, the details of hormonally induced changes in the activity of apical membrane channels involved in sodium reabsorption and potassium secretion are not as well understood.

Elevation of plasma potassium has long been regarded as a means of inducing increases in the levels of the mineralocorticoid hormone aldosterone, in both mammals [20, 26, 36] and amphibia [12, 27, 35]. The elevation of plasma aldosterone has in turn been linked to increased rates of renal sodium reabsorption and potassium secretion [12, 20, 27]. This phenomenon has been shown to increase not only the transport capacity at the basolateral surface [20, 29], but also the number of active sodium channels on the apical surface of the rat collecting tubule [7] and tissue culture analogues of the vertebrate distal nephron [25].

One unique observation of our study is an increase in the density of active large conductance potassium channels in the apical membrane of the initial collecting tubules taken from potassium-adapted animals. The channel conductance, direction of current flow, low open probability at 0 mV voltage clamp, its sensitivity to imposed voltage clamps and its activity when the chloride contents of the pipette are replaced with gluconate all argue that the channel is the so-called maxi K channel. The maxi K channel is ubiquitous in that it appears in many species and in many different cell types.

Of the two types of cells in the apical membrane of the mammalian collecting tubule, only one, the principal cell, is believed to be involved in aldosterone-sensitive potassium secretion and sodium reabsorption [21, 26, 31]. Pacha et al. have studied the presence of maxi K channels in the apical membrane of the rat and observed that most of the maxi K channels they observe are located in the apical membranes of the intercalated cells

Table 3. Summary of 4pS sodium channels in the apical membrane of *Ambystoma* initial collecting duct

Parameter	Environment	
	Tap water	50 mM KCl
Conductance (pS)	3.66 ± 0.20 (22)	3.70 ± 0.20 (36)
Unitary Current (pA)	0.374 ± 0.021 (20)	0.329 ± 0.021 (34)
Pipette reversal potential (mV)	−109.3 ± 7.0 (22)	−76.4 ± 4.7
Mean open time (msec)	567.2 ± 171.2 (14)	432.6 ± 116.6 (8)
Open probability	0.442 ± 0.067 (14)	0.336 ± 0.057 (8)
Number of levels per patch	3.1 ± 0.4 (22)	7.0 ± 0.6 (34)*
Fraction of patches with 4 pS	22/49	36/45^{+}
Density (N/μm^2)	0.99	3.89

The surface area under each patch is assumed to be 1.40 μm^2 for calculation of density. Unitary current, mean open time and open probability were all determined at a voltage clamp of 0 mV. When multiple channel levels were observed mean open time was computed from only single level events.

* *P* less than 0.001 *vs.* tap water, $^{+}$ *P* less than 0.01 *vs.* tap water

Table 4. Characteristics of 4 pS sodium channels in amiloride treated patches (2×10^{-6} M) of apical membrane of everted *Ambystoma* initial collecting duct

Parameter	Value	% change
Conductance (pS)	3.52 ± 0.28 (6)	−3.2
Unitary current (pA)	0.414 ± 0.017 (6)	+10.6
Pipette reversal potential (mV)	−113.6 ± 15.3 (6)	+3.9
Mean open time (msec)	30.5 ± 15.6 (6)*	−94.3
Open probability	0.008 ± 0.004 (6)*	−81.9

Unitary current, mean open time, and open probability were all measured with the clamp voltage at zero.

* *P* less than 0.01 *vs.* Control value.

[21]. In a previous study, we found that in the rat 2/3 of the maxi K channels found were seen to coexist with low-conductance sodium channels [31]. In this study nearly 85% of the patches expressing maxi K channels also contained 4 pS sodium channels. Since it is widely accepted that only principal cells exhibit the 4 pS sodium channels, we conclude that the increase in the density of active maxi K channels is in the principal cells.

To our knowledge, this is the first observation of the increase in density of active maxi K channels in the apical membrane of any distal nephron segment. We do not know whether this upregulation represents the activation of channels already present in the apical membrane, the insertion of channels, or the synthesis of new channels.

As stated in Materials and Methods, we see relatively few maxi K channels in the apical membrane in our animals after four to six days of potassium adaptation. We observe a dramatic increase in the density of active maxi K channels only in animals adapted for seven or more days. While such a slow time course for the increase in channel density is consistent with the synthesis of new channels, other possible scenarios, including a time-dependent activation of quiescent channels, cannot be excluded.

The physiological significance of the upregulation of the number of maxi K channels in the apical membrane of these initial collecting tubule principal cells is not yet clear. One possibility is that they are needed in cell volume regulation. That they are largely quiescent when the cell is voltage-clamped to 0 mV could indicate that they are involved in cell volume regulation as has been proposed for the maxi K channels of rat thick ascending limb [33], native cortical collecting tubule [11, 31] and cultured collecting tubule cells [18].

Another possible explanation for the upregulation of this channel is that it is involved in potassium secretion. Numerous investigators have speculated that the apical maxi K channels could contribute to potassium secretion by the distal nephron [1, 8, 14, 18, 24], however, little evidence exists to support this view. Indeed, under in vitro conditions, the maxi K channels observed in the distal nephron apical membrane are relatively quiescent [13, 21, 31]. This is also true of the channels seen in this study.

In the rat collecting tubule, potassium adaptation causes an upregulation of low-conductance potassium channels known to be involved in potassium secretion [36]. Sodium deprivation does not cause an increase in potassium channel density [6]. Only one group [G. Frindt and L. Palmer, *personal communication*] has looked for an effect of potassium adaptation on the density of maxi K channels in the apical membrane of the cortical rat collecting tubule. They could find no such correlation.

In the amphibia, the upregulation of potassium channels in response to potassium adaptation is not a new observation. Wang and coworkers [35] have reported that aldosterone causes an increase in the density of Type

II potassium channels in apical membrane of the early distal frog tubules. Amphibia have an early distal tubule which shares many of the transport characteristics of the mammalian thick ascending limb [28, 35]. The Type II channel observed has a conductance of 45 pS, is pH sensitive and is inhibited by barium [35]. These authors indicate that after potassium adaptation, this segment of the amphibian nephron does secrete potassium.

In the late distal tubule of amphibia, whose transport properties are similar to those of the mammalian collecting tubule [28], it has been suggested that the mechanism of potassium secretion may differ from that of mammals [12]. Horisberger and his coworkers [12] failed to observe any significant potassium conductance of the apical membrane of *Amphiuma* initial collecting tubule or any increase in the potassium conductance of the apical membrane upon exposing the animals to four days of saline containing 50 mM KCl. They concluded that potassium secretion by this segment of the nephron was electrodiffusive and through the paracellular pathway. It could be that this difference is due to species or that *Amphiuma* lack gills and less potassium enters the animal than when gilled, larval salamanders are used. Alternatively, it could be that potassium adaptation requires more than four days to occur. Thus, the upregulation of maxi K channels we see after seven to nineteen days of adaptation may not really be at variance with those of Horisberger [12].

We found a correlation between the number of sodium channels in a given patch and the pipette voltage at which current reversal of the maxi K channel should occur. In addition, only patches without sodium channels have spontaneously active (secretory) maxi K channels at a voltage clamp of 0 mV. The reversal potentials of the maxi K channels in patches that contain large numbers of sodium channels would indicate that if active at zero mV, they would allow for potassium reabsorption. This condition would not be consistent with either cell volume regulation or secretion. We find this observation puzzling.

One possible explanation for this contradiction is that when large numbers of sodium channels are present in the apical membrane, we never see a condition in which all of the sodium channels are closed and are thus unable to adjust the trace to zero current. The residual sodium current would tend to depolarize the membrane patch and could result in an apparent shift of channel reversal potential.

Another possible explanation is that the reversal potential is different under in vivo conditions. Like most laboratories who study kidney tubules in vitro we use aphysiological conditions. One difference is we expose the apical membrane to a saline containing 109 mM sodium. The sodium concentration of the luminal solution of the in vivo initial collecting tubule is about 20 mM [37]. The high apical sodium concentration would tend to depolarize the membrane much more than the physiological sodium concentration. Thus, under physiological conditions the electrochemical gradient may favor a secretory movement of potassium. Another important difference in conditions is that, potassium-adapted animals express plasma potassium concentrations of nearly 9 mM [27]. It is possible that placing the cells in media which mimic that seen in vivo, low luminal sodium concentration and/or a hyperkalemic basolateral solution may yield maxi K channels that are active at 0 mV voltage clamp and therefore secreting potassium.

In that upregulation of the number of channels due to potassium adaptation could enhance the secretory capacity under some as yet undefined conditions, these results keep open the possibility that potassium secretion after potassium adaptation is achieved by the upregulation of the number of maxi K channels in the apical membrane of the collecting tubule of amphibia. Future studies will undoubtedly consider what conditions activate these channels and are responsible for the regulation of potassium secretion.

It is widely accepted that the distal nephron sodium channels (ENaC), which are the rate-limiting step in sodium reabsorption, are sensitive to low concentrations of amiloride [7, 23]. We recently reported that the apical membrane of the *Ambystoma* initial collecting tubule expressed numerous low-conductance amiloride-sensitive sodium channels [31]. As expected, a dramatic increase in the density of these sodium channels was observed in potassium-adapted animals. As shown in Table 3, there is a highly significant increase in the number of sodium channels and a modest increase in the fraction of patches expressing sodium channels. There were nearly 4 times more sodium channels in the apical membrane of amphibian initial collecting tubule dissected from animals that were exposed to water containing 50 mM KCl (Table 3). We are the first to observe an increase in the density of sodium channels in the native distal nephron of an amphibian in response to potassium adaptation. Others have observed that aldosterone increases the number of active low-conductance sodium channels in amphibian tissue culture lines, such as A-6 cells, thought to represent distal nephron [25].

The shift in pipette reversal potential of the sodium channels from −109 mV in patches on tap water-treated animals to −76 mV in patches on tubules from potassium-adapted animals is also highly significant. Although other factors could contribute, this shift is consistent with the depolarization of the apical membrane expected in animals with high plasma aldosterone.

Potassium feeding of mammals and exposure of amphibia to water containing potassium chloride causes rather extensive changes in the transport characteristics of the late distal nephron [12 and 20]. Importantly, not

only does the rate of entry of sodium into the cell via the apical membrane increase, but also the surface area of the basolateral membrane increases [26], and the metabolic machinery associated with transepithelial transport also is elevated. Increased levels of potassium intake are known to elevate both plasma aldosterone and the number of sodium channels in the distal nephron of mammals [6, 20, 26]. Stiffler and his coworkers [27] previously reported that treatment of larval *Ambystoma* with 100 mM KCl for four days raised their plasma aldosterone levels from 161 pg/ml in tap water to 410 pg/ml. In these same animals, fractional sodium reabsorption increased from 0.942 to 0.991. It seems reasonable to postulate the increased frequency of active sodium channels we observe is a direct result of increased plasma aldosterone levels.

The authors express their appreciation to Miss Heidi Wheeler for her expert technical support and Drs. Mary Ellen Trimble and Peter Holohan for reading the manuscript and their many helpful suggestions during the course of this study. This project was supported by a grant from the National Science Foundation, sponsor ID number, IBN9506128.

References

1. Bolivar, J.J., Cereijido, M. 1987. Voltage and Ca^{2+}-activated channel in cultured epithelial cells (MDCK). *J. Membrane Biol.* **97:**43–51
2. Burg, M.B. Perfusion of isolated renal tubules. 1972. Yale *J. Biol. Med.* **45:**321–326
3. Dube, L., Parent, L., Sauve, R. 1990. Hypotonic shock activates a maxi K^+ channel in primary cultured proximal tubule cells. *Am. J. Physiol.* **259:**F348–F356
4. Engbretson, B.G., Stoner, L.C. 1988. The everted renal tubule: a methodology for direct assessment of apical membrane function. *Am. J. Physiol.* **255:**F1276–F1280
5. Frindt, G., Palmer, L.G. 1987. Ca-activated K channels in apical membrane of mammalian CCT, and their role in K secretion. *Am. J. Physiol.* **252:**F458–F467
6. Frindt, G., Palmer, L.G. 1989. Low-conductance K channels in the apical membrane of rat cortical collecting tubule. *Am. J. Physiol.* **256:**F143–F151
7. Frindt, G., Sackin, H., Palmer, L.G. 1990. Whole-cell currents in rat cortical collecting tubule: low-Na diet increases amiloride-sensitive conductance. *Am. J. Physiol.* **258:**F562–F567.
8. Gitter, A.H., Beyenbach, K.W., Christine, C.W., Gross, P., Minuth, W.W., Fromter, E. 1987. High-conductance K^+ channel in apical membranes of principal cells cultured from rabbit renal cortical collecting tubule anlagen. *Pfluegers Arch.* **408:**282–290
9. Hamill, O.P., Neher, M.E., Sakman, B., Sigworth, F.J. 1981. Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. *Pfluegers Arch.* **391:**85–100
10. Hinton, D.E., Stoner, L.C., Burg, M.B., Trump, B.F. 1982. Structural heterogeneity in the distal nephron of the salamander (*Ambystoma tigrinum*): An electron microscopic study of isolated tubule segments. *Anatomical Record* **204:**21–32
11. Hirsch, J., Leipziger, J., Frobe, U., Schlatter, E. 1993. Regulation and possible physiological role of the Ca(2+)-dependent K^+ channel of cortical collecting ducts of the rat. *Pfluegers Arch.* **422:**492–498
12. Horisberger, J., Hunter, M., Stanton, B., Giebisch, G. 1987. The collecting tubule of Amphiuma II: Effects of potassium adaptation. *Am. J. Physiol.* **253:**F1273–F1282
13. Hunter, M., Lopes, A.G., Boulpaep, E., Giebisch, G. 1986. Regulation of single potassium ion channels from apical membrane of rabbit collecting tubule. *Am. J. Physiol.* **251:**F725–F733
14. Kolb, H.A., Paulmichl, M., Lang, F. Epinepherine activates outward rectifying K channel in Madin-Darby canine kidney cells. 1987. *Pfluegers Arch.* **408:**584–591
15. Lagrutta, A., Shen, K., North, R.A., Adelman, J.P. 1994. Functional differences among alternatively spliced variants of *Slowpoke*, a Drosophila calcium-activated potassium channel. *J. Biol. Chem.* **269:**20347–20351
16. Latorre, R., Miller, C. 1983. Conduction and selectivity in potassium channels. *J Membrane Biol.* **71:**11–30
17. Ling, B.N., Hinton, C.F., Eaton, D.C. 1991. Potassium permeable channels in primary cultures of rabbit cortical collecting tubule. *Kidney Int.* **40:**441–452
18. Ling, B.N., Webster, C.L., Eaton, D.C. 1992. Eicosanoids modulate Ca^{2+}-dependent K^+ channels in cultured rabbit principal cells. *Am. J. Physiol.* **263:**F116–F126
19. Marty, A. 1989. The physiological role of calcium-dependent channels. *Trends in Neurosci.* **12:**420–424
20. Natke, E., Jr., Stoner, L.C. 1982. Na^+ transport properties of the peritubular membrane of the cortical collecting tubule. *Am. J. Physiol.* **242:**F664–F671
21. Pacha, J., Frindt, G., Palmer, L.G. 1991. Apical maxi K channels in intercalated cells of CCT. *Am. J. Physiol.* **261:**F696–F705
22. Palmer, L.G., Frindt, G. 1986. Amiloride-sensitive Na channels from the apical membrane of the rat cortical collecting tubule. *Proc. Natl. Acad. Sci. USA* **83:**2767–2770
23. Palmer, L. 1992. Epithelial Na channels: function and diversity. *Ann. Rev. Physiol.* **54:**51–66
24. Petersen, O.H., Maruyama, Y. 1984. Calcium-activated potassium channels and their role in secretion. *Nature* **307:**693–696
25. Smith, P.R., Stoner, L.C., Viggiano, S.C., Angelides, K.J., Benos, D.J. 1995. Effects of vasopressin and aldosterone on lateral mobility of epithelial Na^+ channels in A6 renal epithelial cells. *J. Membrane Biol.* **147:**195–205
26. Stanton, B.A., Biemisderfer, D., Wade, J.B., Giebisch, G. 1981. Structural and functional study of the rat distal nephron: Effects of potassium adaptation and depletion. *Kidney Int.* **19:**36–48
27. Stiffler, D.F., De Ruyter, M.L., Hanson, P.B., Marshal, M. 1986. Interrenal function in larval *Ambystoma* tigrinum. *Gen. And Comp. Endocrin.* **62:**290–297
28. Stoner, L.C. 1977. Isolated, perfused amphibian renal tubules: The diluting segment. *Am. J. Physiol.* **223:**438–444
29. Stoner, L.C., Natke, E., Jr. 1984. The isolated, collapsed cortical collecting tubule viewed as a basolateral membrane preparation. *In: Progress in Clinical Biological Research: Information and Energy Transduction in Biological Membranes.* C.L. Bolis, E. Helmreich and H. Passow, editors. pp. 421–429. Alan R. Liss, New York
30. Stoner, L.C. 1985. The movement of solutes and water across the vertebrate distal nephron. *Renal Physiol.* **8:**237–248
31. Stoner, L.C., Morley, G. 1995. Effect of basolateral or apical hyposmolarity on apical maxi K channels of everted rat collecting tubule. *Am. J. Physiol.* **268:**F569–F580
32. Stoner, L.C., Engbretson, B.G., Viggiano, S.C., Benos, D.J., Smith, P.R. 1995. Amiloride-sensitive apical membrane sodium channels of everted Ambystoma collecting tubule. *J. Membrane Biol.* **144:**147–156

33. Taniguchi, J., Guggino, W.B. 1989. Membrane stretch: a physiological stimulator of Ca^{2+}-activated K^+ channels in thick ascending limb. *Am. J. Physiol.* **257:**F347–F352
34. Ubl, J., Murer, H., Kolb, H.A. 1988. Ion channels activated by osmotic and mechanical stress in membranes of opossum kidney cells. *J. Membrane Biol.* **104:**223–232
35. Wang, W., Henderson, R.M., Geibel, J., White, S., Giebisch, G. 1989. Mechanism of aldosterone-induced increase of K conductance in early distal renal tubule cells of the frog. *J. Membrane Biol.* **111:**277–289
36. Wang, W., Schwab, A., Giebisch, G. 1990. Regulation of small-conductance K^+ channel in apical membrane of rat cortical collecting tubule. *Am. J. Physiol.* **259:**F494–F502
37. Wiederholt, M., Sullivan, W.J., Giebisch, G. 1971. Potassium and sodium transport across single distal tubules of *Amphiuma. J. Gen. Physiol.* **57:**495–525