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Calcium-Sensitive Nonselective Cation Channel Identified in the Epithelial Cells Isolated from the Endolymphatic Sac of Guinea Pigs

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Abstract. We identified a Ca^{2+}-sensitive cation channel in acutely dissociated epithelial cells from the endolymphatic sac (ES) of guinea pigs using the patch-clamp technique. Single-channel recordings showed that the cation channel had a conductance of 24.0 ± 1.3 pS (n = 8) in our standard solution. The relative ionic permeability of the channel was in the order $K^+ = Na^+ > Ca^{2+} \gg Cl^-$. This channel was weakly voltage-dependent but was strongly activated by Ca^{2+} on the cytosolic side at a concentration of around 1 mM in inside-out excised patches. With cell-attached patches, however, the channel was activated by much lower Ca^{2+} concentrations. Treatment of the cells, under cell-attached configuration, with ionomycin (10 μM), carbonyl cyanide 3-chlorophenylhydrazone (CCCP, 20 μM), or ATP (1 mM), which increased intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$), activated the channel at an estimated $[Ca^{2+}]_i$ from 0.6 μM to 10 μM. It is suggested that some activators of the channel were deteriorated or washed out during the formation of excised patches. Based on this Ca^{2+} sensitivity, we speculated that the channel contributes to the regulation of ionic balance and volume of the ES by absorbing Na^+ under certain pathological conditions that will increase $[Ca^{2+}]_i$. This is the first report of single-channel recordings in endolymphatic sac epithelial cells.

Key words: Nonselective cation channel — Calcium-sensitive channel — Endolymphatic sac — Patch-clamp — Inner ear — Fura-2

Introduction

The endolymphatic sac (ES) epithelium of the mammalian inner ear is believed to absorb endolymphatic fluid produced by marginal cells of the stria vascularis in the cochlea and by the dark cells in the vestibular organ. Morphologically, it has been demonstrated that endolymphatic hydrops was observed by surgical ablation of the endolymphatic duct and sac in mammalian inner ear (Kimura & Shuknecht, 1965; Lundquist, 1965; Bagger-Sjöbäck et al., 1987; Sziklai et al., 1992). Recent electrophysiological studies have revealed various aspects of ion transport in ES epithelial cells. Studies using the whole-cell patch-clamp technique demonstrated the presence of an outward rectified K^+ channel (Wu & Mori, 1996), an amiloride-sensitive Na^+ channel (Mori & Wu, 1996) and an ATP-activated nonselective cation channel (Wu & Mori, 1999). Expression of these channels in ES epithelial cells suggests that the cells regulate ion concentration and volume of endolymph. Regulation of endolymph volume is important for the function of the inner ear, since excess of the endolymph volume induces an enlargement of the endolymphatic compartment called endolymphatic hydrops. Endolymphatic hydrops is thought to induce disorder of inner ear functions resulting in Ménière's disease (Hallpike & Cairns, 1938; Yamakawa, 1938; Kimura & Shuknecht, 1965).

Nonselective cation channels have been identified in the inner ear (Marcus, Takeuchi & Wangemann, 1992; Takeuchi, Marcus & Wangemann, 1992; Sunose et al., 1993; Van den Abbeele, Tran & Teulon, 1994; Yeh et al., 1998) and in a large variety of cells (Gögelein & Greger, 1986; Tatsumi & Katayama, 1994; Korbmacher et al., 1995; Kanzaki et al., 1999). They are permeable to monovalent cations and/or divalent cations (Gustin et al., 1988; Bear, 1990; Filipovic & Sackin, 1991). In the renal epithelia, nonselective cation channels are activated by intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) increase and play a role in sodium reabsorption, calcium entry, cell volume regulation and cell proliferation (Hescheler & Schultz, 1993). Therefore, if nonselective cation chan-

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nels in the ES exist, it would be important to understand the regulation of ionic balance in the ES. Actually, recent electrophysiological studies of ES epithelial cells suggest that extracellular ATP induces an increase in ($[Ca^{2+}]_i$) (Arishige, Takumida & Yajin, 1998), which may in turn increase nonselective cation currents (Wu & Mori, 1999). However, no single-channel analysis of the nonselective cation channel has been done in ES epithelial cells. In the present study, we first identified a Ca^{2+}-sensitive nonselective cation channel in ES epithelial cells, and its electrophysiological properties were directly examined at the single-channel level in cell-attached or excised inside-out patches.

Materials and Methods

Epithelial Cell Preparation

Healthy albino guinea pigs (250–350 g weight, Preyer reflex-positive) of either sex were anaesthetized by inhalation of diethyl ether and decapitated. The temporal bones were quickly removed and placed in oxygenated physiological saline after decapitation. The bony shell between the ES and the endolymphatic duct was peeled off carefully with a microeletric drill, and then the ES and the endolymphatic duct were picked up with fine forceps with the aid of a stereoscopic microscope and kept in physiological saline containing (in mM) 140 NaCl, 5.4 KCl, 1.13 $CaCl_2$, 1.2 $MgCl_2$, 10 *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulphonic acid (HEPES) and 5 glucose (pH = 7.4). Thereafter, the intermediate portion of the ES was separated by cutting the endolymphatic duct and was treated with papain (Worthington Biochemical, Lakewood, NJ; 24 units/ml) in low-Ca^{2+} and low-Mg^{2+} Krebs solution at 37°C for 15 min. The low-Ca^{2+} and low-Mg^{2+} Krebs solution was made by adding 2.5 mM ethylenediaminetetraacetic acid (EDTA) to Krebs solution [in mM: 117 NaCl; 4.7 KCl; 2.5 $CaCl_2$; 1.0 $MgCl_2$; 11 glucose; 25 3-[N-Morpholino] propanesulfonic acid (MOPS); pH = 7.4 (adjusted with NaOH)]. The ES was then transferred to the physiological saline and triturated using pipettes. The resulting dissociated epithelial cells were kept at room temperature (21–24°C) and were used within 2 hr after isolation.

The epithelial cells of the guinea pig are classified into two types according to ultrastructural observation (Fukazawa, Matsunaga & Fujita, 1990). However, as we could not identify the cell types in the dissociated cell preparations with our phase contrast/differential interference microscope, both types of cells were included in this study.

This research was approved by the Animal Care and Use Committee of Nagoya University and Kagawa Medical University.

Single-Channel Recording and Analysis

Patch electrodes were fabricated from glass capillaries (Drummond Scientific, Broomall, PA) with a vertical electrode puller (PP-83, Narishige, Tokyo, Japan) and heat polished with a microforge (MF-83, Narishige, Japan). Pipettes were mounted on a micromanipulator and connected to the probe of a patch-clamp amplifier (model 200B, Axon Instruments, Union City, CA). Most recordings were done with cell-attached and excised inside-out patches. All experiments were conducted at room temperature. Records of the currents were stored on VHS videotapes by means of a PCM recorder (VR-10B, Instrutech, Port Washington, NY). The stored data were replayed through a four-pole Bessel filter (cut-off at 1 kHz) and analyzed using a software (pCLAMP 8, Axon Instruments). Ion permeability ratios were calculated by best-fitting of the current equation of Goldman-Hodgkin-Katz to the *I-V* curve at a given condition using the software Microcal Origin 5.0 (Microcal Software, Northampton, MA). Permeability ratios were presented as means ± fitting error. In inside-out patches, membrane potential was defined as the negative value of the pipette potential; upward current deflections in figures represent cation movement from the cytoplasmic side to the external side of the membrane and vice versa for anions. In cell-attached patches, chemicals were applied by ejection from a glass pipette to the cell positioned in the stream (*see* Fig. 5A).

Ca^{2+} Measurements

Intracellular Ca^{2+} concentrations in ES epithelial cells were monitored by the Fura-2 ratiomety according to the method described previously (Grynkiewicz, Poenie & Tsien, 1985). The cells were incubated in the extracellular solution with 4 μM Fura-2 acetoxymethyl ester (AM) for 15 min at 37°C. Epifluorescent observations were carried out with an inverted microscope (IX 70, Olympus, Tokyo, Japan). The preparations were intermittently exposed to the excitation light; exposure time and excitation wavelength of 340 and 380 nm were controlled by a MetaFluor system (Universal Imaging, Downington, PA). Images of emission fluorescence of Fura-2 were captured with a cooled-CCD camera (Micromax, Princeton Instruments, Trenton, NJ) through a fluorescent objective lens (Olympus UPlan Fl ×60, Olympus, Tokyo, Japan) with an optical filter (cut-off at 420 nm). Each captured image at 340 nm was divided by that at 380 nm to get a ratio image (F_{340}/F_{380}) with the MetaFluor imaging system. The average values (F_{340}/F_{380}) from individual cells were measured during the experiment. Following the fluorescence measurements, $[Ca^{2+}]_i$ was calibrated in vitro after permeabilizing the cell membrane with 5 μM ionomycin at various extracellular Ca^{2+} concentrations as described in a previous report (Soeda, Tatsumi & Katayama, 1997).

Solutions

The standard pipette solution (standard extracellular solution) contained (in mM) 140 NaCl, 5.4 KCl, 1.13 $CaCl_2$, 1.2 $MgCl_2$, 10 HEPES and 5 glucose adjusted to pH 7.40 with NaOH. The standard internal solution (in the bath) facing the cytoplasmic surface of the patch consisted of (in mM) 5.4 NaCl, 140 KCl, 1.13 $CaCl_2$, 1.2 $MgCl_2$, 10 HEPES and 5 glucose adjusted to pH 7.4 with KOH. NMDG solution was made by replacing cations (Na^+, K^+, Ca^{2+} and Mg^{2+}) with NMDG adjusted to pH 7.4 with HCl. The Ca^{2+} concentrations of low-Ca^{2+} solutions (<10 μM) was adjusted with EGTA using the program EqCal (Biosoft, Ferguson, MO) with stability constants (Owen, 1976).

Results

Ion Channels in the ES

Single-channel recordings were performed in excised inside-out or cell-attached patches of the epithelial cells from the endolymphatic sac. Two types of single-channel currents were recorded in inside-out patches, while scarcely any could be observed (<1 event/min) in cell-attached patches (Fig. 1*A,B*). We first characterized the channels in inside-out patches on the basis of their conductances and permeability ratio of Na^+ to K^+ determined from current-voltage (*I-V*) relations under two different ionic conditions: 1) (in mM) 140 NaCl, 5.4 KCl, 1.13 $CaCl_2$, 1.2 $MgCl_2$, 5 D-glucose, 10 HEPES in the

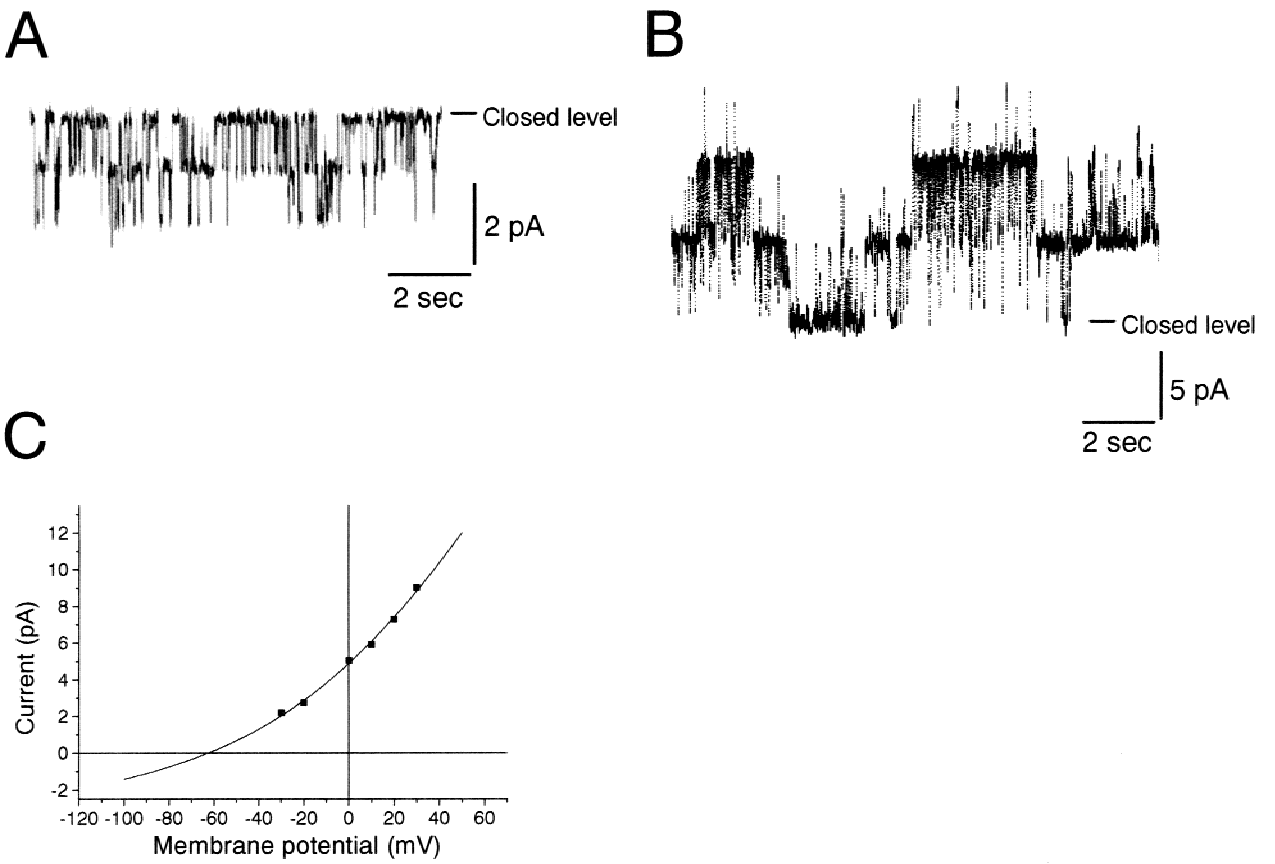

Fig. 1. Typical records of single-channel currents of a nonselective cation channel and K^+ channel. (*A*) 24 pS nonselective cation channel at −50 mV. (*B*) 150 pS K^+ channel at +10 mV. Both traces were obtained from excised inside-out patches with standard extracellular solution in the pipette and standard internal solution in the bath. (*C*) Current-voltage relationship of the K^+ channel in inside-out patches with standard solution. Curve is drawn according to the Goldman-Hodgkin-Katz current equation.

pipette and the bath, or 2) the above solution in the pipette and (in mM) 140 KCl, 5.4 NaCl, 1.13 $CaCl_2$, 1.2 $MgCl_2$, 5 D-glucose, 10 HEPES in the bath. These experiments showed that one channel was a 24 pS cation channel with a permeability ratio of Na^+ to K^+ (P_{Na}/P_K) of 0.94, and the other, a 150 pS K^+ channel with a P_{Na}/P_K of 0.05 (Fig. 1*C*). Single-channel activity of the former nonselective cation channel was observed in 301 out of 1887 patches while the K^+-selective channel was observed only in 25 out of 1887 patches. Because the 24 pS cation channel predominated in the majority of patches examined, we focused on and analyzed this channel.

Ion Selectivity

The ion selectivity of the cation channel was estimated from its *I-V* curves obtained in inside-out patches exposing the cytoplasmic surface of the membrane to various solutions. Figure 2*A* shows single-channel activities of the cation channel in an excised inside-out patch in a standard solution (*see* Methods) at various membrane potentials. Figure 2*B* shows *I-V* relationships of the channel obtained under various ionic conditions. With the standard solution ($n = 8$), a nearly linear *I-V* relationship crossing at near 0 mV with a mean slope conductance of 24 ± 1.3 pS was obtained (Fig. 2*B*, -▲-). All the *I-V* curves in Fig. 2*B* were well fitted by the Goldman-Hodgkin-Katz (GHK) current equation, and the permeability ratios, P_{Na}/P_K and P_{Cl}/P_K, were calculated as 0.94 ± 0.02 ($n = 5$) and 0.13 ± 0.01 ($n = 5$), respectively. Similarly, P_{Ca}/P_K was calculated using the equation, $P_{Ca}/P_K = [K^+]_i\{1+\exp(-E_{rev}F/RT)\}/\{4[Ca^{2+}]_o\exp(-2E_{rev}F/RT)\}$, where E_{rev} denotes the reversal potential; *F*, Faraday's constant; *R*, the gas constant; *T*, absolute temperature (°K); $[K^+]_i$, the bath concentration of K^+; and $[Ca^{2+}]_o$, the concentration of Ca^{2+} in the pipette. P_{Ca}/P_K was 0.49 ± 0.09 ($n = 5$) with 72.5 mM $CaCl_2$ in the pipette and 145 mM KCl in the bath. With this high Ca^{2+} concentration in the pipette, the slope conductance in the positive membrane potential region was smaller than that with standard solution, suggesting a channel blockade by Ca^{2+}. However, as we could observe no apparent increase in the open-channel

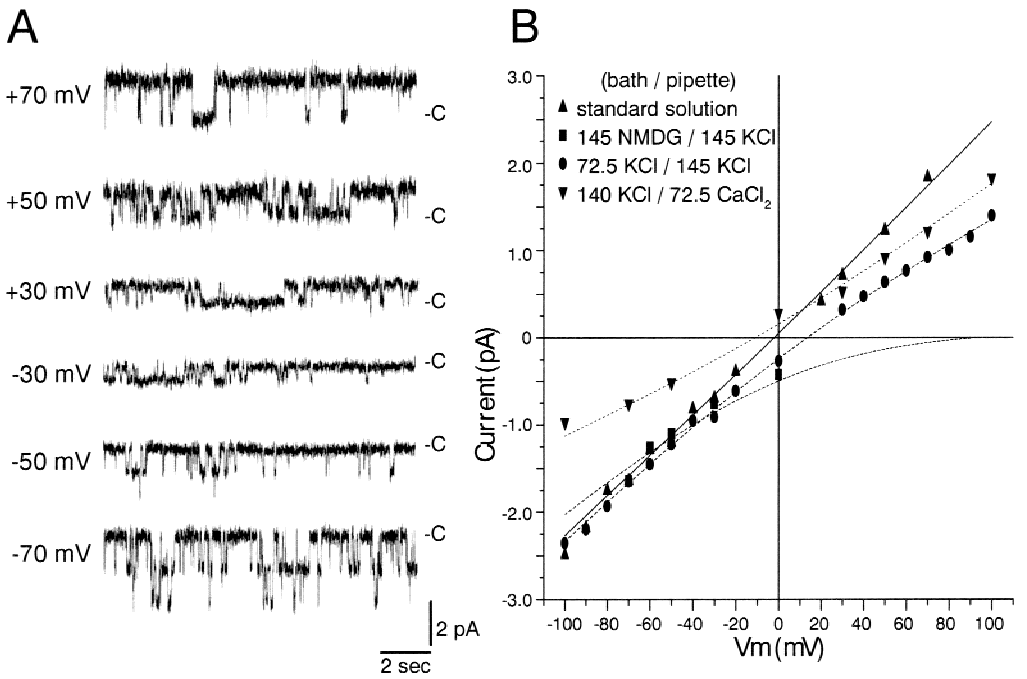

Fig. 2. Current-voltage relationships of the nonselective cation channel. (*A*) single-channel activities in an excised inside-out patch in standard solution at various membrane potentials. (*B*) current-voltage (*I-V*) relationships for nonselective cation channels in inside-out patches with standard solution (▲), 145 mM KCl in pipette and 145 mM NMDG in bath (■), 145 mM KCl in pipette and 72.5 mM KCl in bath (●), and 72.5 mM $CaCl_2$ in pipette and 145 mM KCl in bath (▼). Each data point represents an average value from 5 patches. Curves are drawn according to the Goldman-Hodgkin-Katz current equation.

noise, the possibility of the channel blockade by Ca^{2+} is an open question at present. These results demonstrate that the cation channel was nonselective for mono- and di-valent cations.

Calcium- and Voltage-Dependencies

The effect of cytoplasmic-side Ca^{2+} concentration on the channel activity was studied by exposing the cytoplasmic side of the patch to solutions with various Ca^{2+} concentrations ($[Ca^{2+}]_b$). Figure 3*A* demonstrates Ca^{2+} activation of the nonselective cation channel in an inside-out patch with various $[Ca^{2+}]_b$ at a membrane potential of −50 mV in a standard solution. Although the channel-open probability P_o was extremely low (0.003) at 0.1 μM $[Ca^{2+}]_b$, it increased with $[Ca^{2+}]_b$ (Fig. 3*B*). Activation of the nonselective cation channel required more than 1 μM $[Ca^{2+}]_b$ and the majority of the channels were strongly activated at $[Ca^{2+}]_b$ greater than 1 mM. These data were well fitted by two Hill equations with a low dissociation constant (K_d = 6.6 μM) and a low Hill coefficient (0.74), and with a higher K_d (1.3 mM) and a higher Hill coefficient (1.13) (Fig. 3*B*, inset). These results suggest that the channel has either high- and low-affinity Ca^{2+} binding sites or two types of channels with the same conductance but with different Ca^{2+} sensitivities.

The nonselective cation channel showed voltage dependence. Figure 3*C* shows the mean open probability P_o of the nonselective cation channel against various membrane potentials in a standard solution. As the membrane potential increased, so did P_o (the correlation coefficient was 0.66).

Effect of Flufenamic Acid on the Nonselective Cation Channel

The nonsteroidal anti-inflammatory drug, flufenamic acid, is a potent blocker of certain types of nonselective cation channels (Gögelein et al., 1990). Therefore we tested the effect of flufenamic acid on our nonselective cation channel. Figure 4*A* shows a current trace of the nonselective cation channels recorded in an inside-out patch at −50 mV. Flufenamic acid (100 μM) reversibly inhibited the activity (P_o) of the nonselective cation channel without changing its conductance. Figure 4*B* demonstrates the dose-dependent P_o inhibition of the nonselective cation channel by flufenamic acid, indicating that the half maximum inhibition occurred at 4.0 μM.

Ca^{2+} Dependency in the Cell-attached Configuration

The effect of intracellular Ca^{2+} on the channel activity was studied with cell-attached patches that are physi-

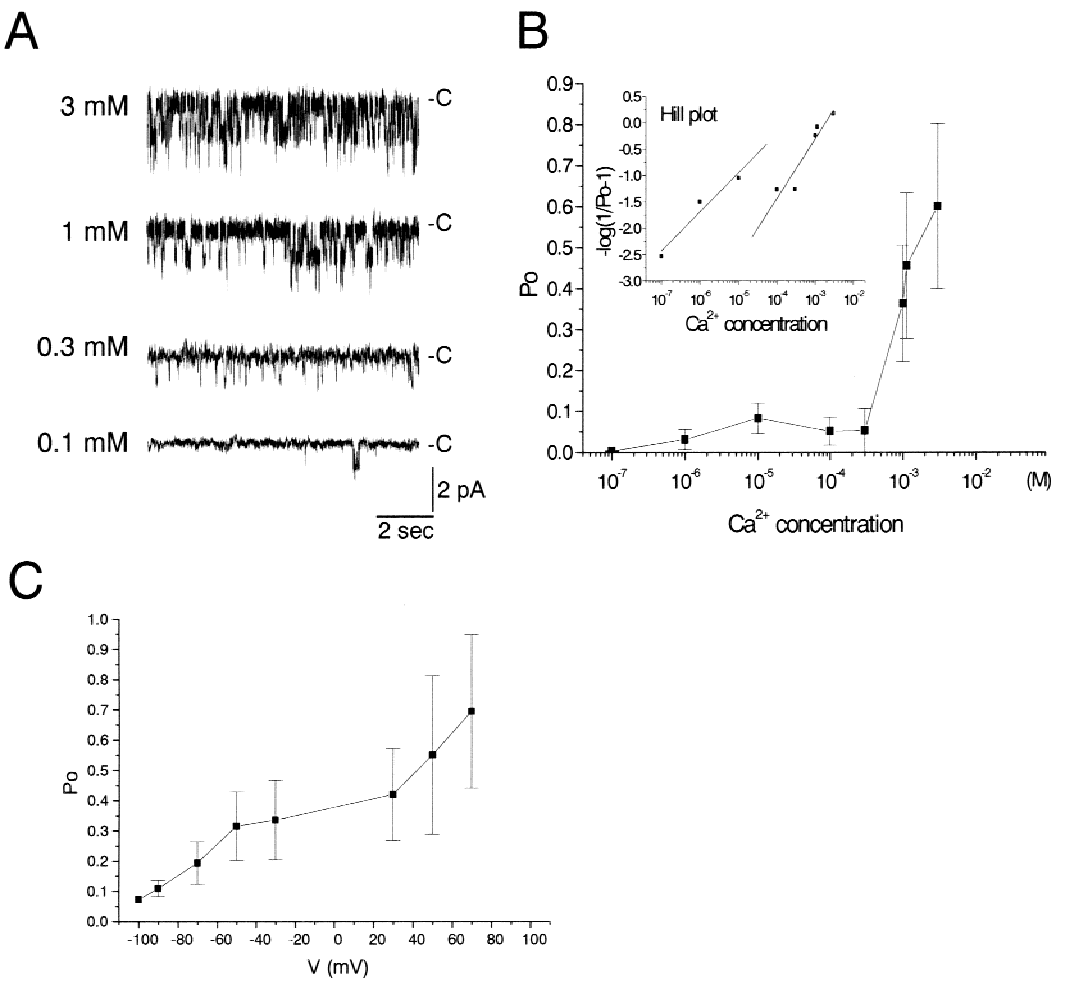

Fig. 3. Calcium- and voltage-dependency of the nonselective cation channel. (*A*) Single-channel current traces in an inside-out patch at various Ca^{2+} concentrations on the cytosolic side and at −50 mV. P_o of the channel increases with Ca^{2+} concentration. (*B*) Relationship between P_o and Ca^{2+} concentration. *Inset:* a Hill plot of open-probability P_o at −50 mV. The data points were well fitted by two separate lines with a high affinity and low cooperativity (K_d = 6.6 μM, Hill coefficient = 0.74), and a low affinity and moderate cooperativity (K_d = 1.3 mM, Hill coefficient = 1.13). (*C*) Voltage dependency of P_o of the nonselective cation channel. Each point represents the mean ± SEM from 5 experiments.

ologically more intact preparations than excised patches. To control $[Ca^{2+}]_i$, we used the Ca^{2+} ionophore ionomycin and measured $[Ca^{2+}]_i$ by the standard fura-2 method. A solution containing 10 μM ionomycin was puffed through the glass pipette to the cells. Figure 5*A* demonstrates the activation of the nonselective cation channels by 10 μM ionomycin, and Figure 5*B* shows the time course of the P_o-increase during exposure to the solution containing ionomycin. The average P_o gradually increased and reached a value of 0.38 ± 0.36 at 170 sec after the onset of ionomycin application. It should be noted that the activation was maintained during drug application.

Upon ionomycin application, $[Ca^{2+}]_i$ in the epithelial cells started to increase from 0.119 ± 0.128 μM and reached a plateau of 13 ± 8.0 μM at 100 sec (n = 5, Fig. 5*D*). Comparing the time courses of P_o (Fig. 5*C*) and $[Ca^{2+}]_i$ (Fig. 5*D*), the $[Ca^{2+}]_i$ that started to activate the nonselective cation channels was estimated to be 3.6 ± 1.2 μM (n = 5).

The effect of intracellular Ca^{2+} on the channel activity was also studied using CCCP (20 μM), an uncoupler of oxidative phosphorylation, which is known to increase $[Ca^{2+}]_i$ (Fig. 5*Bb*). As expected, CCCP increased both P_o (up to 0.45 ± 0.20, n = 3) and $[Ca^{2+}]_i$ (up to 4.2 ± 7.9 μM, n = 5) with an estimated channel activation $[Ca^{2+}]_i$ of 0.60 ± 0.34 μM (n = 5). CCCP may change the intracellular pH in addition to $[Ca^{2+}]_i$ increase, since it is known as an H^+ ionophore. This pH change might modulate the channel-open probability but we did not make a rigorous check for this possibility.

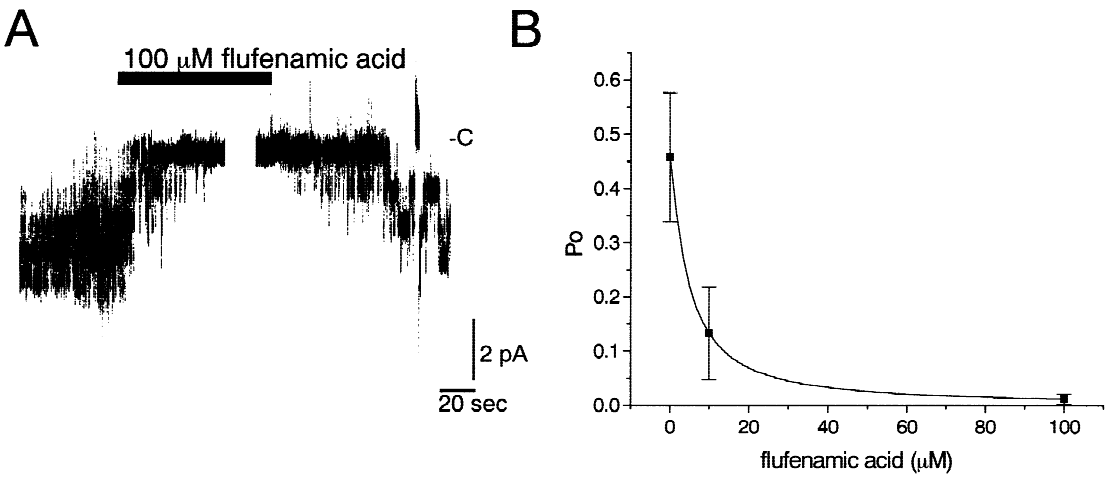

Fig. 4. Effect of flufenamic acid upon nonselective cation channel activity. (*A*) Inhibition of channel activity by flufenamic acid. Flufenamic acid was applied to the cytoplasmic surface of inside-out patches. The horizontal bar indicates the period of drug application. Channel activity is inhibited by the drug without significant reduction in the amplitude of single channel current. (*B*) Dose-dependent inhibition of the channel activity (P_o) by flufenamic acid ($n = 5$).

Activation of the Nonselective Cation Channel by ATP

It was reported that extracellular ATP evoked nonselective cation channel currents that were thought to be mediated by the increased $[Ca^{2+}]_i$ through the activation of P_{2Y} purinoreceptors (Wu & Mori, 1999). We were interested in whether the nonselective cation channel identified in this study is sensitive to ATP. To test this possibility we applied ATP to the cell through the glass pipette which contained 1 mM ATP, while cell-attached patch-recording was made. As shown in Fig. 6*A* and *B*, the average P_o of the nonselective cation channels was transiently increased and peaked (0.53 ± 0.27) 25 sec after an application of ATP. Following these experiments, we excised the patch from the cell and examined the conductance (24 pS) and ion selectivity ($P_{Na^+}/P_{K^+} \simeq 1$) of these channels to confirm that the channels were the same type as those previously identified by excised-patch recordings.

Interestingly, ATP transiently increased the $[Ca^{2+}]_i$ up to 10 ± 4.1 μM with a time course similar to that of P_o ($n = 5$, Fig. 6*B*). The close correlation of the time courses of P_o and $[Ca^{2+}]_i$ changes strongly suggests that the ATP-dependent P_o increase is mediated by $[Ca^{2+}]_i$ increase. The ATP-induced $[Ca^{2+}]_i$ increase exceeded quickly the threshold level (several μM) for channel opening. This might cause a coinciding increase in the channel opening and $[Ca^{2+}]_i$.

Discussion

The present study demonstrated with the patch-clamp technique that a 24 pS nonselective cation channel is expressed in the ES epithelia of guinea pig. The main electrophysiological characteristics of the channel in the ES epithelia are its Ca^{2+}-sensitivity, voltage-dependency, blockade by flufenamic acid, permeability sequence ($K^+ = Na^+ > Ca^{2+} \gg Cl^-$), and single-channel conductance of 24 pS.

Ca^{2+}-sensitivity of the majority of nonselective cation channels in inside-out patches was relatively low; the channels were activated at around 1 mM $[Ca^{2+}]_b$. A nonselective cation channel with a similar low sensitivity to $[Ca^{2+}]_b$ was reported in rat thyroid follicular cells (Maruyama, Moore & Petersen, 1985), rat and mouse salivary endpiece cells (Maruyama, Gallacher & Petersen, 1983), whereas channels with higher Ca^{2+} sensitivity (activated at <1 μM) were found in gerbil outer sulcus epithelial cells (Chiba & Marcus, 2000).

In cell-attached patches, however, the nonselective cation channel was activated by much lower $[Ca^{2+}]_i$ levels that were created by drug treatments (3.6 μM by ionomycin, 0.60 μM by CCCP or <10 μM by ATP, respectively). The discrepancy of the $[Ca^{2+}]_i$-sensitivity of the channel between inside-out patches and cell-attached patches might be explained by washout of putative intracellular activators of the channel during the formation of inside-out patches. Several intracellular activators for nonselective cation channels are reported: multifunctional Ca^{2+} calmodulin-dependent protein kinase in human T84 epithelial cells (Braun & Schulman, 1995) and cyclic AMP-dependent phosphorylation in renal epithelial (A6) cells (Marunaka et al., 1997).

The gating of the nonselective cation channel showed weak voltage-dependence. A similar voltage-dependency of channel-open probability was reported for nonselective cation channels in rabbit proximal tubules (Gögelein & Greger, 1986) and human nasal epithelial

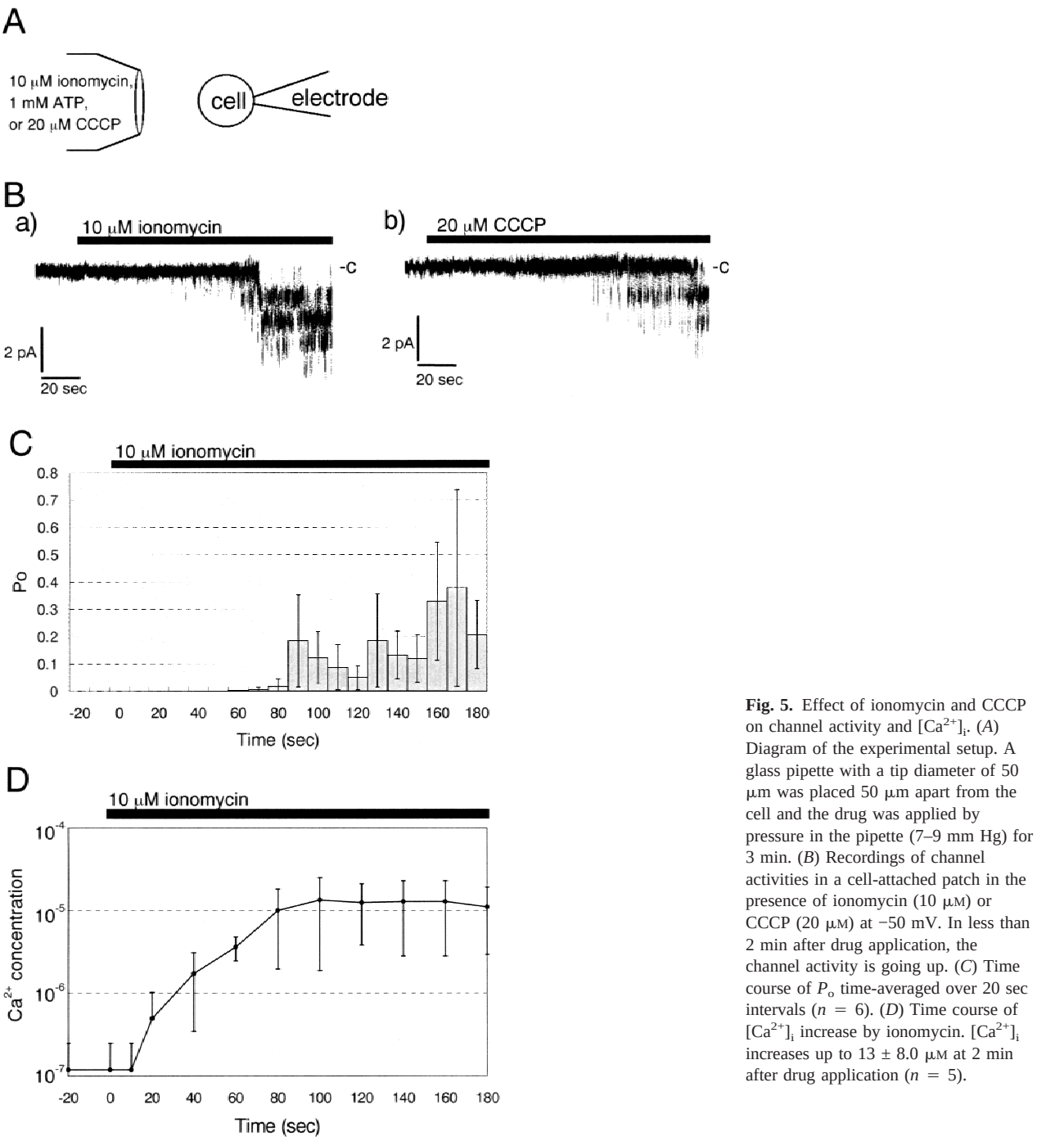

Fig. 5. Effect of ionomycin and CCCP on channel activity and $[Ca^{2+}]_i$. (*A*) Diagram of the experimental setup. A glass pipette with a tip diameter of 50 μm was placed 50 μm apart from the cell and the drug was applied by pressure in the pipette (7–9 mm Hg) for 3 min. (*B*) Recordings of channel activities in a cell-attached patch in the presence of ionomycin (10 μM) or CCCP (20 μM) at −50 mV. In less than 2 min after drug application, the channel activity is going up. (*C*) Time course of P_o time-averaged over 20 sec intervals ($n = 6$). (*D*) Time course of $[Ca^{2+}]_i$ increase by ionomycin. $[Ca^{2+}]_i$ increases up to 13 ± 8.0 μM at 2 min after drug application ($n = 5$).

cells (Popp & Gögelein, 1992). In contrast, voltage-insensitive nonselective cation channels were observed in rat neurons from basal nucleus (Tatsumi & Katayama, 1994) and vestibular dark cells (Marcus et al., 1992).

In the present study, 100 μM flufenamic acid completely blocked the nonselective cation channel in the ES, similar to the nonselective cation channel in exocrine pancreas (Gögelein et al., 1990). On the other hand, 100 μM flufenamic acid partially blocks the nonselective cation channel in outer hair cells (Van den Abbeele et al., 1994) and in outer sulcus epithelial cells (Chiba & Marcus, 2000).

The relative ionic permeability of the channel falls in the sequence, $K^+ = Na^+ > Ca^{2+} \gg Cl^-$, indicating that the nonselective cation channel is permeable to both monovalent and divalent cations. This order of ionic permeability of the channel agrees with the previous whole-cell patch-clamp study (Wu & Mori, 1999) except for the Ca^{2+} permeability. The relative permeability of Ca^{2+} with respect to K^+ in the present study ($P_{Ca}/P_K =$

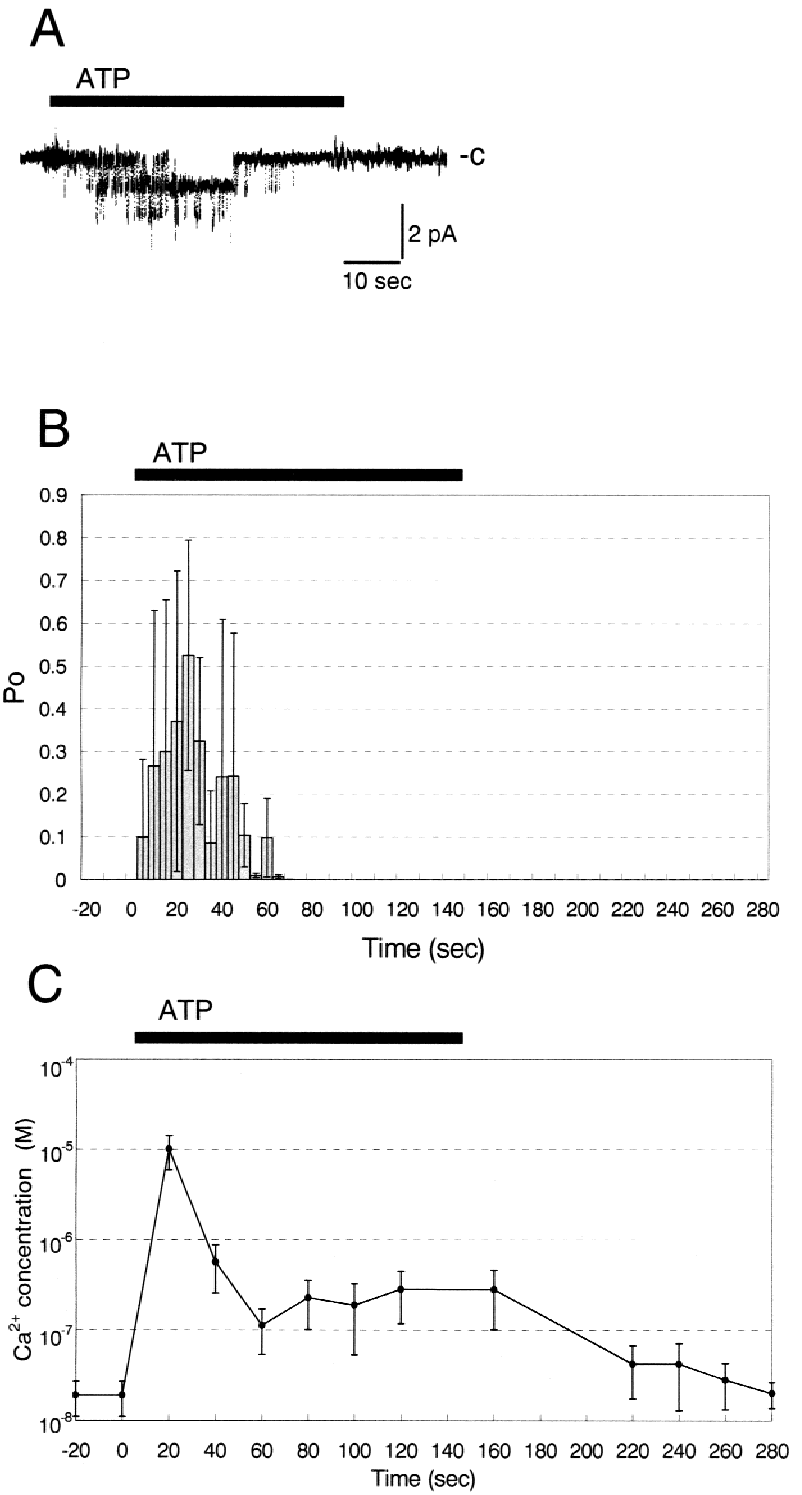

Fig. 6. Activation of the nonselective cation channel by ATP. (*A*) Single-channel current recording obtained from a cell-attached patch in response to ATP at the holding potential of −50 mV. Pipette and bath contained the standard extracellular solution. ATP transiently increased channel activity. (*B*) Time course of the channel activation by ATP ($n = 5$). The channel activity increases rapidly in response to ATP application and peaks at 20 sec after drug application. (*C*) Time course of $[Ca^{2+}]_i$ increase by ATP. $[Ca^{2+}]_i$ increases up to 10 ± 4.1 μM at 20 sec ($n = 5$) after ATP application.

0.49) is much smaller than that ($P_{Ca}/P_K = 3.24$) in a previous report examined by whole-cell patch-clamp configuration (Wu & Mori, 1999). One explanation for this discrepancy may be a contribution to the whole-cell currents of another nonselective cation channel with a small conductance below the resolving power of the single-channel recording system in this study; such a small channel is reported in proximal tubule cells (conductance value, $P_{Ca}/P_{Na} = 4.49$) (Robson & Hunter, 2000).

Nonselective cation channels may play a role in sodium reabsorption from lumen into cells in rabbit cortical collecting tubule (O'Neil & Boulpaep, 1982; Ling, Hinton & Eaton, 1991) and rat inner medullary collecting duct in the kidney (Light et al., 1988), and may play a role in cell volume regulation in the renal epithelia (Gögelein & Greger, 1986; Merot et al., 1988; Filipovic & Sackin, 1991, 1992). The endolymph in the ES contained (in mM) 103.3 Na^+, 11.6 K^+, 0.47 Ca^{2+}, 85.0 Cl^- (Amano, Orsulakova & Morgenstern, 1983; Ninoyu & Morgenstern, 1986; Mori, Ninoyu & Morgenstern, 1987) and the ES epithelial cells have about −60 mV resting membrane potential (Wu & Mori, 1999). In the endolymph of the ES, +15 to +20 mV potential has been recorded (Amano et al., 1983; Mori, Uozumi & Sakai, 1990). Additionally, localization of Na^+-K^+-ATPase on the basolateral side has been demonstrated in the ES epithelial cells (Mizukoshi et al., 1988). Thus, there is an electrical driving force for the entry of Na^+ and Ca^{2+} from the lumen to the intracellular side of the ES epithelial cell. The electrical driving force for K^+ is also from lumen to cells, but it is much smaller than that of Na^+ and Ca^{2+}. Thus, the activation of the nonselective cation channel may mainly induce Na^+ absorption, $[Ca^{2+}]_i$ increase and depolarization in ES epithelial cells. The latter two factors, $[Ca^{2+}]_i$ increase and depolarization, may further activate the channel, forming a self-activation circuit for the nonselective cation channel.

It is known that ATP plays an important role as an extracellular messenger in many cell types. In the renal glomerulus, it is reported that ATP is released from macula densa cells in response to high Na^+ concentration in urine, and the released ATP activates nonselective cation channels to reabsorb Na^+ (Bell et al., 2000). In the inner ear, though ATP secretion is not clarified, the presence of ATP in the endolymph is reported (Munoz et al., 1995). As the P_{2Y} receptor has been demonstrated in the ES epithelial cells (Wu & Mori, 1999), it is possible that extracellular ATP regulates the ion transport of the ES epithelial cell by activating the nonselective cation channel. The nonselective cation channel in the ES cells may also participate in absorbing Na^+ and Ca^{2+} under pathological conditions such as anoxia, which would increase $[Ca^{2+}]_i$ of the ES cells.

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