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A non-selective cation channel in the apical membrane of cultured A6 kidney cells

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The patch-voltage clamp technique was used to investigate the characteristics of a non-selective cation channel (NSCC) identified in the apical membrane of cultured A6 toad kidney cells. The NSCC was present in cell-attached and inside-out membrane patches. The characteristics of this NSCC are as follows: (a) linear current-voltage relationship with a channel conductance of 21 ± 2 pS; (b) a low selectivity between Na^+ and K^+ (1.5:1); (c) a high selectivity of Na^+ to Cl^- (> 45:1); (d) this channel has a single open state and two closed states; (e) the open-time constant and the second closed-time constant of this channel are voltage dependent; and (f) this NSCC is insensitive to amiloride (10^{-7} M). We conclude that the NSCC resembles previously described non-selective cation channels. The NSCC of the apical membrane of A6 cells may aid in the movement of Na^+ and K^+ in response to varying ionic concentrations across the apical membrane.

Introduction

With macroscopic current experiments, it has been demonstrated that A6 toad kidney epithelial cells in culture possess many functional and structural characterisitcs suggesting cortical collecting duct origin (a 'tight' epithelium). These characteristics include: (a) development of a high transepithelial resistance; and (b) generation of a short-circuit current which is stimulated by aldosterone, blocked by amiloride and attributable to sodium transport [1–4]. The advent of patch-voltage clamp technology allows the examination of individual ion channels [5,6].

Patch-clamp experiments of A6 cells (apical membrane) have revealed the presence of various types of channels which have been suggested by macroscopic current experiments of A6 cells [1–4,7] and cortical collecting duct cells [8–11]. Hamilton and Eaton [12–14] have described two types of Na^+ channels based on channel selectivity, conductance, and amiloride sensitivity. Furthermore, Hamilton and Eaton [15] have demonstrated the presence of a cAMP-induced K^+ channel in the apical membrane of A6 cells. Also, a highly conductive Cl^- channel has been identified in nonconfluent A6 cells [16]. As can be seen above, numerous types of channels are present in the apical membrane of A6 cells.

Non-selective cation channels (NSCC) have been identified in a variety of vertebrate cell types [17–25]. These channels allow the passage of Na^+ and/or K^+ without discrimination. Recently, Light et al. [26,27] have described an amiloride-sensitive NSCC from the inner medullary collecting duct of the rat. The physiological role of this NSCC is not clear, but it may be involved in transepithelial Na^+ transport.

In the present study, we report the existence of a non-selective cation channel in the apical membrane of cultured A6 cells. Using the patch-voltage clamp technique, we determined the following characteristics of this channel: (a) single-channel conductance of 21 pS; (b) selectivity of Na^+ to K^+ of 1.5 : 1; (c) selectivity of Na^+ to Cl^- of > 45 : 1; (d) a single open state and two closed states; and (e) this channel is insensitive to amiloride (10^{-7} M). Part of this study was previously reported in abstract form [28].

Abbreviations: Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N'*-tetraacetic acid; cAMP, cyclic adenosine monophosphate; NSCC, non-selective cation channel.

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Materials and Methods

Cell culture

All cells were purchased from American Type Culture Collection (Rockville, MD) in the 68th plating [29]. All experiments were carried out on platings 73–94. Cells were grown to confluence on glass coverslips or fibronectin-coated Millicell (CM-12) permeable supports. Fibronectin-coated supports provided a matrix for cell attachment and also allowed access to either side of the epithelial monolayer. Cells were grown in Dulbecco's modified Eagle Medium (Gibco, Grand Island, NY) supplemented with 10% fetal bovine serum (Hyclone, Logan, UT), 100 units $\cdot$ ml^{-1} penicillin, and 100 μg $\cdot$ ml^{-1} streptomycin (Gibco). Cells were grown at 27°C in a humidified incubator gassed at 1.5% CO_2/98.5% air. Cells grown on glass coverslips were seeded at a density of 10^6 cells/35 mm dish while cells grown on Millicell supports were seeded at a density of 10^4 cells/support. Cover slips or small portions of a Millicell support were transferred to an experimental chamber which allowed solution changes. All experiments were conducted at ambient temperatures of 20–22°C.

Patch recordings

Pipettes were fabricated from WPI TW150 glass (New Haven, CT) or Corning glass 8161 [5] using a two stage Narishige pipette puller (PP-83), fire-polished and coated with Q-dope (GC Electronic, Rockford, IL). When pipettes were filled with a 130 mM NaCl solution (Solution A, Table I), pipettes were measured to have tip resistances of 2–4 MΩ. Cells were visualized with an inverted Nikon Diphot microscope equipped with Hoffman modulation contrast optics. Currents were recorded with an EPC-5 amplifier (Medical Systems, Great Neck, NY) in both the cell-attached and inside-out patch configuration [5]. An Ag-AgCl pellet was used to couple electrically the headstage amplifier and the pipette solution, bridged via a KCl-agar filled tube. The bath electrode was also an Ag-AgCl pellet and was likewise bridged to the bath solution via a 3 M KCl-agar filled tube. Electrical potential difference was referenced to the pipette solution, therefore, outward current was indicative of current flow from the cell (or the cytoplasmic surface) to the pipette. Junction potentials created by changes in Cl^- bath solutions were calculated using the Henderson approximation of the Nernst-Planck equation and found to be less than 0.5 mV. Voltage steps were provided by an S 95 stimulator (Medical Systems). Current outputs were directly digitized with a modified pulse-code-modulation unit (Sony, Vetter Co., Redersburg, PA) and stored on video tape (Sony Beta SL-2700) for later analysis.

Solutions

The standard pipette solution was a 130 mM NaCl saline (pH 7.2 titrated with NaOH; Solution A, Table I). An alternate pipette solution consisted of 140 mM NaCl saline (pH 7.2; Solution C, Table I). The standard bathing solution was the above mentioned 130 mM NaCl saline with the addition of 10 mM glucose. For inside-out patch experiments, the bathing soluton was exchanged with either a K-glutamate saline (Solution B) of pH 7.2 (titrated with KOH); or a 70 mM NaCl saline (Solution D) of pH 7.2 (titrated with NaOH). All pipette solutions were filtered immediately before use (0.2 μm membrane filter, Vangard).

In some experiments, cells were dissociated from Millicell supports with one of the following solutions (in mM) 140 NaCl, 6.8 KCl, 1 EGTA, 5 Hepes, with pH 7.2; 104 NaCl, 25 $NaHCO_3$, 3.4 KCl, 1 EGTA, 5 Hepes, and pH 7.2, or 140 Na-glutamate, 1 EGTA, 5 Hepes, with pH 7.2. Cells were washed and centrifuged once in the dissociation solution and twice in the appropriate experimental bath solution. Subsequently, cells were placed directly into the experimental chamber and allowed to settle to the bottom of the chamber. Cell viability was 85% determined using Trypan-blue exclusion in 12 preparations. Solutions were perfused through the experimental chamber at a rate of 1.5 ml/min with a perfusion pump (Bechler, Saddlebrook, NJ). During Na : K selectivity experiments, generally, Cl^- was the major anionic constituent of the pipette solution rather than glutamate for the following reasons. First, the patch formation rate was reduced when glutamate was present in the patch pipette. Secondly, a successful preliminary experiment with Na-glutamate (140 mM) in the pipette revealed a similar Na : K selectivity as seen with NaCl (140 mM) in the pipette (data not shown). Finally, the premeability of the NSCC to Cl^- was shown to be very low (see Results, ion selectivity), therefore, NaCl solutions were used due to the relative ease of patch formation.

Amiloride sensitivity

To examine the amiloride sensitivity of the NSCC, amiloride (10^{-7} M) was added to the 130 mM NaCl pipette solution (Solution A). With cell-attached patch experiments, mean open and closed times of the channel (as evaluated with frequency distribution histograms) were examined to determine if amiloride affected the open and close constants of the channel. Amiloride was purchased from Sigma Chemical Co. (St. Louis).

Data analysis

Current records were filtered at 300 Hz or 500 Hz with an 8 pole bessel filter (Frequency Devices, 902LPF, Haverhill, MA) and sampled at 1 kHz or 2 kHz with a TL-1 interface-Lab master board (Axon Instruments,

Inc., Burlingame, CA). Analysis of data was executed with the Fetchan portion of pCLAMP hardware and software (Axon Inst., Inc). Current-voltage (*I-V*) relationships were constructed by plotting the mean value of a current amplitude distribution versus the applied voltage from which the distribution was obtained. Frequency distribution histograms of channel open and closed times were constructed with pCLAMP software. Open- and closed-time distributions were best fit by either single or double exponentials. Time constants were determined from these exponential fits. Relative ion permeabilities were calculated using the Goldman-Hodgkin-Katz voltage equation from experimentally determined reversal potentials. Conductance of the NSCC was determined by calculating the slope of the *I-V* relationship. The percent (%) open time of the channel was calculated as the amount of time the channel remained open divided by the total amount of time of the recording period (taking into account the number of channels observed in the patch).

Statistics

Data are reported as means ± one standard deviation. Linear and curvilinear regression analyses were used to determine slope conductance and reversal potentials of *I-V* relationships. Exponential fits of histogram data were executed with a variation of the pSTAT portion of pCLAMP (Ver. 4). Voltage dependence of the NSCC was determined by statistically examining the slope of the linear regression of time constants versus voltage and % open time versus voltage as compared to a slope of zero [41].

Results

Single-channel recordings

Fig. 1 demonstrates a series of current traces of a NSCC from a cell-attached patch of the apical membrane of A6 cells. When the pipette contained 130 mM NaCl (Solution A, Table I), at 0 mV (no applied voltage to the patch pipette, therefore, normal resting membrane potential for the cell), current flow was from the pipette to the cell indicative of movement of cations into the cell. As the cell membrane (i.e., patch potential) was hyperpolarized, the inward unitary current increased in magnitude due to the increased electrical driving force for cation movement. Conversely, as the membrane patch was depolarized, the current flow reversed (outward current flow), indicating current flow from the cell to the pipette. From the current traces, it is apparent that as the membrane patch was depolarized, the channel spent more time in the open state (see below).

To determine the conductance of the NSCC, *I-V* relationships were constructed from the mean current amplitude histograms (gaussian distributions, data not shown) at various holding potentials. Fig. 2A, shows the *I-V* relationship (filled circles) of the NSCC in the cell-attached configuration. With the 130 mM NaCl saline (Solution A) in the patch pipette, the *I-V* relationship was linear with a slope conductance of 21 ± 2

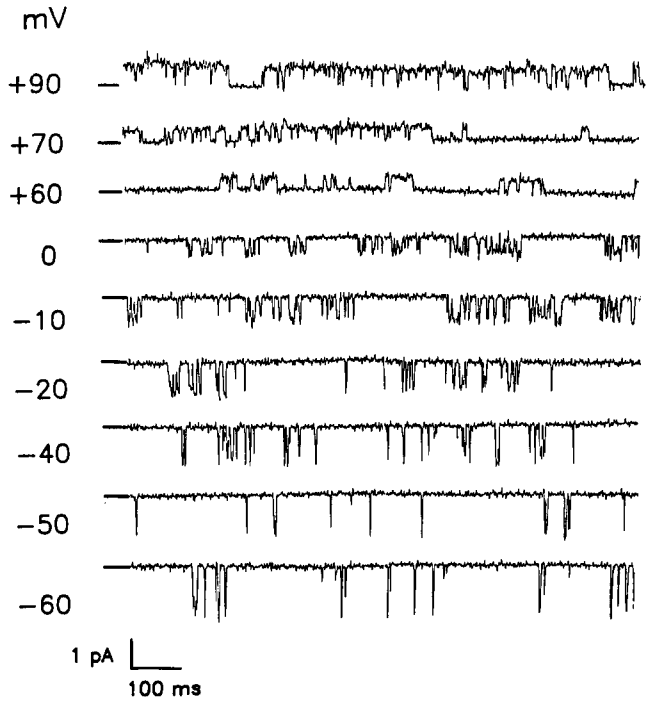

Fig. 1. Single-channel currents from a cell-attached patch of a NSCC recorded from the apical membrane of an A6 kidney cell. At 0 mV, there is no applied potential to the pipette. Therefore, the membrane potential is the cell's normal resting potential. Voltages (mV) added to the membrane potential are shown to the left of the current traces. The zero current level of the channel is shown as a solid line to the left of the current traces. The pipette contained the 130 mM NaCl saline (Solution A, Table I), while the bath contained the 130 mM NaCl saline with 10 mM glucose added. Downward transitions indicate inward current while upward transitions indicate outward current. These current traces are filtered at 300 Hz.

TABLE I

Composition of solutions

All concentrations are in mM. All solutions were buffered to a pH of 7.2.

	Solution			
	A	B	C	D
NaCl	130	0	140	70
KCl	5	0	0	0
$CaCl_2$	2	0	2	1
$MgCl_2$	2	0	2	1
Na-glutamate	0	5	0	0
K-glutamate	0	130	0	0
Ca-gluconate	0	0.5	0	0
Mg-gluconate	0	2	0	0
EGTA	0	1	0	0
Hepes	5	5	5	2.5

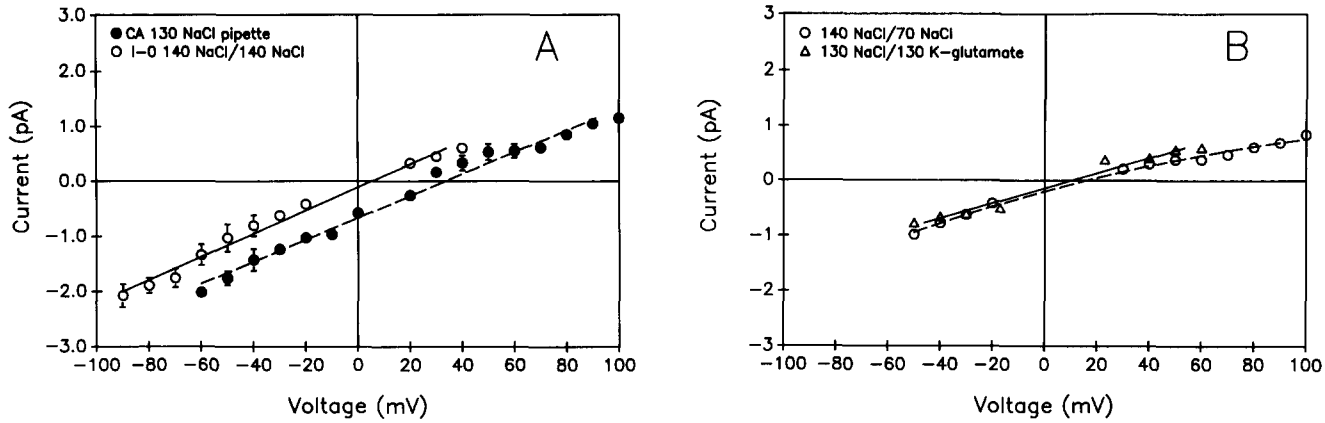

Fig. 2. Current-voltage relationship of the NSCC of the A6 kidney cell. (A) In the cell-attached configuration [130 mM NaCl (Solution A) in the pipette and the bath (with 10 mM glucose)], the conductance of the NSCC was 21 ± 2 pS ($n = 9$) (closed circles). When an inside-out patch (140 mM NaCl, Solution C, in the pipette) was bathed with the 140 mM NaCl saline, the conductance of the channel was 20 pS ($n = 4$) and the reversal potential shifted to near 0 mV (1 ± 2 mV) (open circles). (B) This figure shows the current-voltage relationship of a NSCC when an inside-out patch (130 mM NaCl pipette solution, Solution A) was bathed with 130 mM K-glutamate (solution B) (open triangles). With a reversal potential of 10 mV, the Goldman-Hodgkin-Katz equation was used to calculate an apparent cation selectivity of 1.5 : 1, Na^+ to K^+. The cation to anion selectivity of the NSCC was determined by exposing an inside-out patch (140 mM NaCl pipette, Solution C) to a 70 mM NaCl (Solution D) bath solution (open circles). The reversal potential of the *I-V* relationship shifted from 0 mV (Fig. 2A) to +17 mV (Fig. 2B), the relative ion permeability of Na^+ to Cl^- was calculated to be greater than 45 : 1 (mean ± S.D. shown).

pS ($n = 9$). In the inside-out configuration with symmetrical 140 mM NaCl saline (Solution C) in the pipette and bath, the *I-V* relationship was also linear and had a slope conductance of 20 pS ($n = 4$) (Fig. 2A, open circles). The reversal potential of the *I-V* relationship shifted to near 0 mV (1 ± 2 mV, $n = 4$).

Ion selectivity

To determine the cation selectivity of the NSCC, an inside-out patch (130 mM NaCl saline, solution A in the pipette) was formed and the bath solution was exchanged with the 130 mM K-glutamate saline (Solution B, 5 mM Na-glutamate). If the apical membrane of A6 cells was perfectly selective for Na^+, then, the reversal potential for Na^+ (calculated E_{Na^+}) would be +82 mV for the pipette and bath solutions described above. The E_{K^+} for a *K*-selective membrane was calculated to be −82 mV. However with the above mentioned experimental conditions, the *I-V* relationship of the NSCC was linear (slope conductance of 14 pS) and a reversal potential of 10 mV was observed (Fig. 2B, open triangles). From reversal potential measurements (10 mV), the cation selectivity of the NSCC was determined to be 1.5 : 1, Na^+ over K^+. Therefore, this channel shows little selectivity between Na^+ and K^+.

To determine the cation to anion selectivity of this NSCC, an inside-out patch with 140 mM NaCl in the pipette (Solution C) was exposed to a 70 mM NaCl bath solution (Solution D). If the apical membrane of A6 cells is selective only for cations (Na^+ the only cation in the pipette or bath solutions) the calculated reversal potential for cations was +17.4 mV. For an anion selective membrane (Cl^- as the anion in this experiment) the E_{anion} was calculated to be −17.4 mV. However, with Solution C in the pipette, when the patch was bathed with a 70 mM NaCl bath solution the reversal potential shifted from 0 mV (140 mM NaCl pipette and bath) to +17 mV suggestive that the apical membrane is selective for cations (Fig. 2B, open circles). The slope conductance of this channel in the negative voltage range was 19 pS ($n = 1$), while the slope conductance in the positive voltage range was 12 pS. From the reversal potential measurements, the relative ion permeability of Na^+ to Cl^- was calculated (with the Goldman-Hodgkin-Katz equation) to be greater than 45 : 1, Na^+ over Cl^-. Therefore, this NSCC shows a greater selectivity for cations over anions.

Channel kinetics

Patches which possessed only one channel (only one channel observed at both depolarizing and hyperpolarizing potentials) were used for kinetic analysis. To determine the time constants (mean open and closed times) for this NSCC, frequency distribution histograms of channel open and closed times were constructed. A representative set of histograms are shown in Fig. 3 for a cell-attached patch held at 0 mV (i.e., no applied potential, therefore, normal resting membrane potential of the cell; Solution A in the pipette). The open time frequency distribution had a mean open time of 6 ms (Fig. 3A, $n = 3$). The open time distribution was fit with a single-exponential function suggestive of one single open state of the channel [30]. However, the closed-time frequency distribution was fit with a double-exponential function with the first mean closed time of 3 ms and a second closed time of 19 ms (Fig. 3B, $n = 3$). The double-exponential fit of the closed-time frequency distribution suggests two closed states of the channel [30].

Fig. 4 illustrates of the open- and closed-time constants as a function of voltage for the NSCC of A6 cells. If voltage of the membrane affects the open-time constants of the NSCC, then the slope of this relationship should be significantly different from a slope of zero. In cell-attached patches ($n = 3$) with 130 NaCl saline (Solution A) in the patch pipette, as the patch potential was depolarized, i.e., holding potential made more positive, the open-time constant increased (Fig. 4, open circles). In fact, comparison showed that the slope of the relationship of open-time constants as a function of voltage, as determined by linear-regression analysis, was significantly different from a slope of zero suggesting that voltage affects the open-time constants of the NSCC ($F = 43.6$, $P < 0.05$). This also can be noted by the channel spending longer periods of time open as the cell potential is depolarized (Fig. 1). Conversely, as the cell potential was depolarized, the second closed-time constant decreased (Fig. 4, closed circles). The slope of the relationship of the second-time constant versus voltage was significantly different from a slope of zero, likewise, suggesting that voltage affects the second time constants of the NSCC ($F = 18.2$, $P < 0.05$). The first closed-time constant appeared unaffected by membrane potential (Fig. 4, open triangles). Therefore, the open-time constant and the second closed-time constant of the NSCC appeared to be voltage dependent. In other words, the NSCC spent longer periods of time in the open state as the membrane potential was depolarized.

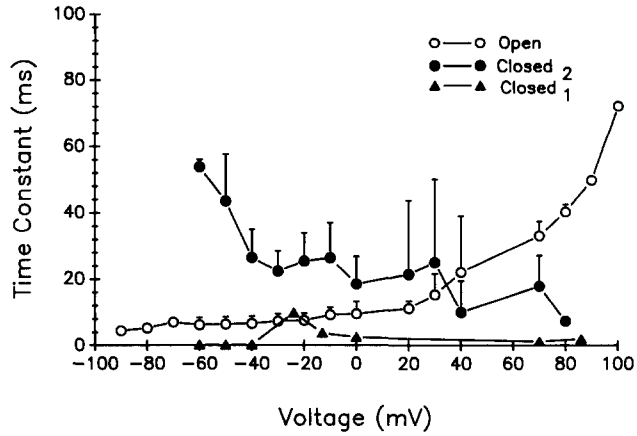

Fig. 4. Open- and closed-time constants of the NSCC are shown. The open time-constant increased as the membrane potential was depolarized (open circles). The first closed-time constant (closed triangles) was not affected by membrane potential. However, the second closed time constant (closed circles) decreased as the membrane potential was depolarized. Therefore, the open-time constant and second closed-time constant of the NSCC were voltage dependent ($n = 3$).

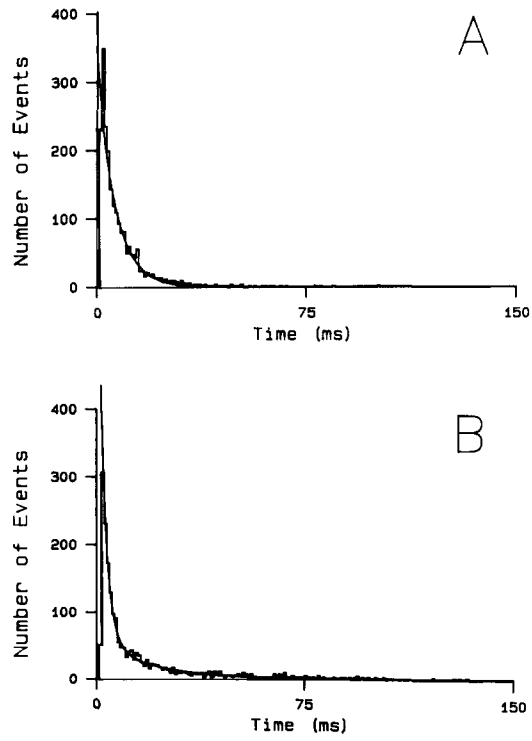

Fig. 3. Frequency distribution histograms of the open and closed times of the NSCC when a cell-attached patch was held at 0 mV (no applied potential). The pipette solution was the 130 mM NaCl saline (Solution A) and the cells were bathed with the identical solution with 10 mM glucose added. (A) This figure shows the open-time distribution of the NSCC (2192 events). These data are fit by a single-exponential function with a mean open time of 6 ms ($n = 3$). The single-exponential fit is suggestive of a single open state of the channel. (B) This figure shows the closed-time distribution at 0 mV (2131 events). These data are fit with a double-exponential function with the first mean closed time of 3 ms and a second mean closed time of 19 ms ($n = 3$). The double-exponential fit of the distribution of closed times is suggestive of two closed states of the channel. One closed state can be noted as an interburst closed state, as can be seen in Fig. 1, while the second closed state of the channel can be seen within the burst activity of the channel.

Another way to determine whether this NSCC is voltage-dependent is to examine the relationship between the percent open time of the channel and applied voltage. If voltage affects the percent open time of the channel, then the slope of that relationship would be significantly different from a slope of zero. For cell-attached ($n = 7$) patches, as the cell potential (or cytoplasmic surface of the patch) was depolarized, the channel spent more time in the open state (Fig. 5). Similarly, the slope of the relationship of percent open time versus voltage was significantly different from a slope of zero ($F = 132.9$, $P < 0.05$) suggesting that voltage influenced the percent open time of the NSCC.

Amiloride sensitivity

Recently, Light et al. [26] have described a non-selective cation channel in the apical membrane of the inner medullary collecting duct of the rat which is sensitive to amiloride ($5 \cdot 10^{-7}$ M) (e.g., decreased mean open time of the channel). Hamilton and Eaton [12] have reported the presence of a low selective Na^+ channel (3–4 : 1, Na^+ over K^+) in the apical membrane of A6 kidney cells which is likewise sensitive to amiloride ($5 \cdot 10^{-7}$

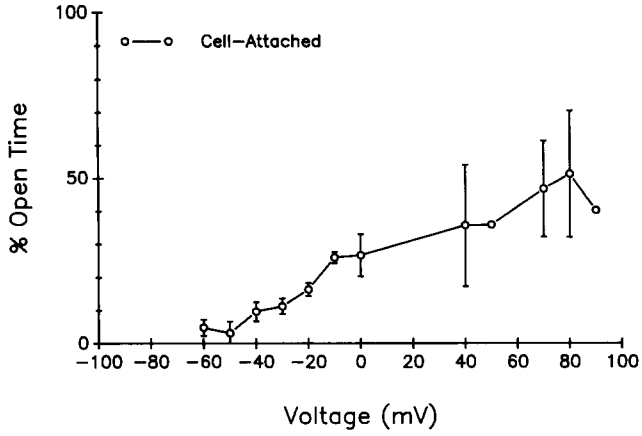

Fig. 5. The percent open time of the NSCC is plotted versus the membrane potential of the patch. For cell-attached patches (130 mM NaCl (Solution A) or 140 mM NaCl (Solution C) salines) the percent open time of the channel increased as the membrane potential was depolarized (open circles, $n = 7$).

M). Therefore, we investigated whether this NSCC possessed any sensitivity to amiloride. With amiloride (10^{-7} M) present in the patch pipette (130 mM NaCl saline, Solution A) in cell-attached experiments, we were unable to detect any effect of amiloride on channel kinetics (mean open or closed times) at either depolarizing or hyperpolarizing potentials (data not shown, $n = 2$).

Discussion

In this report, we describe a NSCC present in the apical membrane of the A6 toad kidney cells. The characteristics of this channel are as follows: (a) a linear *I-V* relationship with a single channel conductance of 21 pS; (b) a high selectivity of Na^+ to Cl^- ($> 45:1$); (c) a low selectivity of Na^+ over K^+ (1.5:1); (d) a single open state and two closed states of the channel; (e) the open-time constant and second closed-time constants are voltage dependent; and (f) insensitivity to amiloride, at least to 10^{-7} M. Similarities and differences exist between the channel characteristics described for this NSCC and other NSCCs from other cell types [17–28,31–34] (see Table II).

Role and function of the non-selective cation channel

Several investigators have demonstrated a physiological role of a NSCC. Maruyama and Petersen [21] suggested the NSCC, identified in pancreatic acinar cells, might play a role in the neural or hormonal control of membrane conductance which is responsible for the Ca^{2+}-dependent acinar fluid secretion. Recently, Frace and Gargus [31] have demonstrated the activation of a NSCC (mouse fibroblasts) by platelet-derived growth factor. These authors suggest the participation of this channel in a growth factor response. Moody and Bosma [34] have reported a NSCC activation by membrane deformation (i.e., stretch) in oocytes of the ascidian *Boltenia villosa.* They suggest this channel may play a vital role in the physiological response of cells during early embryonic development and cell cleavage. It appears NSCCs possess multiple functions in different cell types.

Role and function of the non-selective cation channel of A6 cells

The cortical collecting duct is the primary segment of the nephron where there is considerable hormonal control over the rate of reabsorption of Na^+ and, consequently, maintenance of Na^+ homeostasis [9,10,35].

TABLE II

Characteristics of non-selective cation channels from various cell types

Cell type	Number of states of the channel		Volt. dep.	Amil. sens.	Selectivity		Cond. (pS)	Ref.
	open	closed			Na^+/K^+	$+/-$ [a]		
A6 kidney cells	1	2	yes	no	1.5:1	>45:1 [b]	21	present
Rabbit proximal tubule	2	2	yes	– [c]	1:1	2:1 [b]	28	24
Rat insulinoma	2	2	yes	– [c]	1:1	– [c]	25	18
Mouse neuroblastoma	– [c]	– [c]	no	– [c]	1:1	+ ≫ – [d]	22	20
Rabbit urinary bladder	2	2	no	– [c]	1:1.6	12:1 [e]	20	33
Rat inner medullary collecting duct	– [c]	– [c]	no	yes	1:1	13:1 [b]	28	27
Mouse fibroblast	2	≥2	no	no	1:1	– [f]	28	31
Ascidian oocyte	≥2	2	– [c]	– [c]	1:1	– [c]	11 [g]	34

[a] Cation to anion selectivity.
[b] Na^+ to Cl^- selectivity.
[c] Not reported.
[d] No chloride conductance observed.
[e] K^+ to Cl^- selectivity.
[f] No anion conductance observed.
[g] Chord conductance at −100 mV.

However, the cortical collecting duct is also known to play a major role in K^+ balance [9,10,36]. Therefore, because the A6 cell line is a model of the cortical collecting duct [1–4], it is not surprising that both Na^+ and K^+ channels have been identified in the apical membrane of A6 cells [12–15]. How might the NSCC identified from the apical membrane of A6 cells participate in the overall Na^+ and K^+ transport of A6 epithelial tissue?

At present, the function of the NSCC of A6 cells is not well understood. However, evidence from other cell types (i.e., rabbit urinary bladder) and A6 cells suggests this NSCC may be a down regulated Na^+ channel and/or that this channel participates with the movement of Na^+ and K^+ across the apical membrane of A6 cells.

Lewis and co-workers [33,37,38] have suggested that a NSCC (leak channel) identified from the urinary bladder epithelium of the rabbit is a down-regulated Na^+ channel which has a low selectivity to cations (0.7 : 1, Na^+ to K^+) and is no longer amiloride-sensitive. The model proposed by Lewis and co-workers [33,37,38] describes a Na^+ channel turnover mechanism in which vesicles containing amiloride-sensitive Na^+ channels are inserted into the apical membrane of the bladder cell. These channels are subsequently degraded by serine proteinases (i.e., kallikrein) resulting as non-selective cation leak channels [37]. Zweifach and Lewis [33] have successfully incorporated this NSCC leak channel into a bilayer membrane, and they showed that this channel exhibits similar channel characteristics as a channel observed in the whole tissue preparation [37,38]. Recently, Lewis and co-workers [39,40] have documented the appearance of kallikrein in the mucosal solution of A6 cells grown to confluency on permeable supports. Therefore, one possible explanation for the NSCC described here in the apical membrane of A6 cells is that this NSCC is a degraded form of the low-conductance amiloride-sensitive Na^+ channel which has been previously reported for A6 cells [12–14]. However, the possibility remains that the NSCC reported here is a completely independent protein from the channel protein described by Lewis and co-workers [33,37–40].

Recently, Thomas and Mintz [7] have demonstrated a time-dependence of ion selectivity of the apical membrane of A6 cells. They showed, with microelectrodes, that K^+ channels dominated the apical membrane conductance of non-confluent A6 cells. Furthermore, Thomas and Mintz [7] demonstrated that the apical membrane of A6 cells undergoes a transition from being K^+ to Na^+ selective as the cell layer becomes confluent (approx. 10 days). When the apical membrane is selective for Na^+, the resting potential across the apical membrane was determined to be -51 mV [7]. If we assume such a resting membrane potential for A6 cells in the present study, then the NSCC would mediate a small absorption of Na^+ due to the large electrochemical gradient for Na^+ entry into the cells (Fig. 1). Perkins and Handler [2] have reported in A6 cells that amiloride (10^{-4} M) blocked 93% of the short-circuit current, therefore, the remaining short-circuit current could result from Na^+ moving through the NSCC.

Membrane potentials of -60 to $+30$ mV have been recorded for A6 cells (Thomas, S.R., personal communication). Likewise, outward currents were observed when cell-attached patches were depolarized by $+60$ mV ($+10$ mV membrane potential if the resting potential was -50 mV) (Fig. 1). Under such depolarized conditions (though not a typical physiological state), K^+ secretion could occur [26]. Therefore, the NSCC could play a role in both Na^+ and K^+ transport in response to changes in apical membrane potential created by varying ionic concentrations and correlated with the total physiological state of the epithelium.

Thus, we describe a NSCC which is present in the apical membrane of A6 cells. This channel is highly selective for cations over anions, but has a low selectivity between cations. The NSCC is responsible for the movement of Na^+ and K^+ across the apical membrane of A6 cells. At the present time, the function of the NSCC is not well established, however, additional experiments are necessary to confirm the function of the NSCC of the apical membrane of A6 kidney cells.

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