Minireview

Functional Properties of the Epithelial Ca^{2+} Channel, ECaC

R Vennekens, G Droogmans and B Nilius

Department of Physiology, Campus Gasthuisberg, KU Leuven, Leuven, Belgium

Abstract. ECaC is the first member of a new subfamily of Ca^{2+} channels embedded in the large TRPC family that includes numerous channel proteins The channel has been proposed as the main gatekeeper of transcellular Ca^{2+} transport in kidney and intestine The functional characterization of this channel is evolving rapidly and may have far reaching consequences for other channels of the TRPC family The goal of this mini-review is to summarize the major functional and structural characteristics of ECaC, including (i) its proposed functional role, (ii) its channel structure and expression pattern, (iii) its main electrophysiological characteristics and (iv) its regulation

Key words: Ca^{2+} channels — Ca^{2+} reabsorption — TRP channel family

Introduction

Extracellular Ca^{2+} concentrations ($[Ca^{2+}]_e$) in the body are tightly regulated through the coordinated action of parathyroid glands, kidneys, intestine and bone, which is the repository for stored calcium In the kidney Ca^{2+} is reabsorbed throughout the nephron, the principal sites being proximal tubules, thick ascending limbs and distal tubules The magnitude and mechanisms of this process differ importantly between one segment and another Likewise, the sites of hormonal and drug effects on calcium reabsorption are spatially separated The largest amounts of calcium are reabsorbed in the proximal tubules In this part of the nephron, the main route of calcium reabsorption is mediated through the paracellular pathway between adjacent cells In medullar and cortical ascending limbs, where 20% of filtered calcium is reabsorbed, this process occurs by a combination of paracellular and transcellular routes In the distal part of the nephron paracellular calcium reabsorption is absent All calcium reabsorption in this segment follows a transcellular route (Friedman 2000) This process of transcellular Ca^{2+} transport is generally envisaged as a three step operation consisting of passive apical Ca^{2+} entry, followed by cytosolic diffusion facilitated by calbindins, and active extrusion across

Correspondence to Prof , Dr med Bernd Nilius, Ph D , Laboratorium voor Fysiologie, KU Leuven, Campus Gasthuisberg, B-3000 Leuven, Belgium
E-mail Bernd.Nilius@med kuleuven ac be

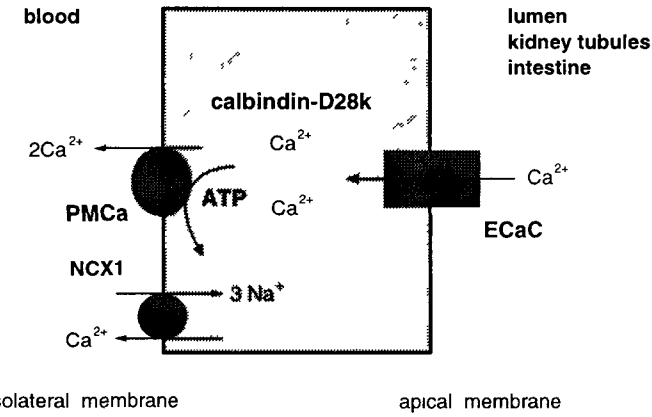


Figure 1. Schematic representation of the three-step process of transcellular Ca^{2+} reabsorption Ca^{2+} reabsorption in the kidney and intestine is generally envisioned as a three-step process Ca^{2+} enters the cell from the luminal fluid passively through ECaC Inside the cell free Ca^{2+} is bound by calbindin-D28k which supposedly facilitates diffusion of Ca^{2+} towards the basolateral membrane where Ca^{2+} is extruded actively by the plasma membrane Ca^{2+} ATP-ase (PMCa) and/or the Na^{+}/Ca^{2+} exchanger type 1 (NCX1) From an energetic point of view, the passive influx through the apical membrane will be the rate-limiting step of transcellular Ca^{2+} transport

the basolateral membrane by a high affinity Ca^{2+}-ATPase and/or a Na^{+}-Ca^{2+} exchanger (Fig. 1) (Hoenderop et al. 1998, 2000c). Although the smallest amounts of Ca^{2+} are reabsorbed in the distal segments of the nephron, it is generally accepted that the regulatory effects on Ca^{2+} absorption are achieved in this part of the nephron (Friedman 2000). The absorption in this part of the nephron is under the hormonal control of the parathyroid hormone (PTH), 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) and calcitonin. The passive influx of Ca^{2+} across the apical membrane is the rate-limiting step in this process and therefore a prime regulatory target for stimulatory and inhibitory hormones. Until 1999, the molecular entity responsible for the apical influx of Ca^{2+} was unknown. Recently the channel responsible for the passive apical Ca^{2+} influx has been identified from rabbit kidney tissue and was called ECaC, for Epithelial Ca^{2+} Channel (Hoenderop et al. 1999).

Structural features and expression pattern of ECaC

The structure and the expression pattern of ECaC have been extensively reviewed elsewhere (Hoenderop et al. 2000b). In short, ECaC represents a new member of a large family of Ca^{2+} permeable cation channels sharing homology with the transient receptor potential channel (TRPC) (Hoenderop et al. 1999). On the basis of sequence homology this large group has been subdivided in 3 groups, i.e. short TRPCs, long TRPCs and Osm-9 like TRPCs. ECaC represents a new member of the

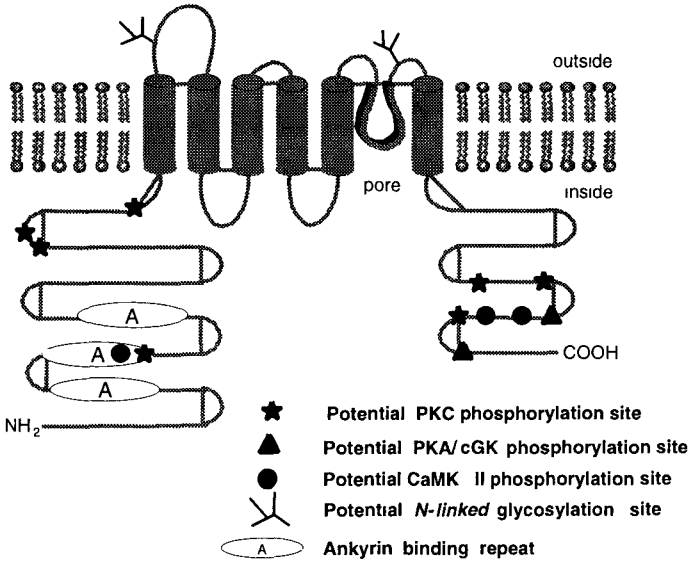


Figure 2. Schematic representation of the Epithelial Calcium channel, ECaC Schematic representation of the membrane topology and domain structure of rabbit ECaC (adapted from (Hoenderop et al 1999)) The channel is 730 amino acids long with a predicted relative molecular mass of 83 kDa (M_r 83,000) Indicated structural components include six transmembrane domains (TM's), a putative pore region between TM5 and TM6, several putative phosphorylation sites, three ankyrin-binding domains and multiple potential N-linked glycosylation sites Inner and outer side of the membrane is indicated (PKC – Ca^{2+} dependent protein kinase C, PKA/cGK – cAMP dependent protein kinase A and cGMP dependent protein kinase, CaMKII – Ca^{2+} calmodulin dependent protein kinase II)

latter group (Harteneck et al. 2000). This group also includes the vanilloid receptor 1 (VR1) and the vanilloid receptor-like 1 (VRL1), but their homology with ECaC is low (30%), indicating that ECaC may form another subgroup within this family of proteins. To date, ECaC has been cloned from three different species, including rabbit, rat and human. The obtained sequences exhibit an overall homology of approximately 85% (Hoenderop et al. 2000b). Recently two new members of the ECaC family have been identified, i.e. Ca^{2+} transporter 1 (Cat1) and Ca^{2+} transporter 2 (Cat2) (Peng et al. 1999, 2000), which were isolated from rat intestine and rat kidney cortex respectively. All these channels consist of 6 trans-membrane segments including a short hydrophobic stretch between transmembrane segment 5 and 6, predicted to be the pore-forming region (Fig. 2). Importantly, some domains, including the pore region, are completely identical between the members of this ECaC family in different species (Hoenderop et al. 2000b). The predicted channel structure shares similarities with the core-structure of the pore-forming subunits of voltage gated Ca^{2+}, Na^+ and K^+ channels and with those of cyclic nucleotide

gated channels (CNG), hyperpolarization-activated cyclic-nucleotide-gated channels (HCN) and polycystins (PKDs) (Harteneck et al 2000) The core-structure of some of these channels is formed by the co-assembly of four subunits (e g , the shaker potassium channel family and the inward rectifying K-channel) or through assembly of four internal repeats (e g , voltage gated Ca^{2+} and Na^{+} channels) It is tempting therefore to speculate that functional ECaC channels also consist of four sub-units, although this remains to be demonstrated

Rabbit ECaC was originally detected in $1,25(OH)_2D_3$-responsive epithelia including intestine, kidney and placenta More detailed studies on the cellular localization of the channel revealed that rbECaC protein was exclusively present at the apical domain of the connecting tubule in the kidney Rat ECaC on the other hand, could also be detected in the medullary thick ascending limb of the nephron (Suzuki et al 2000) In intestine, rbECaC was present in a thin layer along the apical membrane of the duodenal villus tip Importantly, rbECaC completely co-localized in both cases with calbindin-D (the 28Kb isoform in the kidney and the 9Kb isoform in intestine), the plasma membrane Ca^{2+}-ATPase (PMCA, present in kidney and intestine) and/or the Na^{+}-Ca^{2+} exchanger (NCX, not present in intestine) (Hoenderop et al 2000a) This finding together with the apical localization of ECaC further substantiates the postulated function of ECaC in transcellular Ca^{2+} reabsorption

Recently the human homologue of ECaC was cloned and found to be highly expressed in kidney, small intestine, and pancreas Less intense expression was detected in testis, prostate, placenta brain, colon, and rectum (Muller et al 2000) Importantly, ECaC co-localized also in all these tissues with calbindins At the moment, no data are available concerning a role for the simultaneous presence of ECaC and calbindins in non-epithelial tissues such as the brain and pancreas In pancreas, calbindin-D28K and vitamin D_3 receptors have been localized to the β cell (Reddy et al 1997, Sooy et al 1999) It has been suggested that vitamin D_3 through its effect on calbindin expression can regulate cytosolic Ca^{2+} levels and in this way can modulate the depolarization-stimulated insulin release (Sooy et al 1999) ECaC could participate in this control mechanism by facilitating an additional calcium influx pathway, but it is clear that further studies are necessary to delineate the physiological role of ECaC in these organs

Electrophysiological properties of ECaC

In order to functionally characterize ECaC channel properties we have overexpressed rabbit ECaC in human embryonic kidney cells (HEK293), and analyzed channel events using the patch-clamp technique Evidence that ECaC forms a constitutively active Ca^{2+} channel was obtained from experiments showing a close correlation between the level of intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) and the electrochemical Ca^{2+} gradient in ECaC expressing HEK293 cells The driving force for Ca^{2+} entry was either manipulated by administration of 30 mmol l^{-1} extracellular Ca^{2+} at a holding potential of -80 mV (Fig 3A,B) or by clamp-

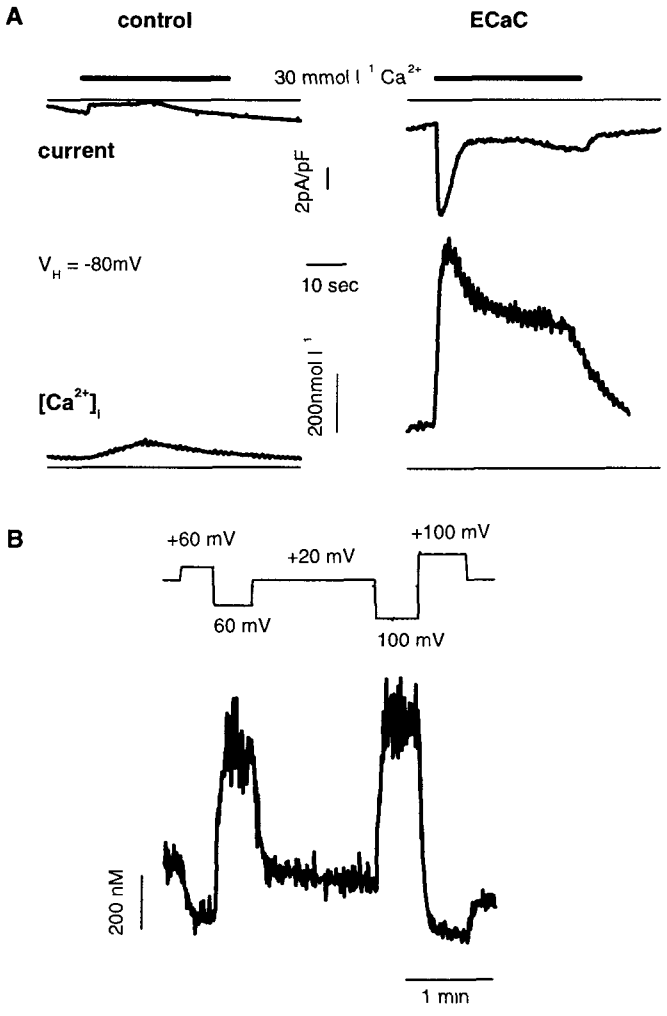


Figure 3. Effect of the electrochemical driving force for calcium on $[Ca^{2+}]_i$ in control and ECaC expressing HEK293 cells **A.** Representative traces of the changes in membrane current and $[Ca^{2+}]_i$ in non-transfected (control) and ECaC expressing (ECaC) HEK293 cells Cells were voltage clamped at −80 mV and exposed to 30 mmol l^{-1} $[Ca^{2+}]_e$ A rapid rise in $[Ca^{2+}]_i$ due to Ca^{2+} permeation through the constitutive open ECaC upon administration of 30 mmol l^{-1} Ca^{2+} is followed by a rapid decrease to a plateau level, while in control cells the current is lowered and $[Ca^{2+}]_i$ slightly enhanced Notice the enlarged basal $[Ca^{2+}]_i$ and current levels in ECaC expressing cells **B.** Changes in $[Ca^{2+}]_i$ in ECaC expressing HEK293 cells clamped at various membrane potentials, as indicated, at an extracellular Ca^{2+} concentration of 1 5 mmol l^{-1}

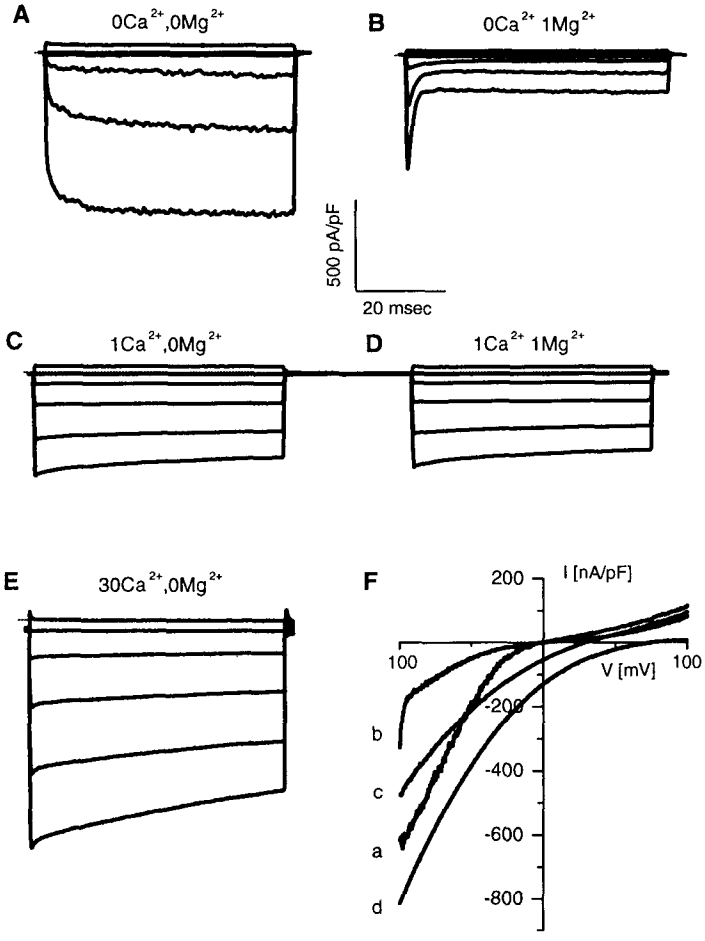


Figure 4. Currents through ECaC **A.–E.** Representative current traces from ECaC expressing HEK293 cells in response to voltage steps at various extracellular Ca^{2+} and Mg^{2+} concentrations Step protocol consisted of 60 ms steps from +60 mV to −140 mV with 40 mV decrement from a holding potential of +20 mV (Sampling rate = 5 kHz and cut-off frequency = 2 kHz in all experiments) Extracellular solution contained (in mmol l^{-1}) 150 NaCl, 6 CsCl, 10 HEPES and 10 glucose, pH 7 4 with CsOH Ca^{2+} and Mg^{2+} concentrations were varied as indicated Pipette solution contained (in mmol l^{-1}) 20 CsCl, 100 Cs-aspartate, 1 $MgCl_2$, 10 BAPTA, 4 Na_2ATP, 10 HEPES, pH 7 2 with CsOH **F.** Current traces in response to a ramp protocol from −100 to +100 mV (duration of ramps is 400 ms, V_H = +20 mV) in various extracellular Ca^{2+} and Mg^{2+} concentrations (in mmol l^{-1}) from ECaC expressing HEK293 cells a) 0 Ca^{2+}, 0 Mg^{2+}, b) 0 Ca^{2+} 1 Mg^{2+}, c) 1 Ca^{2+}, 0 Mg^{2+}, d) 30 Ca^{2+}, 0 Mg^{2+} Intra- and extracellular solutions as above For details on methods see (Vennekens et al 2000)

ing the cell at various holding potentials in the presence of 1.5 mmol·l^{-1} $[Ca^{2+}]_e$ (Fig. 3B). Further analysis showed that ECaC expressing HEK293 cells loaded with 10 mmol·l^{-1} BAPTA through the patch pipette, unlike control cells, display large inwardly rectifying currents in the presence of extracellular Ca^{2+} and Mg^{2+} (see Fig. 4C–F). These currents show high Ca^{2+} dependence and very positive reversal potentials. From the reversal potentials obtained by measurements in 30 mmol·l^{-1} extracellular Ca^{2+}($[Ca^{2+}]_e$) a permeability ratio $P_{Ca} : P_{Na}$ of more than 100 could be calculated (Vennekens et al. 2000). Furthermore, when Ca^{2+} currents were measured in the absence of extracellular Na^+ it was shown that the reversal potential of the current had shifted by 21 mV per 10-fold change in extracellular Ca^{2+} concentration, which is in fairly good agreement with the theoretical value of 29 mV predicted by the Nernst equation (Vennekens et al. 2001). These findings are indicative of a highly Ca^{2+} selective current. The divalent permeability sequence of this current is $Ca^{2+} > Ba^{2+} \sim Sr^{2+} > Mn^{2+}$ (Vennekens et al. 2000). Ca^{2+} currents in 100 μmol·l^{-1} $[Ca^{2+}]_e$ are blocked by extracellular Mg^{2+} with an IC_{50} of 328 ± 50 μmol·l^{-1} (mean ± S.E.M.). The trivalent cations lanthanum and gadolinium block 30 mmol·l^{-1} barium currents through ECaC with an IC_{50} value of 1.1 ± 0.2 μmol·l^{-1} and 4.6 ± 0.4 μmol·l^{-1} respectively (Vennekens et al. 2001).

Despite the channel's ability to select Ca^{2+} over Na^+ in physiological conditions, it becomes permeable to monovalent cations in the absence of extracellular divalent cations. This kind of behavior is indicative for the anomalous mole fraction behavior which was described before for voltage gated Ca^{2+} channels (Almers and McCleskey 1984; Hess et al. 1986) and will be discussed later. Monovalent currents through ECaC in the absence of extracellular divalent cations (nominal divalent cation free conditions) are large inwardly rectifying currents, with a mean reversal potential of +1.1 ± 0.8 mV (see Fig. 4A and F). The permeability sequence for monovalent cations was $Na^+ > Li^+ > K^+ > Cs^+ >>$ N-methyl-D-glucamine ($NMDG^+$), which is the Eisenmann X sequence, indicating a strong field strength binding site (Nilius et al. 2000a). Monovalent ECaC currents are completely blocked with 1 μmol·l^{-1} of either lanthanum or gadolinium. Magnesium blocks these monovalent currents with an IC_{50} value of 62 ± 9 μmol·l^{-1}, which is 100-fold less than the IC_{50} value derived in the presence of 100 μmol·l^{-1} Ca^{2+} (Vennekens et al. 2001). Mg^{2+} block in nominally Ca^{2+} free solutions is voltage dependent (see Fig. 4B and F). A standard Woodhull-analysis of this block indicates that Mg^{2+} binds to a single site located superficially (31% from outside) within the membrane electrical field. Block is dramatically increased at negative and weakened at positive potentials (Nilius et al. 2000a).

In contrast to a previous study on Cat1 (Peng et al. 1999) we were able to show single channel currents in cell attached and inside-out patches with a conductance of 77.5 ± 4.9 pS and a reversal potential of +14.8 ± 1.6 mV in the absence of divalent cations and in the presence of 150 mmol·l^{-1} Na^+ and 0.1 mmol·l^{-1} EGTA extracellularly (Nilius et al. 2000a). This reversal potential nicely fits that of the whole cell current under the same conditions (14.3 ± 1.8 mV, see also (Vennekens et al. 2000)). Unfortunately these results could not be obtained in the presence of

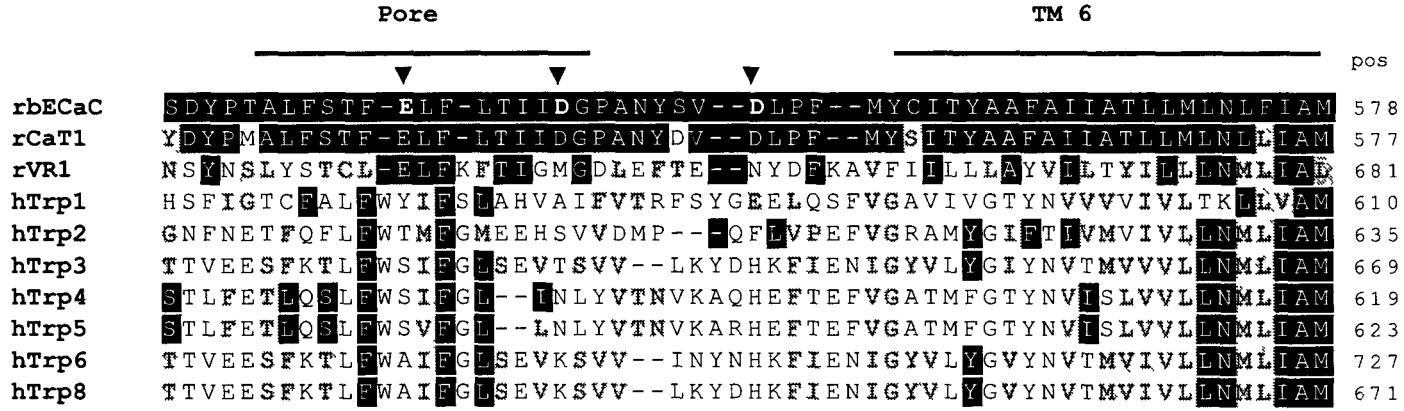
extracellular divalent cations However, these single channel data clearly underscore the channel nature of ECaC

Recently, a report was published concerning the electrophysiologic properties of the mouse homologue of ECaC overexpressed in CHO cells (Suzuki et al 2000) The overall properties reported there are fairly similar to our data, including high Ca^{2+} selectivity and Na^{+} permeation in the absence of extracellular divalents However, kinetic properties of the reported currents, including the rectification of the currents, are dramatically different from our data Whether this is due to the inherent differences between rabbit and mouse ECaC or the different expression system is unclear at the moment

The single pore residue Asp^{542} and anomalous mole fraction behaviour: a permeation model for ECaC

The pore region of ECaC (and of the highly homologous channel CaT1) contains a unique and highly conserved assembly of residues compared with VR1 and TRPC channels (Table 1) Most striking is the presence of three negatively charged amino acids in the pore, consisting of a glutamate at position 535 and aspartates at position 542 and 550 respectively (Table 1, see also Nilius et al 2000b) Through the analysis of point mutants of these residues we have found that the single aspartate residue at position 542 is responsible for the key features of ECaC including high Ca^{2+} permeability, block of currents by Mg^{2+} and Ca^{2+} dependent current decay (Nilius et al 2000b) Analogous to the pore structure of L-type voltage gated Ca^{2+} channels and since functional ECaC channels supposedly are formed by 4 subunits, it is tempting to speculate about a ring of four aspartate residues that forms a single high affinity Ca^{2+} binding site within the ECaC pore Permeation through ECaC shares some properties, such as high affinity block of monovalent currents by Ca^{2+} and other divalents, anomalous mole-fraction behaviour and high Ca^{2+} selectivity at higher Ca^{2+} concentrations with other Ca^{2+} selective channels, e g , voltage-gated Ca^{2+} channels (Almers and McCleskey 1984, Hess et al 1986) and the Ca^{2+} release activated Ca^{2+} channel (I_{CRAC}) (Lepple-Wienhues and Cahalan 1996) We have shown that ECaC displays anomalous mole-fraction behaviour when mixtures of Na^{+} and Ca^{2+} or of Ba^{2+} and Ca^{2+} are applied (Vennekens et al 2001) In the case of Na^{+} and Ca^{2+} this behaviour is exemplified (see Fig 5A) when inward current is measured as a function of the external Ca^{2+} concentration in a standard bath solution containing 150 mmol l^{-1} Na^{+} At very low Ca^{2+} concentrations (<100 nmol l^{-1}) a large inward current is apparent which is carried by Na^{+} When $[Ca^{2+}]_i$ is raised up to 100 μmol l^{-1} current densities will decrease, but as $[Ca^{2+}]_i$ increases further into the millimolar range inward current densities will rise again and their reversal potential shifts towards the equilibrium potential for Ca^{2+}, indicating that it is now mainly carried by Ca^{2+} This anomalous mole-fraction behaviour is generally accepted as evidence for a channel pore containing multiple binding sites occupied by permeant ions moving in single-file manner through the channel (Hille 1992) Two distinct kinetic models have been

Table 1. Alignment of the putative pore region of ECaC with that of homologues channels Identical residues are in black boxes, conservative substitutions are in gray boxes and non-conserved amino acids are in white boxes The GenBank accession numbers of the rabbit ECaC, rat CaT1, rat capsaicin receptor and other members of the TRPC channel family are AJ133128 (rbECaC), AF160798 (rCaT1), AF029310 (rVR1), X89066 (hTrp1), X89067 (hTrp2), U47050 (hTrp3), AF175406 (hTrp4), AF054568 (hTrp5), AF080394 (hTrp6), NM012035 (hTrp8)



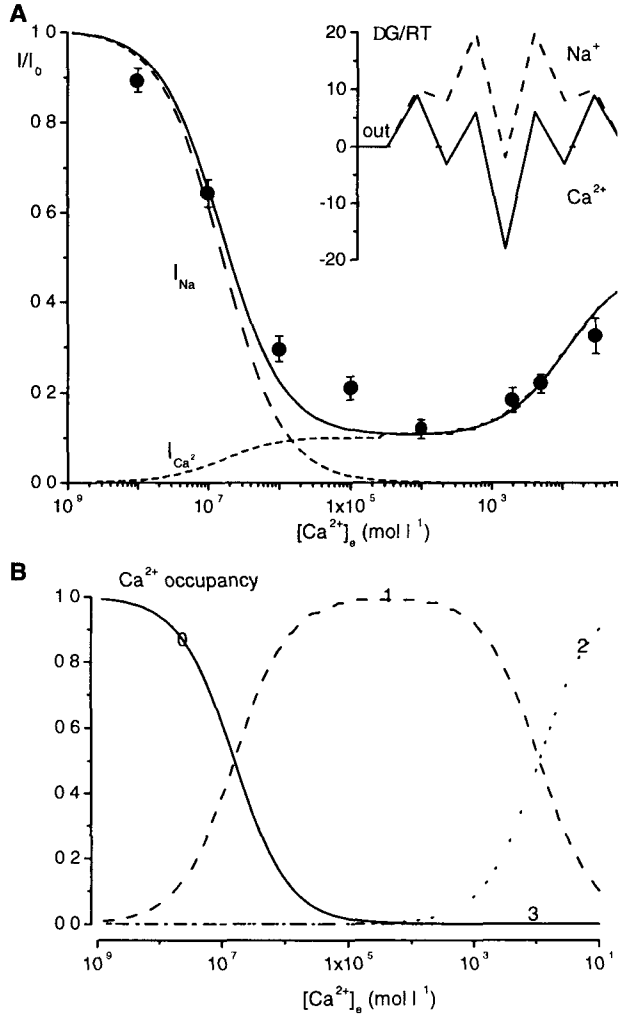


Figure 5. Anomalous mole-fraction behavior between Ca^{2+} and Na^{+} the step model **A.** Mean normalised current values measured at −80 mV during linear voltage ramps at various $[Ca^{2+}]_e$ Currents were normalised to the current value for the same cell in a buffered divalent cation free solution (1082 ± 164 pA/pF (mean ± SE) ranging between 295 and 3000 pA/pF, n = between 10 and 13) The solid line represents the current densities as predicted by a model with one high affinity binding site flanked by a low affinity binding site at each side, using the energy profiles for Ca^{2+} and Na^{+} depicted in the inset The dashed and dotted lines represent the fractions of the current carried by Ca^{2+} and Na^{+} respectively The inset shows an energy profile of the ECaC pore along the path of the pore for either Ca^{2+} or Na^{+} (in RT values, for details see (Vennekens et al 2001)) This illustrates the schematic structure of the ECaC pore, with one high affinity binding site flanked by two low affinity binding sites **B.** The predicted occupation of the ECaC pore by Ca^{2+} as a function of the Ca^{2+} concentration i e the chance to find 1, 2, 3 or no Ca^{2+} bound within the pore (For details see (Vennekens et al 2001))

developed that can describe ion permeation and that can account for this anomalous mole-fraction behaviour and the high ion transfer rate of the Ca^{2+} channel pore in the case of L-type voltage gated Ca^{2+} channels, i e the 'repulsion model' (Almers and McCleskey 1984, Hess et al 1986) and the 'step model' (Dang and McCleskey 1998) These models provide the opportunity to deduce general principles of ion permeation through the channel pore in the absence of an exact knowledge of the underlying molecular structure We have tried to describe our ECaC data with both models but found only a fairly good description with a reasonable set of parameters for the step model (the solid line in Fig 5A) The step model envisions a channel pore in which two low affinity binding-sites flank a central high affinity binding-site (see the inset in Fig 5A) Applied to ECaC we propose a ring of four aspartates as the central high affinity Ca^{2+} binding site As is discussed in Dang and McCleskey (1998) the low-affinity binding sites need not to be explicit binding sites built into the pore It is clear that every intermediate step in the stepwise replacement of water molecules by coordinating ligands (the 4 aspartates) and *vice versa* will provide a step of potential energy in the Ca^{2+} ion's travel across the pore

In the absence of extracellular Ca^{2+} the channel pore is available for monovalent cation permeation However in the presence of $[Ca^{2+}]_e$ the binding-sites within the pore will preferentially bind Ca^{2+}, as a result of the higher binding affinity of the binding sites for Ca^{2+} compared to Na^+ From the Ca^{2+} occupancy plot (Fig 5B) it is clear that block of monovalent currents in nanomolar $[Ca^{2+}]_e$ occurs through binding of a single Ca^{2+} ion in the pore when $[Ca^{2+}]_e$ rises to the micromolar range From this blocking effect one can calculate an IC_{50} and estimate thereby the affinity of the central binding site for Ca^{2+} In the case of ECaC we found that 50 percent current inhibition occurs at a Ca^{2+} concentration of about 0 2 μmol l^{-1}, a value that is comparable to that of L-type Ca^{2+} channels (0 7 μmol l^{-1} (Almers and McCleskey 1984)) The Ca^{2+} flux at higher $[Ca^{2+}]_e$ is generated in parallel with multiple occupancy of the channel pore, although the chance to find the ECaC pore in the triple Ca^{2+} occupied state is very low Put in a more direct way, Ca^{2+} flux in this model parallels the occupancy of the internal low affinity binding-site The drive for ion permeation results from the steps in binding affinity provided by the low affinity sites, as if the flanking sites provide stair steps for the ion to mount out of the channel pore (Dang and McCleskey 1998)

Regulation of the ECaC channel

As much as is known about the electrophysiological properties of ECaC, as little is known about its regulation The data obtained with our expression system indicate that ECaC is a constitutively open channel Thus, a substantial Ca^{2+} influx will flow through ECaC into distal nephron cells with a membrane potential around – 70 mV under non stimulated physiological conditions (Friedman and Gesek 1995) In order to protect cells from Ca^{2+} overload it is therefore of crucial importance that the ECaC channel is subject to some kind of feedback inhibition to limit

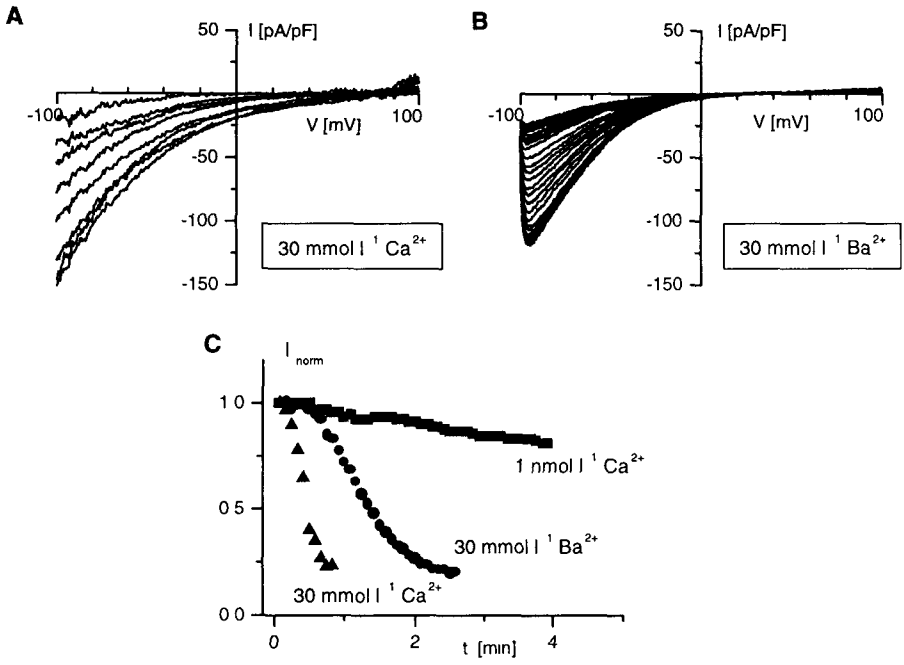


Figure 6. Rundown of ECaC currents during repetitive stimulation **A.** Rundown of the current through ECaC in the presence of 30 mmol l^{-1} $[Ca^{2+}]_e$ Voltage ramps from −100 to +100 mV (V_H = +20 mV, duration of ramps = 400 ms were applied every 5 s, sampling rate = 5 kHz and cut-off frequency = 2 kHz in all experiments) **B.** Rundown of the current through ECaC in the presence of 30 mmol l^{-1} $[Ba^{2+}]_e$ Voltage ramps, as above, were applied every 5 s **C.** Representative examples of the time course of the current rundown from three single cells in either Ca^{2+} free medium, 30 mmol l^{-1} Ba^{2+} or 30 mmol l^{-1} Ca^{2+} containing medium, as indicated All extracellular solutions contained 1 mmol l^{-1} Mg^{2+}, Intra- and extracellular solutions are as described in Fig 4 For details on methods see (Vennekens et al 2000)

the amount of calcium entering the cell. Indeed, during repetitive stimulation in Ca^{2+} containing solutions, the channel activity will run down (Vennekens et al 2000) (Fig. 6A) This process is clearly dependent on Ca^{2+} permeation, because it is significantly slower in low Ca^{2+} containing solutions or when Ba^{2+} is used as the charge carrier (Fig. 6B). When monovalent cations permeate through the channel, the rundown process is virtually absent (Vennekens et al 2000) (Fig. 6C) The nature of this process remains however unclear. Apparently elevated levels of intracellular Ca^{2+}, presumably in the immediate vicinity of the ECaC pore, are involved since loading ECaC expressing HEK293 cells with 1 μmol l^{-1} Ca^{2+} significantly lowers current densities, independently on whether Ca^{2+} or monovalent cations are the charge carriers (Vennekens et al. 2000) Ca^{2+} dependent feedback

inhibition can also be identified from the Ca^{2+} current response to hyperpolarizing voltage steps (see appropriate traces in Fig 4) These responses are characterised by a rapid but incomplete inactivation, consisting of a fast and a slow component This inactivation is absent in divalent cation free conditions, and the fast component disappears when Ba^{2+} or Sr^{2+} is used as a charge carrier (Vennekens et al 2000)

As postulated for other ion channels, it is possible that the above processes involve phosphorylation and/or dephosphorylation of the channel or associated proteins (Hille 1992, Levitan 1994) In this respect, it is intriguing to speculate on the role of the calcium-calmodulin-dependent protein kinase II for which functionally conserved consensus sites are present in ECaC (S142, S693) (Hoenderop et al 1999, Peng et al 1999) It has recently been demonstrated that this kinase is involved in the Ca^{2+}-dependent regulation of channel activity (Chao et al 1995, Shen and Meyer 1999) 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 depolymerization of the actin cytoskeleton (Price et al 1999) Together with the co-localization of ECaC and calbindin-D28K 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 long-term activation

Perspectives

In the current minireview we presented an overview of the functional data that is currently available on the epithelial Ca^{2+} channel ECaC It is clear however that many questions remain unsolved Perhaps the most important issue to be elucidated concerns the regulation of ECaC, including the underlying mechanism of the Ca^{2+} dependent decay process Furthermore it will be crucial to explore the effect of pharmacological agents, which have been shown to regulate transcellular calcium transport in the distal part of the nephron More specifically the direct and/or indirect effect of factors such as hormones (e g , parathyroid hormone), protein kinase C, cAMP and cGMP dependent protein kinase, calcitonin and vitamin D_3 on the properties of ECaC should be examined Furthermore little or no data is available concerning the pharmacology of ECaC At the moment no specific blockers of ECaC are available, but it is clear that the availability of these compounds would provide the important possibility to study ECaC function *in vivo* An interesting lead in this regard could be the arginine-rich peptides (such as dynorphin A) that have been shown to block VR-1 channels with submicromolar efficiency (Planells-Cases et al 2000) From a clinical point of view, it is clear that understanding of the ECaC permeation and regulation of its gating will be crucial to understand the pathophysiology of several hypercalciuremic diseases and to develop novel pharmaceutical tools Furthermore, from our knowledge of structure function relation, especially for the channel pore, novel functional defective mutants can be predicted

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