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Apical Nonspecific Cation Channels in Everted Collecting Tubules of Potassium-Adapted *Ambystoma*

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Abstract. We observed intermediate conductance channels in approximately 20% of successful patch-clamp seals made on collecting tubules dissected from *Ambystoma* adapted to 50 mM potassium. These channels were rarely observed in collecting tubules taken from animals which were maintained in tap water. Potassium-adaptation either leads to an increase in the number of channels present or activates quiescent channels.

In cell-attached patches the conductance averaged 30.3 ± 2.4 (9) pS. Since replacement of the chloride in the patch pipette with gluconate did not change the conductance, the channel carries cations, not anions. Notably, channel activity was observed at both positive and negative pipette voltages. When the pipette was voltage clamped at 0 mV or positive voltages, the current was directed inward, consistent with the movement of sodium into the cell. The pipette voltage at which the polarity of the current reversed (movement of potassium into the pipette) was −29.6 ± 6.5(9) mV.

Open probability at 0 mV pipette voltage was 0.08 ± 0.03 and was unaffected when the apical membrane was exposed to either 2×10^{-6} or 2×10^{-5} M of amiloride. Exposure of the basolateral surface of the tubule to a saline containing 15 mM potassium caused a significant increase (*P* less than 0.001) in the open probability of these channels to 0.139 ± 0.002 without affecting the conductance of the apical channel.

These data illustrate the presence of an intermediate conductance, poorly selective, amiloride-insensitive cation channel in native vertebrate collecting tubule. We postulate that, at least in amphibia, this channel may be used to secrete potassium.

Key words: Nonspecific Cation Channels — Everted collecting tubule — *Ambystoma* — Potassium adaptation — Sodium reabsorption — Potassium secretion

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Introduction

The amphibian distal convoluted tubule shares many of the structural and functional characteristics of the mammalian distal nephron [6, 13, 14, 25, 27, 28, 37]. The initial collecting tubule (late distal convolution) of amphibia has been shown to express a lumen-negative voltage, actively reabsorb sodium (which is inhibitable by amiloride) and to secrete potassium [1, 14, 23, 28, 37]. We are able to evert and perfuse in vitro nephron fragments of *Ambystoma tigrinum* [7, 9, 30, 31]. Patch-clamp studies of the apical membrane reveal the presence of highly selective [28], low-conductance (4pS), amiloride-sensitive sodium channels [29, 30] on the apical membrane of this tubule fragment. Potassium adaptation of the animals prior to experimental study results in an increased density of this channel type [30]. Since under control conditions, the 4 pS, amiloride-sensitive channel is the only sodium channel observed in native tissue [8, 22, 29, 30], it has been suggested that this is the predominate channel explaining sodium reabsorption [2, 33].

Other sodium carrying channels have been reported in both renal tissue culture lines [4, 5, 12, 15, 16, 17, 19, 21, 35] and nonrenal epithelial cell transport systems [9, 18]. Cultured amphibian distal nephron cells (A-6) express at least three types of channels which carry sodium ions [5, 10, 12, 19]. They include a highly selective 4 pS channel [5], a moderately-selective 9 pS channel [19] and a nonselective 28 pS channel [10, 12, 19], which some investigators report as being less sensitive to amiloride than the 4 pS channel [10]. Similar nonselective cation channels have been reported in cell lines de-

rived from cortical collecting tubule [17] medullary collecting duct cell line [16] and type II nasal epithelial cells [18, 21, 35]. In addition, reverse transcriptase polymerase chain reaction techniques (RTPCR) and *in situ* hybridization experiments have demonstrated the presence of the mRNA which codes for this channel in a number of renal tubule segments including the distal tubule, cortical collecting tubule and inner medullary collecting duct [3]. The presence of the mRNA suggests the channel may be present in these tissues. On the other hand, the failure to observe this channel in native distal nephron epithelia, using patch-clamp methodology, has raised questions about the physiological significance of the 28 pS channel in renal tissue. Thus, the function of this channel is a matter of speculation.

Using established patch-clamp methodology [11] we have identified an intermediate conductance (28 pS) amiloride-insensitive cation channel in the apical membrane of the everted amphibian collecting tubule. We observe this channel most frequently in tissue dissected from animals maintained for a week or more in a 50 mM KCl saline. The channel's electrical characteristics appear identical to those of the 28 pS channels reported for the rat collecting tubules [8, 22], cultured cells derived from nasal epithelium [18, 21, 35], cortical collecting tubule [15, 17], medullary collecting ducts [16] and A-6 cells [12, 19]. Doses of amiloride as high as 20 μM did not inhibit our channel, a dose nearly one-hundredfold higher than that shown to inhibit the 4 pS sodium channel. With the exception of the nasal epithelial cells [4], the channels found in the preparations listed above are all more sensitive to amiloride.

While a channel of similar size has been reported in vasopressin-treated toad bladder epithelial cells [9] and native nasal epithelial cells [18], this study is the first to report the expression of the 28pS channel in native distal nephron cells. This channel is most often expressed in tissue taken from animals exposed to 50 mM environmental potassium for a week or more. Exposure of the basolateral membrane to saline containing 15 mM KCl increases the open probability of the channels. This together with the fact that, in amphibia, little sodium remains in the luminal fluid by the time filtrate arrives in the late distal nephron [1, 37] leads us to speculate that the physiological significance of this channel could be related to potassium transport.

Materials and Methods

Neonatal tiger salamanders, *Ambystoma tigrinum*, were obtained from Charles Sullivan (Nashville, TN). Animals were kept in aquaria (Aquaratron, Westminster Scientific, Westminster, MD) containing circulating tap water at 50° F. Salamanders were fed crickets daily. To expose animals to 50 mM KCl, animals were transferred to plastic cages containing two inches of KCl solution. These cages were kept in the same aquaria. The duration of KCl exposure ranged from 8 to 60 days.

Solutions

Ambystoma were doubly pithed immediately prior to removal of the kidneys. Slices of kidney several millimeters thick were cut and 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; and dextrose, 5.5. The mean osmolarity of this solution was 218 mOsm/Kg H_2O. The pH was titrated to 7.6. For dissection, to reduce the tendency of dissected tubules to stick to glass and the dissection instruments, one gm % of Fraction V bovine serum albumin (Sigma, St. Louis, MO) was added to the above saline.

Unless stated otherwise, the above saline solution, minus albumin, was used to bathe both the apical and basolateral surfaces of the everted tubule and to fill the patch pipette. In some experiments, the saline bathing the basolateral surface was exchanged for one in which potassium was used to replace some of the sodium so that the final concentration of potassium was 15 mM.

Patch-Clamp Methods

The detailed methods of everting amphibian renal tubule fragments have been described in a previous publication [7]. A brief description of the technique is presented here.

After transferring the dissected tubules to a setup designed to perfuse kidney tubule fragments *in vitro*, they were everted. 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. 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 was used to gently pull the tubule off the inner perfusion pipette. The inner pipette remained in the lumen of the everted tubule so that perfusate could be supplied to perfuse the basolateral surface of the everted tubule. The general features of the methods used to form a seal betweeen the patch pipette and the apical membrane have been described previously [29, 30, 31].

Patch-clamp pipettes were fabricated by a method modified from those of Hamill et al. [10]. Pipettes were pulled from 100 μL Microcaps (Drummond Scientific, Broomall, PA) on a Brown-Flaming P-80/PC puller (Sutter Instrument, San Rafael, CA). The tips of patch pipettes were firepolished on a Narishige Microforge (Narishige, Tokyo, Japan) to facilitate seal formation and to minimize the capacitative properties of the electrode.

The data presented are all from cell-attached patches. We attempted on numerous occasions to excise patches. This procedure invariably led to rapid inactivation of the nonselective cation channel.

In some experiments we applied amiloride to the outside surface of the patch while recording from channels in a cell-attached patch. Fluid exchange of the patch pipette was achieved with a modification of a system originally developed by Tang et al. [38]. We used 0.01 in. I.D. × 0.03 in. O.D. Microbore tubing (Thomas Scientific, Swedesboro, NJ) fitted with a fine quartz tip to deliver the drug to within 2.0 to 0.5 mm of the tip of the patch pipette. We filled the system initially with mineral oil, then aspirated a column of saline containing 2×10^{-6} or 2×10^{-5} M of amiloride into the tip of the exchange system. The details of this method have been published previously [29, 30].

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 5000 Hz. For analy-

Table 1. The relative abundance of cation channels in the apical membrane of the initial collecting tubule of control and potassium-adapted *Ambystoma*

Channel type	Control			Potassium-adapted		
	g (pS)	Freq.	Rel. abundance	g (pS)	Freq.	Rel. abundance
ENaC*	3.7 ± 0.2	(22/49)	1.40	3.7 ± 0.2	(36/45)	5.60
Maxi K*	103.6 ± 3.2	(3/49)	0.12	97.2 ± 6.0	(22/55)	1.00
Nonselective cation channel	28.0 ± 1.9	(3/60)	0.07	28.0 ± 1.9	(16/56)	0.41

Frequency represents the fraction of successful seals which expressed a given channel type. Relative abundance is computed as the product of the frequence of observance and the number of levels in the patch.
* These values are compiled from studies previously published [29, 30, 31].

sis of nonspecific cation channel records, data was fed into the computer at a sampling rate of 50–200 μsec/point and filtered at 500–1000 Hz. P-CLAMP6 software (Axon Instruments) was used to analyze the data on a Dell Optiplex PC (Dell Computer).

Most of the seals obtained expressed channels. Many of the patches clearly possessed more than one type of channel. The chord conductance of channels was determined from the slope of the *I–V* relationship. Pipette voltages used typically ranged from pipette positive −100 to +100 mV. The signal of an active patch was monitored for 10 to 60 sec at each voltage. Mean open times were computed from the time a single channel spends in the level 1 state. 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.

Results are presented as the mean value ± SEM (number of channels studied). The *t*-test for a difference between two independent means was used to evaluate the effect of amiloride and changes in basolateral potassium on various parameters.

Results

Table 1 presents some information on the relative distribution of cation channels found in the apical membrane of the initial collecting tubule. Values for the frequency of observance, the fraction of successful patch-clamp seals that contain a given type of channel, and the relative abundance of that channel are presented for tissue dissected from control animals and from potassium-adapted animals. We usually observe the nonspecific cation channels in apical patches on collecting tubules taken from potassium adapted animals. As Table 1 indicates we rarely see the nonspecific cation channels in patches on tubules of untreated animals. Thus, potassium-adaptation causes an upregulation of these channels. The upregulation of ENaC and maxi K channels has been previously reported [29, 30, 31].

Table 2 presents the average values of a number of biophysical parameters of the intermediate conductance channel. Values for two conditions are presented. The column labeled "chloride pipette" present data collected when normal saline is present on all surfaces of the tubule, including the patch pipette. The column labeled "gluconate pipette" are data collected when the saline used to fill the patch pipette had much of the chloride replaced with gluconate. The conductance and pipette reversal potential of these channels average 30 pS and −30 mV, respectively, when there was chloride in the pipette. The channel is characterized by having a relatively low open probability. Open probability at zero mV averaged 0.08 ± 0.02 (9). Changing the pipette voltage from 0 to 80 mV showed no significant change in open probability ($\Delta = -0.01 \pm 0.03$, 9 patches). Thus, the open probability is independent of voltage. When most of the chloride was replaced with gluconate, the conductance, open probability, mean open time and unitary current were not different from the values obtained from patches with normal saline in the pipette (Table 2).

Table 2. Characteristics of nonspecific cation channels in Ambystoma initial collecting tubule

Parameter	Value	
	Cl pipette	Gluconate pipette
Conductance (pS)	30.3 ± 2.4	28.1 ± 3.3
Pipette reversal potential (mV)	−29.8 ± 6.5	−14.1 ± 5.3
Unitary current (pA)	0.97 ± 0.08	0.85 ± 0.20
Mean open time (msec)	9.3 ± 1.6	11.4 ± 4.2
Open probability	0.08 ± 0.03	0.08 ± 0.02
Number of channels per patch*	1.4 ± 0.2	1.7 ± 0.7

The values for a number of channel parameters are given under each of two conditions. First, when the patch pipette is filled with a chloride containing saline (9 patches) and secondly, when the patch pipette is filled with a solution in which the sodium chloride has been replaced with gluconate as the anion (3 patches). Values for unitary current, mean open time and open probability were determined with the patch voltage-clamped to 0 mV. All values are presented as mean ± SEM.
* The number of channels per patch reflects only the number of levels observed in patches when the 23 pS channel was seen. Therefore, it cannot be used as an index of channel density.

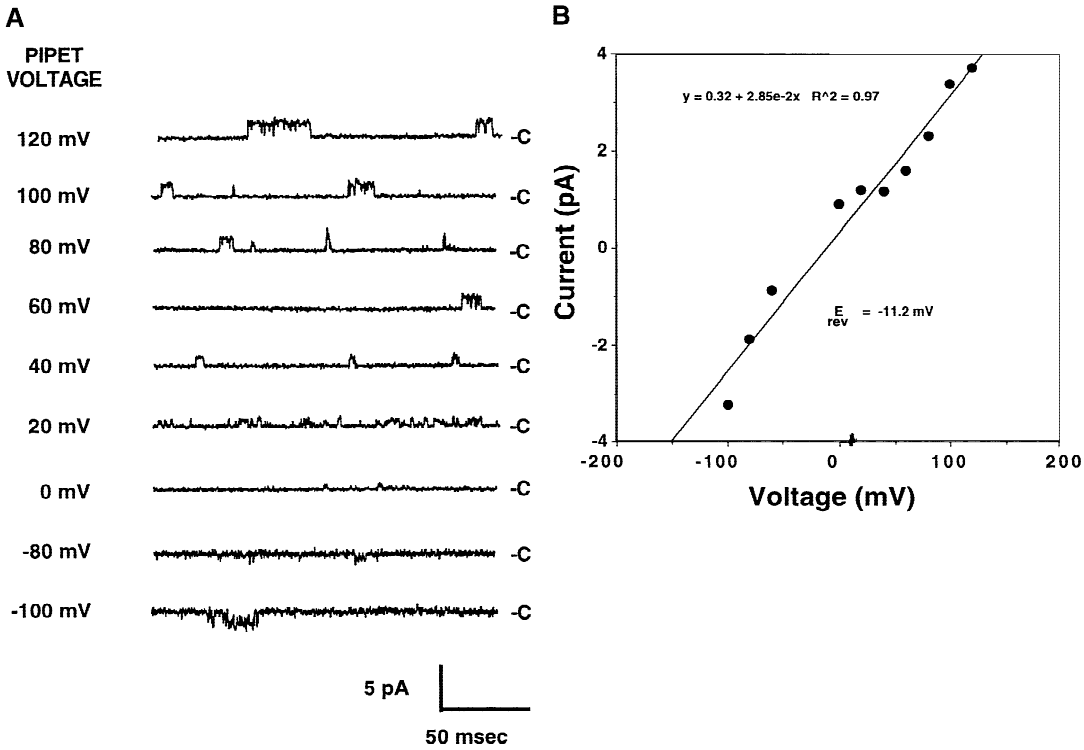

Fig. 1. Panel *A* shows representative traces of intermediate-conductance nonselective cation channels on the apical membrane of *Ambystoma* initial collecting tubule at various voltages. Each trace is 200 msec in length. The trace is filtered at 1500 Hz. The value to which the pipette is voltage clamped is indicated to the left of each trace. **-C** indicates the closed state. Traces at positive voltages show the presence of inward-directed current when the channel is open. Panel *B* is the current-voltage plot of the same patch which is shown in panel *A*.

The lack of effect of anion replacement on these parameters together with the scarcity of other anions in the patch pipette is taken to indicate that the channel is not carrying anions. These data are consistent with the notion that the current is most likely due to cations.

Since chloride replacement had no effect on either conductance or the unitary current the pipette reversal potential data from patches exposed to gluconate were not corrected for junctional potential. While the average reversal potential for channels studied in the presence of gluconate appears smaller, this difference is not statistically significant. Approximately 1.5 channels appeared in each patch. It should be noted that this value may well be an underestimate, since the open probability is so low.

Panel *A* of Fig. 1 shows a series of representative traces of the intermediate conductance channel in a cell-attached patch voltage clamped at a variety of voltages. Normal saline was present on both the apical and basolateral surfaces of the tubule and in the patch pipette. Panel *B* presents the current-voltage plot of the same patch studied in panel *A*. That the data shown for positive and negative voltages are not identical is taken to indicate that this patch is cell-attached, since a cell-detached patch would yield symmetrical data.

One important feature of the data in Fig. 1 is that the direction of current changes to be an inward current at negative pipette voltages greater than −40 mV. The direction of current observed is consistent with the movement of sodium from the pipette into the cell when the pipette is clamped at a positive voltage and, with the movement of potassium from the cell into the pipette when the pipette is clamped at negative voltages.

We studied seven patches exposed to either 2×10^{-6} (three patches) or 2×10^{-5} M amiloride (four patches). Neither dose appeared to alter the open probability of the channels. The data were combined and presented in Table 3. While these data do not exclude the possibility that amiloride may inhibit the channels at a high dose, they certainly do indicate that compared to the 4 pS channel this channel is relatively insensitive to amiloride.

We had recently reported that making the saline on the basolateral surface of the tubule hyperkalemic (15 mM K) activated quiescent maxi K channels into secreting potassium into the luminal fluid [31]. To see if something similar would happen, we exposed this intermediate conductance channel to hyperkalemic conditions. Table 4 shows the results of these experiments. Five patches were studied. When the basolateral surface of the collecting tubule is exposed to a saline containing 15 mM potassium, the open probability and open time

Table 3. Lack of effect of amiloride on nonspecific cation channels in *Ambystoma* initial collecting tubule

Parameter	Value	
	Control	Amiloride
Conductance (pS)	29.5 ± 2.0	31.2 ± 5.1
Pipette reversal potential (mV)	−25.4 ± 6.4	−30.1 ± 8.1
Unitary current* (pA)	1.88 ± 0.18	1.92 ± 0.28
Mean open time (msec)	9.2 ± 1.9	18.6 ± 11.3
Open probability	0.06 ± 0.01	0.07 ± 0.02

The control column represents the mean of the eight patches in Table 1. Seven patches (column 2) were treated with amiloride, 4 at a dose of 2×10^{-5} (M) and 3 patches at 2×10^{-6} (M). None showed an effect of the drug so the data were combined. The values for mean open time and open probability are for 0 mV voltage clamp.
* To facilitate measurement the values for unitary current were taken at voltage clamp of +40 mV. All values are presented as mean ± SEM.

Table 4. Characteristics of nonspecific cation channels in *Ambystoma* initial collecting tubule exposed to 15 mM K saline on the basolateral surface

Parameter	Value
Conductance (pS)	26.6 ± 4.6
Pipette reversal potential (mV)	−17.2 ± 2.3
Unitary current (pA)	0.63 ± 0.13
Mean open time (msec)	17.3 ± 4.6
Open probability	0.14 ± 0.02*
Number of channels per patch	1.8 ± 0.6

The average values for parameters commonly used to characterize channels are given for 5 patches. The basolateral surface was exposed to a saline containing 15 mM K^+. The values for unitary current, mean open time and open probability were determined with the patch voltage-clamped to 0 mV. All values are presented as mean ± SEM.
* Indicates that the value is significantly different from control. $P < 0.01$.

increase significantly. We presume the shift in pipette reversal potential reflects depolarization of the resting membrane potential associated with the change in concentration of environmental potassium.

In three of the five channels studied in Table 4, the basolateral surface of the tubule was first exposed to a saline containing 4.3 mM K. The saline was then changed to one with 15 mM K. The channel activity was monitored for 5 or more min after the fluid exchange was done. Representative traces from such an experiment are presented in Fig. 2. As can be seen little activity exists in the presence of 4.3 mM K and the channel appears to flicker. After several minutes of exposure of the basolateral surface to a saline containing 15 mM K, clear bursts of channel activity can be seen.

Figure 3 shows a representative plot of the time

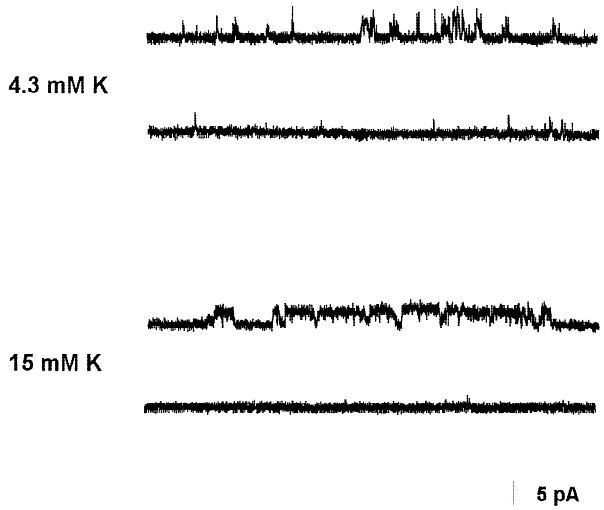

Fig. 2. Representative traces of an intermediate conductance channel when the basolateral membrane is exposed to 4.3 mM K (upper traces) or 15 mM K (lower traces). Pairs of sequential traces are presented. Each trace is 400 msec in duration. The patch was clamped to +100 mV.

course of changes in open time (Panel *A*) and open probability (Panel *B*) after increasing the basolateral potassium concentration. The time necessary to attain a new steady state after changing the potassium level was several minutes. The change in open probability represents an increase of nearly 85% over the control value, while open time increased 75% (Table 4). Thus, the change in activity is best explained by a mechanism which changes the closing rate coefficient of the channel [22].

Discussion

The cloning of the three subunits of the amiloride-sensitive ENaC channel by Rossier and his coworkers [2] together with the observations that this is the only sodium channel generally found in native collecting tubule and its high sensitivity to amiloride [21, 22, 29] have led to the general view that this low-conductance, highly selective sodium channel is responsible for most, if not all of the net sodium reabsorption in the late distal nephron [34].

Some authors have suggested that other channel types may contribute to sodium reabsorption by the late distal nephron. One candidate is the 28–30 pS, nonselective cation channel reported in a number of tissue culture cell lines including the rabbit collecting tubule [17], cells of the rabbit inner medullary collecting duct [6] and A-6 cells [12, 19]. The channel has been reported in nonrenal native tissue such as nasal epithelial cells [18, 20, 21, 35] and vasopressin-treated toad bladder epithelial cells [9]. This channel's poor selectivity

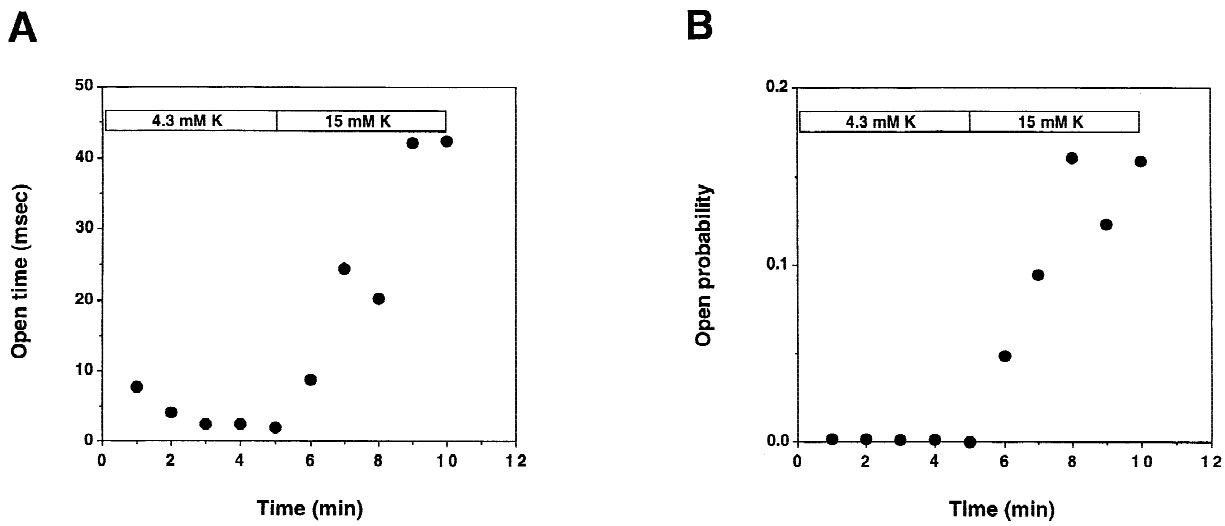

Fig. 3. The time course of the effect of increasing the basolateral potassium concentration from 4.3 to 15 mM on open time (Panel *A*) and open probability (Panel *B*). During the first 5 min the basolateral saline contained 4.3 mM potassium. At 5 min the saline was exchanged for one containing 15 mM potassium.

and questionable inhibition by amiloride have made it difficult to define its physiological importance. Immunocytochemical identification of these channels in several nephron types, including the inner medullary collecting duct of mouse [3], and their reported sensitivity to atrial natriuretic peptide [16] have been taken as argument for their potential involvement in net sodium reabsorption.

This is the first report to identify a 30 pS nonselective cation channel in a native distal nephron segment. Replacement of the chloride in the pipette with gluconate had no effect on the conductance of the channel. We take this to indicate the channel has little, if any, permeability to chloride. We observe this channel under conditions where the animals were pretreated with 50 mM potassium for a week or more prior to the experiment. Animals maintained on tap water rarely express the channel. Upregulation in response to potassium stress suggests that it could be involved in potassium secretion.

The conductance of this channel, nearly 30 pS, and the voltage at which the polarity of current reverses, −30 mV, are virtually identical to those reported for the nonselective cation channel in A-6 cell lines [19]. Nonselective cation channels have in general been found to have P_{Na}/P_K ratios near 1.0 [10]. This is usually evaluated by studying the biophysical properties of the channel in cell-detached patches under conditions where the Na and K activities are precisely controlled. It is not practical to precisely control the ion gradients across the patch in cell-attached patches. It is possible to estimate the relative permeability of sodium to potassium from the voltage at which the polarity reverses [4]. A nonspecific cation channel with equal permeabilities to both sodium and potassium would be expected to reverse current polarity at the apical membrane potential. Thus, the pipette reversal potential should be equal to and of the same polarity as the membrane potential. We observed a pipette reversal voltage of −30 mV. The apical membrane voltage of the collecting tubule has been determined in only one species of potassium-adapted amphibia, *Amphiuma*. It was found to be −15 mV [14]. The value for the transepithelial voltage of this segment in aquatic-phase *Ambystoma* is −35.7 mV [34]. Assuming the basolateral membrane voltage to be −60 mV as measured in *Amphiuma* [14], the value of the apical membrane voltage should be −24 mV. The observed pipette reversal potential (−30 mV) is near this value. An apical membrane voltage between −15 and −24 mV would place the P_{Na}/P_K ratio to be at or slightly below 1.0. Similar nonspecific cation channels have been reported in numerous other systems [4, 5, 9, 12, 15, 16, 17, 18, 19, 21, 35]. The channel was not inhibited by either 2×10^{-6} or 2×10^{-5} M of amiloride, the higher concentration being nearly 100 times the dose known to inhibit the 4 pS sodium channel [12, 22, 29, 30]. Studies of the sensitivity of other nonspecific cation channels to amiloride have produced mixed results. In A-6 cell line, 10 μM amiloride inhibited only 28 pS channels which had been stretch activated prior to the application of the drug [19]. On the other hand, 100 μM amiloride had no effect on control channels that were not stretch activated. Cells derived from rabbit collecting tubule [17] and the inner medullary collecting duct of mouse [16] express intermediate conductance channels that are reported to be sensitive to submicromolar concentrations of amiloride. In nasal epithelial cells a nonspecific cation channel has been found to be sensitive to 1 μM or less amiloride by some authors [18, 21, 35]. More recently, Chinet and

coworkers [4] reported that cell-detached patches from nasal epithelial cells express a nonspecific cation channel that is insensitive to amiloride. In cell-attached patches they observed channels that had the same conductance (28 pS) but were sodium-selective and blocked by 100 μM amiloride. Lower doses of amiloride were found to be ineffective [4]. It is possible that excision of the patch changes the characteristics of the intermediate conductance channels. An alternative explanation is that a number of different, but related, intermediate conductance cation channels exist [4].

Several reports on the transport properties of amphibian distal nephron segments, utilizing both micropuncture [1, 23, 37] and microperfusion techniques [19, 28] have indicated these tubule segments appear to secrete less potassium than their mammalian counterparts [14, 28] where the rate of sodium reabsorption and potassium secretion are of similar magnitudes. This observation could well reflect the dietary input of the animals. Stiffler et al. [26] have shown that exposure of amphibia to large amounts of environmental potassium greatly stimulates the animal's ability to secrete potassium. Others [14] could find no evidence for a conductive pathway for potassium secretion in the *Amphiuma* distal nephron. They proposed that in amphibia, the distal nephron lacks the low conductance potassium channels reported in the distal nephron of the mammal and, that in amphibia potassium secretion occurs through a paracellular pathway.

Recently we demonstrated that exposure of *Ambystoma* to KCl for a period of 7 or more days dramatically increased the estimated density of maxi K channels in the apical membrane of the initial collecting tubule from 0.08 channels per square micron to 0.76 [30]. Subsequent studies showed that under conditions which mimic hyperkalemia the channels activate and secrete potassium [31].

We also exposed collecting tubules containing the nonselective cation channels to hyperkalemic conditions. As reported in Table 4, mimicing hyperkalemic conditions on the basolateral surface of the initial collecting tubule resulted in an increase in channel activity suggesting the possibility that this channel plays a role in potassium secretion. This observation, together with the observation that exposure of the animals to environmental potassium upregulates the number of active channels, argue that the physiological importance of the channel may be related to potassium homeostasis.

Amphibia appear to lack the romK channel important to potassium secretion in mammalian distal nephron [36]. However, like the mammal [25], amphibia do respond to potassium adaptation by dramatically increasing their ability to secrete potassium [14, 26]. This study has shown that potassium-adaptation results in the upregulation of nonspecific cation channels in the apical membrane of the initial collecting tubule. Micropuncture studies indicate that the luminal fluid that normally enters the late distal nephron of amphibia is normally very low in sodium content [1, 37]. The value is probably less than 20 mM. Since the lumen of the amphibian distal nephron is an environment poor in sodium, and the cytosol is rich in potassium, an apical nonselective cation channel should secrete potassium.

We postulate that, at least in amphibia, these nonselective cation channels are normally potassium channels, not sodium channels as postulated for the mammalian inner medullary collecting duct [16]. Their upregulation after potassium adaptation and their activation in conditions that mimic hyperkalemia are both consistent with this hypothesis. This does not preclude the possibility that under conditions of high sodium intake, luminal sodium may be elevated and this channel could well reabsorb sodium.

The intermediate conductance channel we study in *Ambystoma* shares certain properties with the nonspecific channels studied in other species. It is important to note that sequence homology or antibody binding studies needed to demonstrate that these channels are related are lacking. Thus, a number of intermediate conductance channels that we have discussed may represent entirely different proteins with different physiological characteristics. Undoubtedly, future studies will address this question.

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