Permeation and Gating Properties of the Novel Epithelial Ca\textsuperscript{2+} Channel*  

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The recently cloned epithelial Ca\textsuperscript{2+} channel (ECaC) constitutes the Ca\textsuperscript{2+} influx pathway in 1,25-dihydroxyvitamin D\textsubscript{3}-responsive epithelia. We have combined patch-clamp analysis and fura-2 fluorescence microscopy to functionally characterize ECaC heterologously expressed in HEK293 cells. The intracellular Ca\textsuperscript{2+} concentration in ECaC-expressing cells was closely correlated with the applied electrochemical Ca\textsuperscript{2+} gradient, demonstrating the distinctive Ca\textsuperscript{2+} permeability and constitutive activation of ECaC. Cells dialyzed with 10 mM 1,2-bis(2-aminophenoxy)ethane-N\textsubscript{2}N\textsubscript{2}N\textsubscript{2}N\textsuperscript{-}tetraacetic acid displayed large inward currents through ECaC in response to voltage ramps. The corresponding current-voltage relationship showed pronounced inward rectification. Currents evoked by voltage steps to potentials below −40 mV partially inactivated with a biexponential time course. This inactivation was less pronounced if Ba\textsuperscript{2+} or Sr\textsuperscript{2+} replaced Ca\textsuperscript{2+} and was absent in Ca\textsuperscript{2+}-free solutions. ECaC showed an anomalous mole fraction behavior. The permeability ratio \(P_{Ca}/P_{Na}\) calculated from the reversal potential at 30 mM [Ca\textsuperscript{2+}]\textsubscript{i} was larger than 100. The divalent cation selectivity profile is Ca\textsuperscript{2+} > Mn\textsuperscript{2+} > Ba\textsuperscript{2+} > Sr\textsuperscript{2+}. Repetitive stimulation of ECaC-expressing cells induced a decay of the current response, which was greatly reduced if Ca\textsuperscript{2+} was replaced by Ba\textsuperscript{2+} and was virtually abolished if [Ca\textsuperscript{2+}]\textsubscript{i} was lowered to 1 mM. In conclusion, ECaC is a Ca\textsuperscript{2+} selective channel, exhibiting Ca\textsuperscript{2+}-dependent autoregulatory mechanisms, including fast inactivation and slow down-regulation.

The gastrointestinal tract and kidney determine the net intake and output of Ca\textsuperscript{2+} for the entire body and, thereby, maintain together with bone the overall Ca\textsuperscript{2+} balance (1). The most important underlying mechanism is 1,25-dihydroxyvitamin D\textsubscript{3}-(1,25(OH)\textsubscript{2}D\textsubscript{3})-regulated active transport of Ca\textsuperscript{2+} from the lumen to the blood compartment, which occurs primarily in the proximal small intestine and the distal part of the nephron. This process of transcellular Ca\textsuperscript{2+} transport is a three-step operation consisting of passive apical Ca\textsuperscript{2+} entry followed by cytosolic diffusion facilitated by calbindins and active extrusion across the basolateral membrane by a high affinity Ca\textsuperscript{2+}-ATPase and/or a Na\textsuperscript{+}−Ca\textsuperscript{2+} exchanger (2). The apical influx of Ca\textsuperscript{2+} is the rate-limiting step in this process and, therefore, a prime regulatory target for stimulatory and inhibitory hormones. The epithelial Ca\textsuperscript{2+} channel (ECaC), recently cloned from rabbit kidney, is the candidate channel for being the gatekeeper of this apical Ca\textsuperscript{2+} influx mechanism (3).

ECaC is exclusively present in 1,25(OH)\textsubscript{2}D\textsubscript{3}-responsive epithelia, including intestine, kidney, and placenta, and is structurally related to the family of transient receptor potential channels, capsaicin receptors, and the growth factor-regulated channel (4–6). These Ca\textsuperscript{2+}-permeable cation channels contain six putative transmembrane domains, including a pore-forming region, but share only 30% homology that is mainly restricted to the pore-forming region and flanking transmembrane segments. ECaC contains putative phosphorylation sites for protein kinase C, AMP-dependent and cGMP-dependent protein kinase, calcium-calmodulin-dependent protein kinase, and structural domains, such as N-linked glycosylation sites and ankyrin repeats (3).

ECaC-expressing *Xenopus laevis* oocytes mediate a saturable Ca\textsuperscript{2+} uptake determined with tracer studies and a hyperpolarization-stimulated Ca\textsuperscript{2+} influx measured indirectly as Ca\textsuperscript{2+}-induced Cl\textsuperscript{−} current (3, 7). These initial studies indicate that ECaC exhibits a distinct Ca\textsuperscript{2+} permeability. A similar conclusion was reached for a Ca\textsuperscript{2+} transporter (calcium transporter 1) cloned from rat intestine, which shares with ECaC a structural similarity of 75% and several basic functional properties (8).

The identification of ECaC offers for the first time a realistic approach to study the functional and regulatory aspects of this Ca\textsuperscript{2+} influx pathway. Knowledge of ECaC functioning is of vital importance to understand the Ca\textsuperscript{2+} handling by Ca\textsuperscript{2+} absorbing epithelia and will, in particular, provide a molecular basis for achieving a better understanding of Ca\textsuperscript{2+} malabsorption/malreabsorption. The aim of the present study is to functionally characterize ECaC by a combined whole-cell patch-clamp analysis and fura-2 fluorescence microscopy using HEK293 cells heterologously expressing ECaC.

**EXPERIMENTAL PROCEDURES**

* Vector Construction for ECaC-Green Fluorescent Protein (GFP) Expression—The entire open reading frame from rECaC was cloned as a *Pvu*II-* BamH*II fragment in the pcDNA3/IRESP-GFP vector (3, 9). This bicistronic expression vector pcDNA3/IRESP-GFP/rbECaC was co-transfected with rECaC and enhanced GFP.

* Cell Culture and Transfection—Human embryonic kidney cells, HEK293, were grown in Dulbecco’s modified Eagle’s medium containing 10% (v/v) human serum, 2 mM l-glutamine, 2 units/ml penicillin, and 2 mg/ml streptomycin at 37 °C in a humidity-controlled incubator.
Electrophysiology—Electrophysiological methods and Ca$^{2+}$ measurements have been described in detail previously (11). Whole-cell currents were measured with an EPC-9 (HEKA Elektronik, Lambrecht, Germany; sampling rate, 1 ms; 8-pole Bessel filter, 2.9 kHz) or an L/M-EPC-7 (List Elektronik, Darmstadt, Germany) using ruptured patches. Electrode resistances were between 2 and 5 M$\Omega$, and the actual experimental conditions used. 

**Fig. 1.** Effect of $[\text{Ca}^{2+}]_{o}$ and $V_{m}$ on the $[\text{Ca}^{2+}]_{i}$ of control and ECaC-expressing HEK293 cells. $A$, representative traces of the changes in $[\text{Ca}^{2+}]_{i}$ in nontransfected (control) and ECaC-expressing HEK293 cells (identified by their green fluorescence) were voltage-clamped at +20 mV and exposed to different $[\text{Ca}^{2+}]_{o}$, administered at the concentrations indicated by arrows. The bottom panel shows the pooled data from the maximum $[\text{Ca}^{2+}]_{o}$, closed circles, ECaC; $n = 3–5$. $B$, changes in $[\text{Ca}^{2+}]_{i}$ in control and ECaC-expressing HEK293 cells clamped at various potentials at an extracellular Ca$^{2+}$ concentration of 1.5 mM. The top panel shows a typical trace for an ECaC-expressing cell together with the corresponding voltage levels, and the bottom panel shows the pooled data for both cell types obtained from various cells (open circles, control; closed circles, ECaC; $n = 2–8$). The dotted line in the top panels represents the zero Ca$^{2+}$ level.

With 10% CO$_2$, HEK293 cells were transiently transfected with the pCINeo/IRES-GFP/rhECaC vector using methods described previously (10). Transfected cells were visually identified in the patch-clamp setup. GFP-negative cells from the same batch were used as controls.

**Electrophysiology**—Electrophysiological methods and Ca$^{2+}$ measurements have been described in detail previously (11). Whole-cell currents were measured with an EPC-9 (HEKA Elektronik, Lambrecht, Germany; sampling rate, 1 ms; 8-pole Bessel filter, 2.9 kHz) or an L/M-EPC-7 (List Elektronik, Darmstadt, Germany) using ruptured patches. Electrode resistances were between 2 and 5 M$\Omega$, and capacitance and access resistances were monitored continuously. The ramp protocol consisted of linear voltage ramps changing from $-100$ or $-150$ mV to $+100$ mV within 400 ms, applied every 5 s. The step protocol consisted of a series of 400-ms-long voltage steps applied from a holding potential of $+20$ mV to voltages between $-100$ and $+100$ mV, with an increment of 25 mV. The current density was calculated from the size of net current at $-80$ mV during the ramp protocol. The permeability of Ca$^{2+}$, the only other permeable monovalent cation besides Na$^+$ in pipette and bath solution, was measured relative to that of Na$^+$ from the reversal potential in the absence of extracellular Ca$^{2+}$ (1 mM free [Ca$^{2+}]_{o}$, buffered by 5 mM EGTA and 10 mM total [Ca$^{2+}]_{o}$) to Equation 1.

$$P_{\text{Ca}}/P_{\text{Na}} = \frac{[\text{Na}] - [\text{Na}] \exp(V_{m}/RT)}{[\text{Ca}] \exp(V_{m}/RT) - [\text{Ca}]}.$$  

(Eq. 1)

Permeability of the divalent cations Ca$^{2+}$, Ba$^{2+}$, Sr$^{2+}$, and Mg$^{2+}$ relative to Na$^+$ was calculated from the reversal potential measured with 30 mM of the respective cation in the extracellular solution.

$$P_{\text{Ca}}/P_{\text{Na}} = \frac{([\text{Na}] + \alpha[\text{Ca}] \exp(V_{m}/RT)) - [\text{Na}] - \alpha[\text{Ca}]}{4[X]}.$$  

(Eq. 2)

$P_{\text{Ca}}$ represents the permeability of the respective divalent cation; $X$ represents its extracellular concentration; $\alpha$ is $P_{\text{Na}}/P_{\text{Ca}}$, obtained from Equation 1 using divalent cation-free solutions; [Na$^-$], [Na$^+$], [Ca$^{2+}$], and $[\text{Ca}]_{o}$ are the extra- and intracellular concentrations for Na$^+$ and Ca$^{2+}$, respectively; and $V_{m}$ is the reversal potential (10).

**Ca$^{2+}$ Measurements**—ECaC-expressing cells were loaded with fura-2 via the patch pipette and excited alternately at wavelengths of 360 and 390 nm through a filter wheel rotating at 2 cycles/s. The fluorescence emitted at each excitation wavelength was measured at 510 nm using a photomultiplier. Autofluorescence was subtracted. Apparent free [Ca$^{2+}$] was calculated from the fluorescence ratio $R$ by $[\text{Ca}^{2+}]_{o} = K_{\text{app}} (R - R_{\text{eff}})/(R_{\text{eff}} - R)$, where $K_{\text{app}}$ is the effective binding constant, $R_{\text{eff}}$ is the fluorescence ratio at zero Ca$^{2+}$, and $R_{\text{eff}}$ that at high Ca$^{2+}$. These calibration constants were determined experimentally for the given set-up and the actual experimental conditions used.

**Solutions and Experimental Procedures**—The standard extracellular solution (Krebs) contained 150 mM NaCl, 6 mM CsCl, 1 mM MgCl$_2$, 10 mM HEPES, and 10 mM glucose, pH 7.4, with CsOH, and the concentration of Ca$^{2+}$, Ba$^{2+}$, Sr$^{2+}$, or Mn$^{2+}$ was varied between 1 and 30 mM as indicated in the text. Nominally free Ca$^{2+}$ concentration was estimated at 10 mM. Ca$^{2+}$-free solutions were buffered by 5 mM

$[\text{Ca}^{2+}]_{o}$ consisted of linear voltage ramps changing from $-20$ mV to voltages between $-60$ and $-100$ mV, with an increment of 20 mV within 400 ms, applied every 5 s. The step protocol consisted of a series of 400-ms-long voltage steps applied from a holding potential of $+20$ mV to voltages between $-100$ and $+100$ mV, with an increment of 25 mV. The current density was calculated from the size of net current at $-80$ mV during the ramp protocol. The permeability of Ca$^{2+}$, the only other permeable monovalent cation besides Na$^+$ in pipette and bath solution, was measured relative to that of Na$^+$ from the reversal potential in the absence of extracellular Ca$^{2+}$ (1 mM free [Ca$^{2+}]_{o}$, buffered by 5 mM EGTA and 10 mM total [Ca$^{2+}]_{o}$) to Equation 1.
EGTA at a free [Ca\textsuperscript{2+}] below 1 mM, as calculated by the CaBuf program (G. Droogmans, KU Leuven). The internal (pipette) solution contained 20 mM CsCl, 100 mM Cs-aspartate, 1 mM MgCl\textsubscript{2}, 10 mM BAPTA, 4 mM Na\textsubscript{2}ATP, 10 mM HEPES, pH 7.2, with CsOH. For simultaneous measurement of the membrane current and [Ca\textsuperscript{2+}], the same extracellular solution was used, and the internal (pipette) solution contained 20 mM CsCl, 100 mM Cs-aspartate, 1 mM MgCl\textsubscript{2}, 0.1 mM EGTA, 10 mM HEPES, 4 mM Na\textsubscript{2}ATP, and 0.5 mM fura-2 (pentapotassium salt), pH 7.2, with CsOH.

Cells were kept in a nominally Ca\textsuperscript{2+}-free medium to prevent Ca\textsuperscript{2+} overload and exposed for a maximum of 5 min to a Krebs solution containing 1.5 mM Ca\textsuperscript{2+} before sealing the patch pipette to the cell. All experiments were performed at room temperature (20–22 °C).

Statistical Analysis—In all experiments, the data are expressed as the mean ± S.E. Overall statistical significance was determined by analysis of variance. In the case of significance (p < 0.01), individual groups were compared by Student’s t test. Experimental data were fitted to multiple exponentials using the fitting routine of the ASCD program (G. Droogmans).

RESULTS

Evidence that ECaC forms a constitutively active Ca\textsuperscript{2+} entry pathway was obtained from experiments showing a close correlation between the level of intracellular calcium ([Ca\textsuperscript{2+}]) and the electrochemical Ca\textsuperscript{2+} gradient in ECaC-expressing HEK293 cell (Fig. 1). The driving force for Ca\textsuperscript{2+} entry was therefore modified either by changing extracellular ([Ca\textsuperscript{2+}], Fig. 1A) or the membrane potential (V\textsubscript{M}) (Fig. 1B). In Fig. 1, the top panels show some representative traces obtained by the changes in [Ca\textsuperscript{2+}] induced by changes in [Ca\textsuperscript{2+}] and V\textsubscript{M}, respectively. Increasing [Ca\textsuperscript{2+}] from 0 to 30 mM in cells clamped at +20 mV markedly increases [Ca\textsuperscript{2+}], in ECaC-expressing cells, whereas the effect was much less pronounced in nontransfected cells (Fig. 1A).

Another experiment, we varied the membrane potential at a constant [Ca\textsuperscript{2+}], of 1.5 mM. Depolarization from +20 to +60 mV decreased [Ca\textsuperscript{2+}], whereas hyperpolarization from +20 to −100 mV increased [Ca\textsuperscript{2+}], (Fig. 1B). The pooled data from several cells are summarized in the bottom panels of Fig. 1, which represent the maximal values of [Ca\textsuperscript{2+}], observed at each [Ca\textsuperscript{2+}], or V\textsubscript{M}. It is obvious that intracellular [Ca\textsuperscript{2+}], is closely correlated with either [Ca\textsuperscript{2+}], or V\textsubscript{M} and that the corresponding changes of [Ca\textsuperscript{2+}], are much smaller in nontransfected cells.

The characteristics of the Ca\textsuperscript{2+} entry pathway in ECaC-expressing cells were further investigated in electrophysiological whole-cell experiments in cells diazylized with 10 mM BAPTA and using the described ramp and step protocols. Under these circumstances, voltage ramps induced large inward currents in ECaC-expressing HEK293 cells with amplitudes that were obviously enhanced by increasing [Ca\textsuperscript{2+}], (Fig. 2B). Nontransfected HEK293 cells exhibited under these experimental conditions only a small background current, which was in contrast with ECaC-expressing cells inhibited by increasing [Ca\textsuperscript{2+}], (Fig. 2A). Because of the strong inward rectification, it was difficult to obtain reliable values of the reversal potential. Moreover, the reversal potential of +54 ± 4 mV (range, 30–77; n = 13) at 30 mM [Ca\textsuperscript{2+}], is probably an underestimation due to the presence of background currents. Currents in response to voltage-steps to potentials more negative than −40 mV showed time-dependent inactivation, whereas currents at more positive potentials did not. Inactivation was manifest at 30 mM [Ca\textsuperscript{2+}], but was also present at 1 mM (Fig. 2, C and D). Buffering [Ca\textsuperscript{2+}], at 1 nM abolished inactivation and enhanced the current amplitude (Fig. 2E) compared with that at 1 mM [Ca\textsuperscript{2+}],. The fit of the inactivation time course with a sum of exponentials disclosed two kinetically distinct components at 30 mM [Ca\textsuperscript{2+}], or Mn\textsuperscript{2+} (Fig. 3, A and B) but a single slow component if extracellular Ca\textsuperscript{2+} was replaced with Ba\textsuperscript{2+} or Sr\textsuperscript{2+} (Fig. 3, C and D).

To corroborate our insight in the mechanism of permeation through ECaC, we have measured current densities at −80 mV as a function of [Ca\textsuperscript{2+}], in ECaC-expressing cells, as shown in
respectively. Extracellular Ca\(^{2+}\) channel derived from the current densities at The conductance sequence for divalent cations of the ECaC underestimates the real ratio because of the mentioned under-

2, was 107\( ^{6} \)6.4. \( \text{charged carrier through ECaC. Inactivation was delayed compared with Ca}^{2+} \) than Ca\(^{2+}\) as in 

FIG. 3. Inactivation of ECaC-mediated currents. A, currents at steps from +20 mV holding potential to -100, -75, and -50 mV, respectively. Extracellular Ca\(^{2+}\) concentration was 30 mM. The decay of the currents was fitted with two exponentials. At potentials more positive than -50 mV, only non-inactivating current could be recorded. The fitted lines completely matched the current traces. Inactivation time constants are plotted against the respective voltages (n = 4-9). Note that two time constants can be clearly separated. Calibration is identical for all examples shown. B, currents measured with 30 mM Mn\(^{2+}\) showed a similar inactivation, with a fast and a slow component. The protocol used was the same as in A. Decay was fitted by two exponentials (n = 3). C, current steps and inactivation time constant versus voltage plotted for Ba\(^{2+}\) being the charge carrier through ECaC. Inactivation was delayed compared with Ca\(^{2+}\) (n = 3). D, when Sr\(^{2+}\) was substituted for Ca\(^{2+}\), currents through ECaC were still inactivated. Decay was faster than in Ba\(^{2+}\) but slower than Ca\(^{2+}\) (n = 7).

Fig. 4A. At extracellular Ca\(^{2+}\) levels above 1 mM, the current density strongly increased with [Ca\(^{2+}\)]. However, the current density also increased if [Ca\(^{2+}\)] was buffered at 1 mM. Under the latter conditions, the channel becomes apparently permeable for monovalent cations. The [Ca\(^{2+}\)] dependence of the ECaC-specific current density, calculated as the difference current in ECaC-expressing and control cells, shows the typical anomalous mole fraction behavior known for highly Ca\(^{2+}\)-selective channels (Fig. 4B). Note also that the current density in control cells in contrast with ECaC-expressing cells diminished with increasing [Ca\(^{2+}\)], (Fig. 4A).

We have also compared the current densities at -80 mV in ECaC-expressing cells at different [Ca\(^{2+}\)], i.e. in cells buffered with 10 mM BAPTA at extremely low values or at 1 mM (Fig. 4C). It is obvious that an increase in [Ca\(^{2+}\)] down-regulates the current.

Current densities and inactivation pattern of ECaC was different for the various divalents (Fig. 5, A and B, and Fig. 3). The conductance sequence for divalent cations of the ECaC channel derived from the current densities at -80 mV is Ca\(^{2+}\) > Ba\(^{2+}\) > Sr\(^{2+}\) > Mn\(^{2+}\) (Fig. 5C); the values for Sr\(^{2+}\) and Ba\(^{2+}\) are not statistically different (p > 0.01). The permeability ratio of Ca\(^{2+}\) to Ba\(^{2+}\), determined from the reversal potentials at 30 mM [Ca\(^{2+}\)], and in Ca\(^{2+}\)-free solution according to Equations 1 and 2, was 107 ± 32 (range, 7-420; n = 13). This value probably underestimates the real ratio because of the mentioned under-

estimation of the reversal potential. The permeabilities of Mn\(^{2+}\), Ba\(^{2+}\), and Sr\(^{2+}\) relative to that of Na\(^{+}\) were 20, 5.6, and 4, respectively. ECaC is thus despite the smaller current densities observed with Mn\(^{2+}\) as the charge carrier, more permeable for Mn\(^{2+}\) than for Ba\(^{2+}\) and Sr\(^{2+}\) (Fig. 5D). In 1 mM [Ca\(^{2+}\)], we calculated a P\(_{Ca}P_{Na}\) of 0.84, indicating that ECaC is more permeable for Na\(^{+}\) than for Cs\(^{+}\).

Another feature of the current through ECaC is its decay during repetitive stimulations (Fig. 6), with voltage ramps from -100 to +100 mV applied with an interval of 5 s from a holding potential of +20 mV. In the presence of 30 mM [Ca\(^{2+}\)], the current practically disappeared after 8 ramps (Fig. 6A). In contrast, more sweeps were necessary to down-regulate the current when Ba\(^{2+}\) was the charge carrier (Fig. 6B). Decreasing [Ca\(^{2+}\)], to 1 mM virtually abolished the rundown phenomenon (Fig. 6C). The time constants of half-maximal decay of the ECaC currents are summarized in Fig. 6D, showing the dramatic lowering of ECaC currents when Ca\(^{2+}\) is the charge carrier. This finding indicates that the decay process may be associated with a Ca\(^{2+}\)-dependent mechanism.

**Discussion**

The present study describes functional characteristics of the recently cloned epithelial Ca\(^{2+}\) channel, ECaC, that are consistent with its putative role as apical Ca\(^{2+}\) entry channel that mediates transcellular Ca\(^{2+}\) transport in 1,25(OH)\(_{2}\)D\(_{3}\)-respon-
Dependence of currents through ECaC on the extracellular Ca\(^{2+}\) concentration. A, current densities measured at \(-80\) mV in control (open circles) and ECaC-expressing (closed circles) HEK293 cells as a function of [Ca\(^{2+}\)]. The number of cells is indicated in parentheses near the error bars for each data point. B, difference current densities between control and transfected cells as a function of [Ca\(^{2+}\)]. Note the anomalous mole-fraction behavior. C, current densities at \(-80\) mV in ECaC-expressing cells at two [Ca\(^{2+}\)] concentrations, i.e. cells buffered with 10 mM BAPTA or at 1 \(\mu M\).

An additional feature of the gating mechanism is that the current response slowly disappears during successive voltage ramps, generally referred to as rundown or decay. The rundown was significantly diminished when Ca\(^{2+}\) was replaced by Ba\(^{2+}\) as charge carrier and abrogated when extracellular Ca\(^{2+}\) was lowered to 1 \(\mu M\), indicating, as suggested for the inactivation process, that a Ca\(^{2+}\)-operated process inhibits ECaC activity during repetitive stimulation. This form of regulation could have a significant impact on the amount of Ca\(^{2+}\) that enters the cell during repetitive activation. As postulated for other ion channels, it is possible that this rundown involves phosphorylation and/or dephosphorylation of the channel or associated proteins. In this respect, it is tempting to explore the role of a calcium-calmodulin-dependent protein kinase II for which functionally conserved consensus sites are present in ECaC (Ser-142 and Ser-693) (3, 8), because it has recently been demonstrated that this kinase is involved in the Ca\(^{2+}\)-dependent regulation of channel activity (22, 23). Alternatively, calbindin-D28k has been implicated in the regulation of the rundown process of \(N\)-methyl-D-glucamine receptor channel activity, possibly through buffering local Ca\(^{2+}\) elevations and thereby preventing calcium-induced polymerization of the actin cytoskeleton (24). Together with the striking co-localization of ECaC and calbindin-D in Ca\(^{2+}\)-transporting cells, a calbindin-mediated rundown process represents a possible mechanism that could adjust the amount of Ca\(^{2+}\) that enters the cell during repetitive activation.

The present data unambiguously demonstrate that ECaC possesses a high selectivity for Ca\(^{2+}\), illustrated by \(P_{\text{Ca}}/P_{\text{Na}}\) values of more than 100. This implies that under physiological conditions when Ca\(^{2+}\) ions present in the pro-urine or intestinal fluid are largely outnumbered by Na\(^{+}\) ions, ECaC can efficiently discriminate between Ca\(^{2+}\) and other cations, thereby specifically controlling the Ca\(^{2+}\) permeability of the apical membrane of Ca\(^{2+}\)-transporting epithelial cells. This restriction toward monovalent cations was eliminated in the absence of extracellular Ca\(^{2+}\) ions, which can be explained by the observed anomalous mole-fraction behavior of ECaC, indicating multiple ion binding sites in ECaC (16).

The cation selectivity of ECaC is distinguishably different
FIG. 5. Monovalent and divalent cation selectivity of ECaC. A, currents at steps of −100 mV from a holding potential of +20 mV showing representative size and pattern of inactivation for different divalent cations being the charge carrier through ECaC. The step protocol is the same as in Figs. 3 and 4. B, IV curves obtained from voltage ramps for different divalent cations (ramps from −100 to +100 mV, holding potential +20 mV). C, current densities for divalent cations. Concentration is always 30 mM. The number of cells is indicated near the error bars. Currents were measured from voltage ramps at −80 mV. D, permeation of mono- and divalent cations through ECaC. The relative permeation to Na⁺ was calculated according to Equations 1 and 2. The $P_{Cs}/P_{Na}$ permeation ratio was used to calculate permeation of the bivalents by Equation 2. See under “Experimental Procedures” for details.

FIG. 6. Current through ECaC induced by repetitive stimulation exhibits rundown. A, rundown of the current through ECaC in the presence of 30 mM Ca²⁺. Voltage ramps were applied every 5 s (ramps of 400 ms, from −100 to +100 mV; holding potential, +20 mV). B, if Ba²⁺ (30 mM) is the charge carrier, rundown is strikingly delayed. The interval between the ramps is 5 s (same protocol as in A). C, time course of the current rundown from three single cells. D, pooled data representing the time to half-maximal decay from all cells. Note that in 1 nM [Ca²⁺]₀, the estimated time for rundown is longer than 4 min. Current values were normalized to the maximal current value in the specific condition (I₉₀).
from that observed for homologous Ca\(^{2+}\) channels, including members of the Trp family and the ligand-operated vanilloid receptor and growth factor-regulated channels, and is exemplified by an eminent selectivity for Ca\(^{2+}\) over monovalent cations and a permeability sequence for divalent cations of Ca\(^{2+}\) > Ba\(^{2+}\) > Sr\(^{2+}\) > Mn\(^{2+}\). Furthermore, the current-voltage relationship of vanilloid receptor-like 1, vanilloid receptor 1, and growth factor-regulated channels reveals dominant outward rectification of the corresponding current, allowing us to discriminate these channels from the ECaC current (2–6, 25). ECaC, however, resembles several features typical for store-operated Ca\(^{2+}\) entry. The Ca\(^{2+}\)-release activated Ca\(^{2+}\) current (I\(_{\text{CRAC}}\)) is remarkably selective for Ca\(^{2+}\), with a divalent cation selectivity profile similar to that of ECaC, and gives rise to prominent inward rectification at negative voltages (26, 27). Despite these similarities, it could not be demonstrated that ECaC is activated by store depletion (data not shown), indicating that ECaC does not encode I\(_{\text{CRAC}}\). However, it remains intriguing to consider channels homologous to ECaC as candidates for store-operated Ca\(^{2+}\) entry.

A homologue of ECaC was recently cloned from rat duodenum and named calcium transporter 1 (8). The main part of this protein is highly identical (>90%) to ECaC, whereas the last part of the C-tail is structurally different. Based on its macroscopic kinetic properties, it was suggested that this protein represents a unique transition between a channel and a macroscopic kinetic properties, it was suggested that this protein represents a unique transition between a channel and a transporter. The current paper, however, clearly demonstrates that ECaC exhibits defining characteristics typical for a Ca\(^{2+}\) channel, including a positive reversal potential and anomalous mole fraction behavior.

The present study has established the basic kinetic aspects of ECaC, which are important to understanding the control of apical Ca\(^{2+}\) entry in Ca\(^{2+}\)-transporting epithelial cells. It has been shown previously that this process of transcellular Ca\(^{2+}\) transport is under the regulation of multiple signaling pathways involving protein kinase C and cAMP- and cGMP-dependent kinase (13, 28, 29). Together with the observation that relevant consensus sites for these kinases are present in ECaC (3), these findings warrant further investigations to unravel the hormone-activated mechanisms regulating the kinetic properties of ECaC.

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