Phenotype of a recombinant store-operated channel: highly selective permeation of Ca^{2+}

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1. Genes related to *trp* (*transient receptor potential*) are proposed to encode store-operated channels. We examined the ionic permeation of recombinant channels formed by stable and transient expression of the TRP homologue bCCE1 in Chinese hamster ovary (CHO) cells (CHO(CCE1)) and rat basophilic leukaemia (RBL) cells, respectively.
2. Store-operated currents were activated in CHO(CCE1) cells by internal dialysis of IP_3 under strong buffering of intracellular Ca^{2+}. The action of IP_3 was mimicked by thapsigargin but not by IP_4.
3. With extracellular Ca^{2+}, Na^+ and Mg^{2+}, the store-operated currents of CHO(CCE1) rectified inwardly in the presence of internal Cs^+. Outward currents were not detected below +80 mV. Identical currents were recorded with external Ba^{2+} and also with no external Na^+ and Mg^{2+}. In the absence of external Mg^{2+}, the inward currents showed an anomalous mole fraction behaviour between Ca^{2+} and Na^+. Half-maximal inhibition of Na^+ currents was observed with ~100 nM and full block with 2–5 μM external Ca^{2+}.
4. In the parental CHO(–) cells, IP_3 dialysis evoked inward currents that also displayed anomalous mole fraction behaviour between Ca^{2+} and Na^+. However, half-maximal block of Na^+ currents required 5 times higher Ca^{2+} concentrations in CHO(–) cells. Additionally, the density of Ca^{2+} and Na^+ currents at –80 mV was 5 and 2 times larger in CHO(CCE1) cells, respectively.
5. In RBL cells, dialysis of IP_3 evoked store-operated currents that showed 1·4-fold larger densities at –80 mV in cells expressing bCCE1.
6. The enhanced density of store-operated currents in CHO(CCE1) cells and in bCCE1-transfected RBL cells probably reflects the phenotype of CCE1. These results suggest a highly selective permeation of Ca^{2+} through recombinant channels formed by CCE1 either alone or in combination with endogenous channel proteins.

The mobilization of calcium generated by hormones and growth factors underlies vital functions in virtually all cells. In non-excitable cells, Ca^{2+} ions are released from intracellular stores and also enter the cell through capacitative calcium entry (CCE) channels, which open upon depletion of Ca^{2+} stores (Berridge, 1995; Putney, 1997). Due to the link between Ca^{2+} entry and Ca^{2+}-store depletion, CCE channels are also called store-operated channels. Although the ionic currents through CCE channels have been extensively characterized (see Putney, 1997), the molecular basis for capacitative calcium entry remains elusive. A hint was provided by the finding that the *transient receptor potential* (*trp*) gene of *Drosophila* (Montell & Rubin, 1989) encodes proteins that form recombinant store-operated channels (Vaca *et al.* 1994; Gillo *et al.* 1996; Xu *et al.* 1997). Expression studies with the TRP homologues bCCE1 (originally bCCE; Philipp *et al.* 1996), rCCE2 (also called TRP5; Philipp *et al.* 1998) and TRPC1A (Zitt *et al.* 1996) revealed in mammals a family of ionic channels, which have in common a store-operated activation. Surprisingly, the activation of recombinant channels formed by TRPC3 and mTrp6 may not require depletion of intracellular Ca^{2+} stores (Zitt *et al.* 1997; Boulay *et al.* 1997). It is therefore likely that not all genes related to *trp* encode CCE channels. Furthermore, TRPC1A appears to form non-selective recombinant cation channels (Zitt *et al.* 1996) and could be therefore subunits of non-selective native CCE channels (e.g. Lückhoff & Clapham, 1994; Vaca & Kunze, 1994; Krause *et al.* 1996). By contrast, bCCE1 apparently forms Ca^{2+}-selective channels (Philipp *et al.* 1996). Additionally, antisense directed to the CCE1 orthologue mTrp4 suppresses the capacitative calcium entry in L cells (Birnbaumer *et al.* 1996). Thus, CCE1 or another closely related TRP homologue could be implicated in the formation of Ca^{2+}-selective CCE channels (e.g. Hoth & Penner, 1993; Parekh *et al.* 1993; Zweifach & Lewis, 1993; Yao & Tsien, 1997). It is not known, however, whether the mechanism of ion permeation through recombinant CCE1 channels accounts for a selective Ca^{2+} inflow, as is the case with native Ca^{2+}-selective CCE channels (Hoth, 1995; Lepple-Wienhues & Cahalan, 1996).

The construction of a cell line stably expressing bCCE1 allowed us to study the permeation of Ca^{2+} and Na^+ through recombinant CCE1 channels. We found that the currents of CHO cells expressing CCE1 display an anomalous mole fraction behaviour between Ca^{2+} and Na^+. As expected for a Ca^{2+}-selective entry pathway, micromolar external Ca^{2+} was sufficient to block CCE1 channel currents carried by Na^+. Additionally, we expressed bCCE1 in the RBL cell line, which is used as a model in studies of store-operated currents.

METHODS

The bovine CCE1 cDNA (originally bCCE, Philipp *et al.* 1996) was subcloned into the expression vector pMT3 (Genetics Institute, Cambridge, MA, USA) containing the dihydrofolate reductase (DHFR) gene as a selection marker. Stable expression was carried out in a CHO(−) cell line deficient in DHFR using media and conditions previously described (Bosse *et al.* 1992). For detection of bCCE1 and DHFR transcripts by RT-PCR, 1 μg total RNA was reverse transcribed using oligodeoxythymidine as primer. bCCE1 cDNA fragments (293 bp) were amplified with primers 5′GGG-TCAAGTATCACAACC3′ and 5′GCTGGAGTCCCGAACTC3′ and DHFR cDNA fragments (334 bp) with primers 5′CAAGAACGGA-GACCTACC3′ and 5′TGGTTGATTCATGGCTTCC3′. For Northern blot analysis, 10 μg poly(A)$^+$ RNA isolated from CHO(−) and CHO(CCE1) cells was electrophoresed and blotted as described previously (Philipp *et al.* 1996). The complete coding bCCE1 cDNA was used as a probe for hybridization. To control the integrity of the transferred poly(A)$^+$ RNA, the same filter was stripped and rehybridized with a 239 bp cDNA fragment of the human glyceraldyde-3-phosphate dehydrogenase (GAPDH). For recombinant expression of bCCE1 in RBL-1 cells, we constructed the dicistronic expression plasmid pdiCCE1. This dicistronic plasmid is based on the eukaryotic vector pCAGGS (Niwa *et al.* 1991) and contains the bCCE1 cDNA (Philipp *et al.* 1996) downstream of the β-actin promoter followed by the internal ribosome entry site (IRES) of the encephalomyocarditis virus and the cDNA of the green fluorescent protein (GFP). Approximately 1×10^5 RBL cells were seeded in 35 mm dishes 1 day before transfection. The transfection mixture consisted of 5 μl SuperFect (Gibco) and 3 μg pdiCCE1. In mock transfections, we used the pCAGGS vector containing only the GFP cDNA. The IRES sequence of the pdiCCE1 vector allows the simultaneous translation of bCCE1 and GFP from one transcript and, accordingly, cells expressing GFP must also express bCCE1. Current recordings were performed only in GFP expressing cells 48–72 h after transfection.

Membrane currents were recorded in the whole-cell mode of the patch-clamp technique (Hamill *et al.* 1981). Since CHO cells express various ionic currents, we established conditions that favour the recording of nearly pure CCE currents (Fig. 1*C*). Basically, K^+ channel currents were inhibited by internal Cs^+ and Ca^{2+}-activated Cl^- currents by internal EGTA. Volume-regulated currents were not observed. The standard pipette solution used for internal dialysis contained (mM): 115 CsCl, 10 EGTA, 4 $MgCl_2$ and 10 Hepes; pH 7·2 (CsOH). When indicated, BAPTA (10 mM) was used instead of EGTA in the dialysate (pH 7·2). The concentrations of 400 nM and 1 μM internal Ca^{2+} ($[Ca^{2+}]_i$) were obtained by adding 6·9 and 8·5 mM $CaCl_2$ to the standard pipette solution (10 mM EGTA) before adjusting the pH to 7·2, respectively. To achieve rapid cell dialysis, patch pipettes had resistances of 2–3 MΩ. The external solution contained (mM): 115 NaCl, 5 KCl and 10 Hepes; pH 7·2 (NaOH). The final extracellular Na^+ concentration ($[Na^+]_o$) was 120 mM. The concentrations of external free Ca^{2+} ($[Ca^{2+}]_o$) were adjusted to 2 and 5 μM with 1 mM HEDTA mixed with 0·39 and 0·61 mM $CaCl_2$ (pH 7·2), respectively. The external Ca^{2+} concentration of 100 nM was obtained with 1 mM EGTA and 0·4 mM $CaCl_2$ (pH 7·2). A Ca^{2+} concentration of 1 nM was assumed for the solution containing only 1 mM EGTA (pH 7·2). $[Ca^{2+}]_o$ is given throughout as pCa_o. In the external solutions containing EGTA or HEDTA, the concentration of NaCl was reduced to maintain a final Na^+ concentration of 120 mM after adjustment of the pH to 7·2 (NaOH). The extracellular concentration of Mg^{2+} ($[Mg^{2+}]_o$) represents the total amount of added $MgCl_2$. The Ba^{2+} solution was prepared by adding 10 mM $BaCl_2$ and 2 mM $MgCl_2$ to the external solution. In some experiments, extracellular Na^+ was replaced with $NMDG^+$ (mM): 115 NMDG, 10 Hepes; pH 7·2 (HCl).

Whole-cell currents were elicited every 5 s by voltage-clamp ramps (400 ms) from −100 to +100 mV delivered from a holding potential of 0 mV. Series resistances (R_s) and whole-cell membrane capacitance (C_m) were read from the settings provided by the patch-clamp amplifier (EPC-7, List Electronic). Data were analysed from experiments with R_s below 7 MΩ. C_m was $17·93 \pm 4·58$ pF ($n = 131$). Electronic compensation of 30–50 % was used to reduce charging time. Currents were sampled at 10 kHz and filtered at 3 kHz. Liquid junction potentials between −10 and +5 mV measured as described by Neher (1992) were not corrected. Current traces were leak subtracted using an average of three to five traces collected within the first 60 s and 6 s of recordings in CHO and RBL cells, respectively. For analysis, the maximal current observed during CCE activation was normalized to cell size using C_m. Our limit of resolution was 0·1 pA pF^{-1}, which corresponds to inward currents of 1·5–2·5 pA at −80 mV in leak-subtracted traces. Accordingly, cells showing current densities below this limit of resolution were not included in further calculations. Experiments with different concentrations of external Ca^{2+} were carried out in separated cells. Data were analysed with Student's *t* test and are given as the mean ± S.D.

RESULTS

Stable expression of CCE1 in CHO cells

The bovine CCE1 (originally bCCE, Philipp *et al.* 1996) was stably expressed in CHO cells. As illustrated in Fig. 1*A*, RT-PCR analysis confirmed the expression of bCCE1 in two selected clones, CHO(CCE1) and CHO(CCE1a). Accordingly, Northern analysis (Fig. 1*B*) revealed the expression of ∼3·9 kb transcripts corresponding to the expected size of bCCE1 transcripts expressed from the pMT vector. Additional 1·5 kb transcripts may arise from rearrangements or deletion events during selection of stable cell lines (Kaufman *et al.* 1991). No expression of CCE1-related transcripts was detected in parental CHO(−) cells (Fig. 1*A* and *B*).

In the presence of extracellular Ca^{2+}, Na^+ and Mg^{2+}, internal dialysis with 10 μM IP_3 and 10 mM EGTA evoked in CHO(CCE1) cells inward currents that differed from native currents in CHO(−) cells (Fig. 1*C*). The leak-subtracted currents of CHO(CCE1) cells showed prominent inward rectification and reversed at potentials > +40 mV. In fact, no outward current was observed below +80 mV with Cs^+ in the dialysate. For comparison, we calculated the densities of leak-subtracted currents at −80 mV. On average, the density of inward currents was $1{\cdot}63 \pm 0{\cdot}85$ pA pF^{-1} in 17 out of 18 CHO(CCE1) cells and statistically higher ($P < 0{\cdot}001$) than in CHO(−) cells ($0{\cdot}32 \pm 0{\cdot}23$ pA pF^{-1}, $n = 18$). Similar currents were recorded in 5 out of 6 cells of the CHO(CCE1a) line ($1{\cdot}25 \pm 0{\cdot}68$ pA pF^{-1}) dialysed with IP_3, suggesting that the enhanced densities of inwardly rectifying currents are due to the expression of bCCE1 and

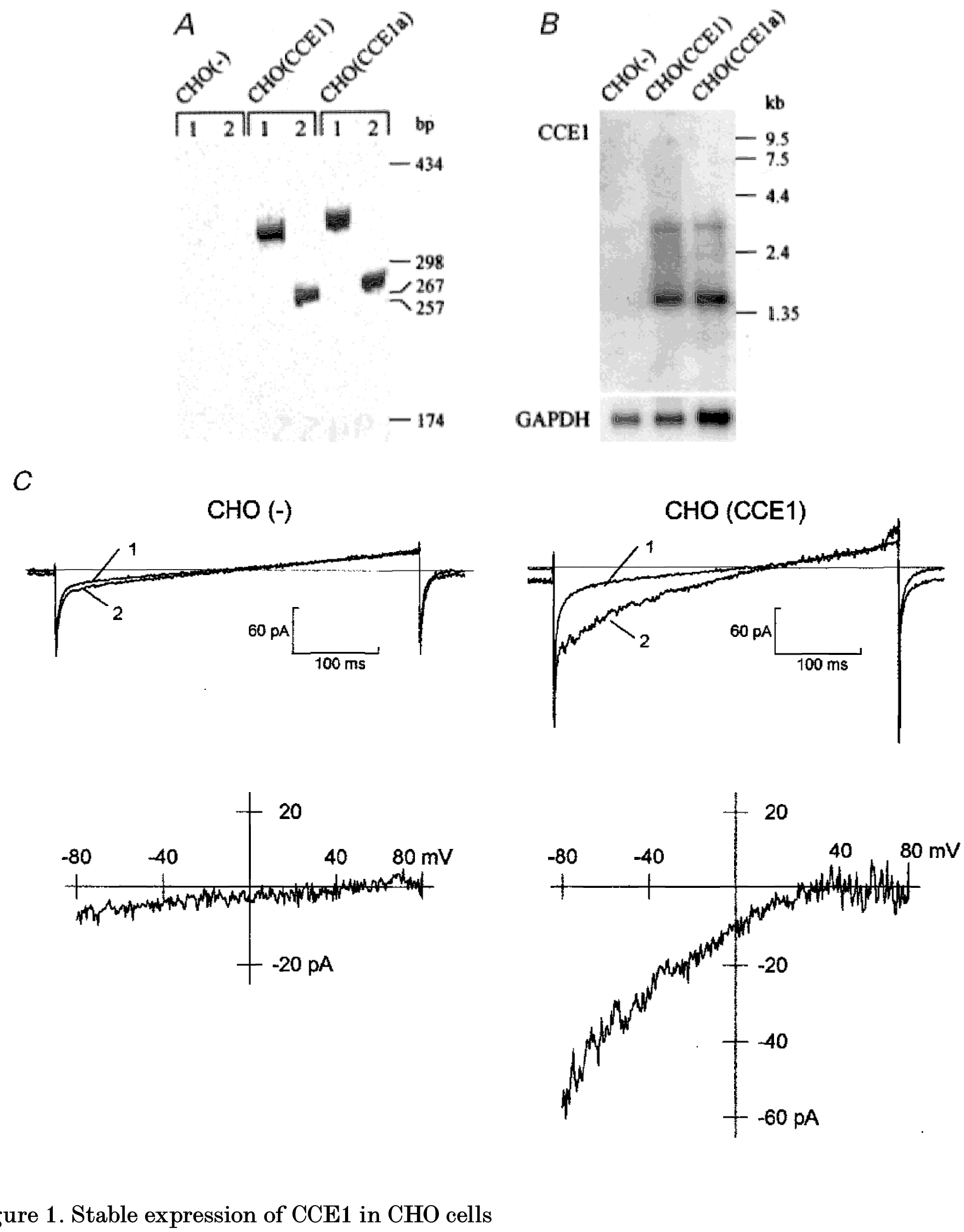

Figure 1. Stable expression of CCE1 in CHO cells

A, RT-PCR analysis of parental (CHO(−)) and bCCE1-transfected (CHO(CCE1), CHO(CCE1a)) cells. PCR primers were specific for the selection marker DHFR (1) and for bCCE1 (2). *B*, Northern blot analysis of CCE1 and GAPDH (glyceraldehyde-3-phosphate-dehydrogenase) expression. *C*, activation of ionic currents in CHO(−) and CHO(CCE1) cells by internal dialysis of 10 μM IP_3. Ionic currents were elicited every 5 s by voltage-clamp ramps (400 ms) from −100 to +100 mV delivered from a holding potential of 0 mV. Leak currents (1) were collected at the start of dialysis and enhanced currents (2) after IP_3 dialysis. Current–voltage relationships (lower panels) were obtained by subtracting leak currents from activated currents. C_m: 15 pF (left) and 20 pF (right). The internal dialysate contained 115 mM Cs^+ and 10 mM EGTA. The external solution contained 120 mM Na^+, 10 mM Ca^{2+} and 2 mM Mg^{2+}.

not to selection of a CHO clone with high density of endogenous currents. With 10 mM BAPTA instead of EGTA (Fig. 2*A*), the IP_3-induced currents of CHO(CCE1) cells showed similar densities ($1{\cdot}36 \pm 0{\cdot}48$ pA pF^{-1} in 8 out of 9 cells). When the dialysate contained 10 μM IP_3 and 400 nM free Ca^{2+} (Fig. 2*B*), however, the inward currents attained densities of $0{\cdot}62 \pm 0{\cdot}24$ pA pF^{-1} in 5 out of 18 cells. No inward current was detected upon dialysis of 10 μM IP_3 in the presence of 1 μM free Ca^{2+} ($n = 5$), suggesting that the action of IP_3 required strong buffering of intracellular Ca^{2+}.

As previously observed in transient expression experiments (Philipp *et al.* 1996), the activation of inward currents in CHO(CCE1) cells was detected 140–330 s after the onset of IP_3 dialysis and the inward currents remained enhanced for $\leqslant 40$ s. In CHO(−) cells, the endogenous inward currents (Fig. 1*C*) activated also with a delay of 49–189 s. In order to ensure that the inwardly rectifying currents were activated by store depletion, intracellular Ca^{2+} stores were emptied in an IP_3-independent fashion by the Ca^{2+}-ATPase inhibitor thapsigargin (Thastrup *et al.* 1990). After application of 1–2 μM thapsigargin to CHO(CCE1) cells dialysed with 10 mM EGTA (Fig. 2*C*), we observed inward currents with densities of $1{\cdot}19 \pm 0{\cdot}40$ pA pF^{-1} ($n = 7$) similar to those induced by IP_3 (see Fig. 1*C*). Dialysis of 20 μM IP_4 and 10 mM EGTA induced currents with densities of $0{\cdot}30 \pm 0{\cdot}12$ pA pF^{-1} in 4 out of 6 CHO(CCE1) cells (Fig. 2*D*). Thus, the action of IP_3 in the CHO(CCE1) cell line required strong buffering of intracellular Ca^{2+} and was mimicked by thapsigargin but not by IP_4. These data

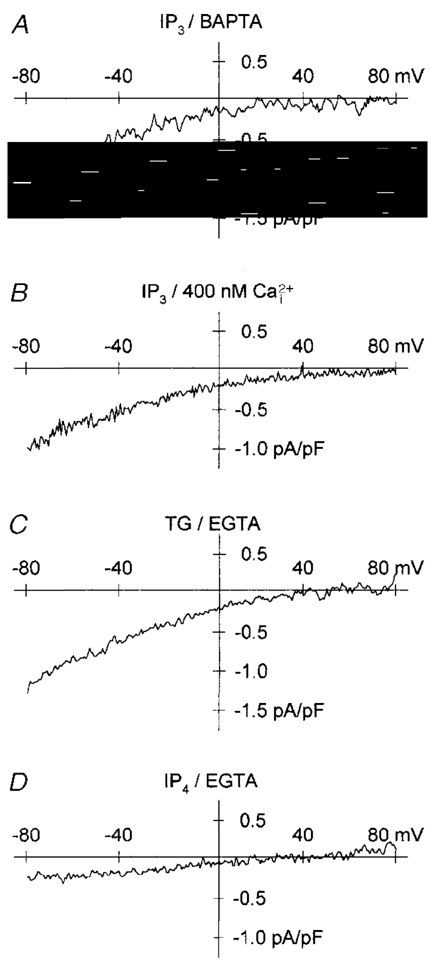

Figure 2. Activation of ionic currents by IP_3, IP_4 and thapsigargin in CHO(CCE1) cells

The internal solution contained 115 mM Cs^+. IP_3 (10 μM) was dialysed in the presence of 10 mM BAPTA (*A*, IP_3/BAPTA) and 400 nM free Ca^{2+} (*B*, IP_3/400 nM Ca_i^{2+}). Thapsigargin (1 μM) was applied to cells dialysed with 10 mM EGTA (*C*, TG/EGTA). IP_4 (20 μM) was dialysed with 10 mM EGTA (*D*, IP_4/EGTA). Same external solution as in Fig. 1.

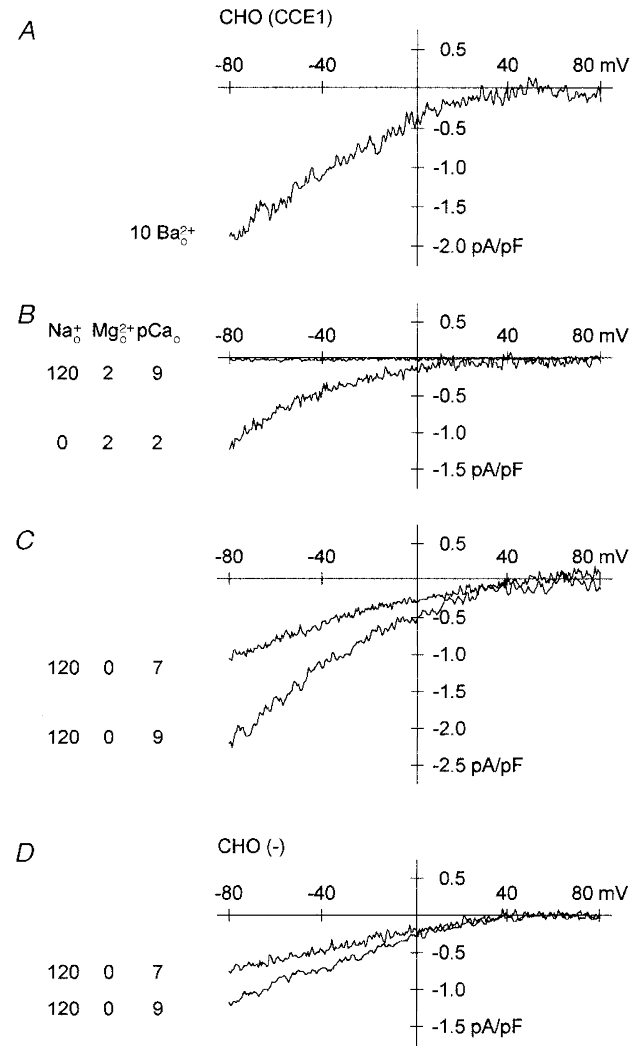

Figure 3. Permeation of monovalent and divalent cations through channels activated by dialysis of IP_3 in CHO(CCE1) and CHO(−) cells

Ionic currents were recorded in CHO(CCE1) cells in the presence of extracellular Ba^{2+} (*A*) and with either external Na^+ or Ca^{2+} in the presence of Mg^{2+} (*B*). Currents recorded with external Na^+ in CHO(CCE1) (*C*) and CHO(−) (*D*) were blocked by extracellular Ca^{2+} in the absence of Mg^{2+}. External concentrations of Ba^{2+} (Ba_o^{2+}), Na^+ (Na_o^+) and Mg^{2+} (Mg_o^{2+}) are millimolar. The external concentration of Ca^{2+} is given as pCa_o. The intracellular concentration of Cs^+ was 115 mM.

support the idea that recombinant channels are activated via a store-operated mechanism in cells expressing bCCE1 (Philipp *et al.* 1996).

Ion permeation in cells expressing CCE1

Native CCE channels expressed in a number of cell types are characterized by a high selectivity for Ca^{2+} over Na^{+} (Hoth & Penner, 1993; Parekh *et al.* 1993; Zweifach & Lewis, 1993; Yao & Tsien, 1997). In some other instances, store-operated channels seem to be less Ca^{2+} selective (Lückhoff & Clapham, 1994; Vaca & Kunze, 1994; Krause *et al.* 1996). If CCE1 encodes channel proteins that mediate a capacitative entry pathway selective for Ca^{2+}, it can be expected that the ion permeation through recombinant CCE1 channels shares basic properties with the permeation through Ca^{2+}-selective channels (see Almers & McCleskey, 1984; Hess & Tsien, 1984; Hoth, 1995; Lepple-Wienhues & Cahalan, 1996). We examined whole-cell currents of CHO cells expressing bCCE1 under various ion conditions to outline the permeation of monovalent and divalent cations through recombinant CCE1 channels.

The ability of external Ca^{2+}, Ba^{2+} and Na^{+} to act as charge carriers in the presence of internal Cs^{+} is illustrated in Fig. 3. Like currents recorded in external Ca^{2+} (Fig. 1*C*), the store-operated currents of CHO(CCE1) cells displayed inward rectification and densities of $1{\cdot}88 \pm 0{\cdot}51$ pA pF^{-1} ($n = 3$) in external Ba^{2+} (Fig. 3*A*). Thus, as reported for native CCE channels (Hoth, 1995), Ca^{2+} and Ba^{2+} function as charge carriers in CHO(CCE1) cells. Removal of external Na^{+} had no effect on store-operated currents recorded in CHO(CCE1) cells but, conversely, no current flow was detected when extracellular Ca^{2+} was reduced (Fig. 3*B*). Since external Mg^{2+} was present in the experiments of Fig. 3*B*, the permeation of Na^{+} was probably blocked by Mg^{2+}. In fact, we succeeded in recording Na^{+} currents in CHO(CCE1) cells after removal of external Mg^{2+} (Fig. 3*C*). In addition, the complete lack of current flow in experiments with CHO(CCE1) cells (Fig. 3*B*) revealed also a blockade of native CCE channel currents. Accordingly, Na^{+} currents were also detected in CHO(−) cells after removal of Mg^{2+} (Fig. 3*D*). Thus, as in other cell systems (Lepple-Wienhues & Cahalan, 1996), Na^{+} acts as a charge carrier in CHO(CCE1) and CHO(−) cells only in the absence of external divalent cations. The blockade of Na^{+} currents by Ca^{2+} was assayed in the absence of external Mg^{2+}. Under these conditions, an increase of extracellular Ca^{2+} strongly reduced Na^{+} currents in CHO(CCE1) and CHO(−) cells (Fig. 3*C* and *D*).

The ability of external Ca^{2+} to block the movements of other permeant cations is a distinct feature of the ionic

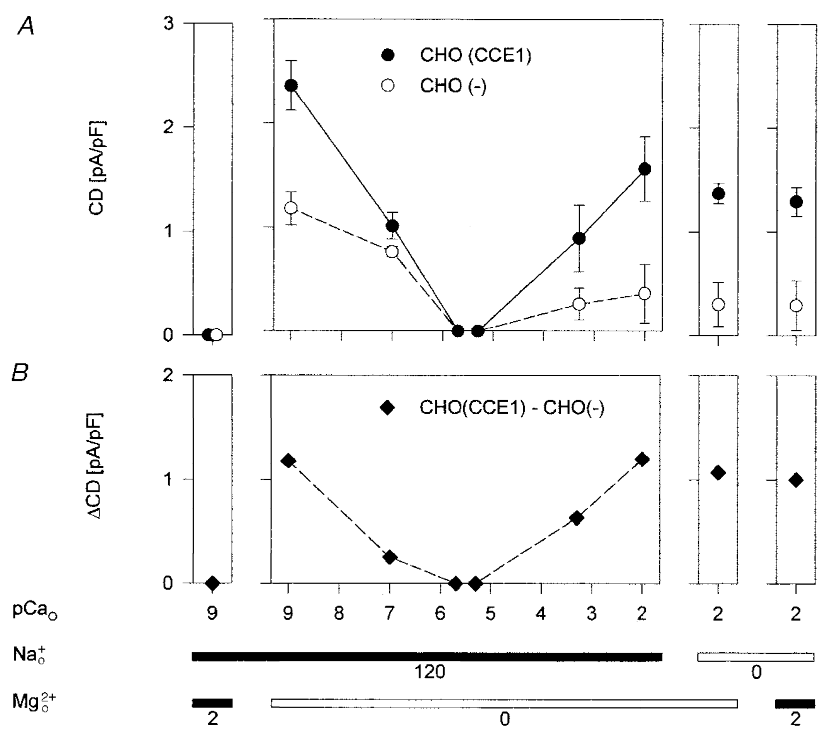

Figure 4. Anomalous mole fraction dependence of ionic currents activated by depletion of Ca^{2+} stores in CHO(CCE1) and CHO(−) cells

A, total current density (CD) measured at −80 mV. $n = 3$–8. Current densities (*A*) were statistically different ($P < 0{\cdot}001$) at pCa_o 9 and 2. *B*, density of difference currents (ΔCD) calculated by subtracting mean current densities of CHO(−) cells from those of CHO(CCE1) cells. The internal dialysate contained 115 mM Cs^{+}. The ionic composition of the external solution is shown under the panels, where Na^{+}_o and Mg^{2+}_o are millimolar. The external Ca^{2+} concentration is given as pCa_o.

permeation through voltage-dependent and store-operated Ca^{2+}-selective channels (Almers & McCleskey, 1984; Hess & Tsien, 1984; Hoth, 1995; Lepple-Wienhues & Cahalan, 1996). Since models that include Ca^{2+} binding sites within the pore of Ca^{2+}-selective channels predict an anomalous mole fraction dependence of ionic currents (Dang & McCleskey, 1998), we examined the currents of CHO(CCE1) and CHO(−) cells in various mixtures of monovalent and divalent cations. When the external concentration of Ca^{2+} (pCa_o) was varied almost 7 orders of magnitude and the extracellular Na^+ concentration maintained constant, the store-operated currents measured at −80 mV in the absence of Mg^{2+} approached a minimum at Ca^{2+} concentrations in the micromolar range (Fig. 4*A*). On the other hand, removal of external Na^+ and Mg^{2+} had no effect on currents measured with millimolar Ca^{2+} (Fig. 4*A*), indicating that Ca^{2+} rather than Na^+ supports CCE currents at high Ca^{2+} concentrations. Mg^{2+} was unlikely to support current flow because no current was measured in solutions with reduced Ca^{2+} (Fig. 3*B*). Thus, like Ca^{2+}-selective channels (Almers & McCleskey, 1984; Lepple-Wienhues & Cahalan, 1996), the CCE channels of CHO(CCE1) and CHO(−) cells display anomalous mole fraction behaviour between Ca^{2+} and Na^+. Moreover, we found different current–pCa_o curves in CHO(CCE1) and CHO(−) cells (Fig. 4*A*). The difference that arose from the expression of bCCE1 in CHO cells is illustrated in Fig. 4*B*. Ca^{2+} currents ($pCa_o = 2$) in CHO(CCE1) cells were 5 times larger than in CHO(−) cells and Na^+ currents ($pCa_o = 9$) were 2 times larger. Additionally, the ratio of Na^+ currents to Ca^{2+} currents was 1·5 in CHO(CCE1) cells and 3·5 in CHO(−) cells. These different current–pCa_o relationships indicate that the expression of bCCE1 in CHO cells enhanced preferentially store-operated currents carried by Ca^{2+}. Additionally, the Ca^{2+} sensitivity of currents carried by Na^+ was also enhanced in CHO cells expressing bCCE1 (Fig. 3*C* and *D*). The descending limbs of the current–pCa_o relationships shown in Fig. 4*A* indicate that ~100 nM and ~500 nM external Ca^{2+} inhibited Na^+ currents half-maximally in CHO(CCE1) and CHO(−) cells, respectively. Full block of Na^+ currents was obtained with Ca^{2+} concentrations higher than 1 μM, as with Na^+ currents through Ca^{2+}-selective channels (see Almers & McCleskey, 1984; Lepple-Wienhues & Cahalan, 1996). In conclusion, CCE currents of CHO(−) and CHO(CCE1) cells showed an anomalous mole fraction behaviour between Ca^{2+} and Na^+ in the absence of Mg^{2+}.

Expression of CCE1 in RBL cells

Since the store-operated currents of CHO(CCE1) cells were larger and slightly more sensitive to external Ca^{2+} than native currents of CHO cells, we expressed bCCE1 in RBL cells. Upon dialysis of 10–20 μM IP_3 and 10 mM EGTA in mock-transfected RBL cells (Fig. 5*A*), the inwardly rectifying currents increased to attain densities of $3·7 \pm 0·68$ pA pF^{-1} within 32–45 s. The expression of bCCE1 had no effect on the reversal potential (> +40 mV) and rectification properties of the store-operated currents in RBL cells (Fig. 5*A*). However, the density of the inward

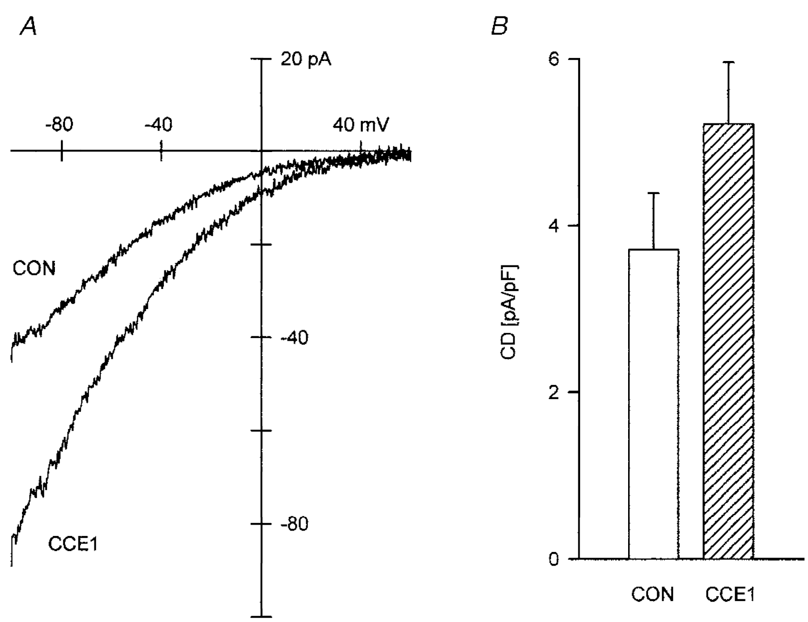

Figure 5. Expression of bCCE1 in RBL cells

A, representative current traces evoked by dialysis of 10 μM IP_3 and 10 mM EGTA in mock-transfected (CON) and bCCE1-transfected (CCE1) RBL cells. C_m: 11 pF, CON; 12 pF, CCE1. Same internal and external solutions as in Fig. 1. *B*, density of inward currents at −80 mV in mock- and bCCE1-transfected RBL cells. The difference is statistically significant ($P < 0·001$). $n = 10$ for CON and 11 for CCE1.

currents at −80 mV was 1·4 times larger in bCCE1-transfected RBL cells. These results support the previous finding in CHO cells, that the expression of bCCE1 enhances the density of Ca^{2+}-selective store-operated currents.

DISCUSSION

Previous expression experiments showed that HEK cells transiently transfected with bCCE1 develop a store-operated conductance that appears to be Ca^{2+} selective (Philipp *et al.* 1996). Using CHO cells that stably express bCCE1, we confirmed the functional expression of store-operated currents and studied the ionic permeation of underlying recombinant channels. Basically, we found that the store-operated currents of CHO(CCE1) cells display an anomalous mole fraction dependence on extracellular Ca^{2+} and Na^+. Na^+ currents were detected in the absence of external divalent cations and were blocked by micromolar Ca^{2+}. Removal of external Na^+ had no effect on currents recorded in millimolar Ca^{2+}. Similarly, we found an enhancement of inward currents evoked by IP_3 in RBL cells expressing bCCE1.

Considering that recombinant TRPC1 and TRPC3 co-immunoprecipitate (Xu *et al.* 1997), it is likely that members of the TRP family assemble into heteromultimeric channels and, in principle, native TRP proteins and CCE1 may form chimeric channels in CHO(CCE1) and RBL cells. Furthermore, since internal dialysis with IP_3 evoked inward currents that displayed an anomalous mole fraction behaviour even in non-transfected cells, native and recombinant currents may also aggregate to build up whole-cell currents in RBL and CHO(CCE1) cells. The idea that the properties of store-operated currents in cells expressing recombinant bCCE1 reflect the phenotype of CCE1 is underlined by previous expression studies with various TRP homologues that revealed functionally distinct currents. For instance, the mammalian TRPC1A (Zitt *et al.* 1996) and the dipterian TRPL (Zimmer *et al.* 1997) appear to form non-selective cation channels, which support linear and outwardly rectifying currents, respectively. Since TRPL and TRPC1A were expressed in CHO cells as was CCE1 in the present study, the functionally diverse currents are likely to reflect the properties not of native but of recombinant TRP channels. Similarly, the properties of store-operated currents in CHO(CCE1) cells probably represent the phenotype of bCCE1.

The recombinant channels formed in CHO cells after expression of bCCE1 parallel Ca^{2+}-selective channels at least in two basic features. (i) Na^+ permeates when Ca^{2+} is absent but Na^+ currents are blocked by micromolar Ca^{2+}. (ii) With millimolar concentrations of Ca^{2+}, CCE1 currents are carried by Ca^{2+} and no Na^+ flux occurs at these concentrations. Hence, monovalent and divalent cations apparently interact within the pore of recombinant CCE1 channels. We predict that recombinant CCE1 channels form multi-ion pores with high-affinity binding site(s) for Ca^{2+}. Due to the high-affinity binding of extracellular Ca^{2+} and, probably, due to a poor permeation of intracellular Cs^+, the ionic currents through CCE1 channels display inward rectification. As expected for a capacitative entry pathway selective for Ca^{2+}, the high-affinity binding of Ca^{2+} prevents the permeation of monovalent cations under physiological Ca^{2+} concentrations and confers to recombinant CCE1 channels a Ca^{2+} selectivity much higher than previously expected (Philipp *et al.* 1996).

In conclusion, both native (Hoth & Penner, 1993; Parekh *et al.* 1993; Zweifach & Lewis, 1993; Lückhoff & Clapham, 1994; Vaca & Kunze, 1994; Krause *et al.* 1996; Yao & Tsien, 1997) and recombinant (Zitt *et al.* 1996; Philipp *et al.* 1996) store-operated channels appear to be functionally diverse. If members of the TRP family co-assemble to form store-operated channels, CCE1 is an appropriate candidate to play the role of a dominant subunit in terms of Ca^{2+} selectivity.

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