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A mammalian capacitative calcium entry channel homologous to *Drosophila* TRP and TRPL

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Intracellular Ca^{2+} signalling evoked by Ca^{2+} mobilizing agonists, like angiotensin II in the adrenal gland, involves the activation of inositol(1,4,5)trisphosphate-($InsP_3$)-mediated Ca^{2+} release from internal stores followed by activation of a Ca^{2+} influx termed capacitative calcium entry. Here we report the amino acid sequence of a functional capacitative Ca^{2+} entry (CCE) channel that supports inward Ca^{2+} currents in the range of the cell resting potential. The expressed CCE channel opens upon depletion of Ca^{2+} stores by $InsP_3$ or thapsigargin, suggesting that the newly identified channel supports the CCE coupled to $InsP_3$ signalling.

Keywords: calcium signalling/calcium spiking/capacitative calcium entry/$InsP_3$ signalling/store depletion

Introduction

Hormones, growth factors and neurotransmitters activate membrane receptors linked to G proteins or tyrosine kinases and generate inositol(1,4,5)trisphosphate ($InsP_3$), which in turn releases Ca^{2+} from the endoplasmic reticulum. Ca^{2+} release is invariably followed by Ca^{2+} entry across the plasma membrane that appears to be regulated by the Ca^{2+} content of intracellular Ca^{2+} stores (Putney, 1990; Berridge, 1993). The resulting oscillations of the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) underlie cellular events such as, for instance, steroidogenesis in adrenal gland (Burnay *et al.*, 1994), prolactin secretion in pituitary cells (Carew and Mason, 1995), fluid secretion in exocrine pancreas (Kasai and Augustine, 1990) and, eventually, migration of nerve growth cones (Gomez *et al.*, 1995). Depending on the cell type, the functional role of the capacitative Ca^{2+} influx is to support $[Ca^{2+}]_i$ elevations during receptor stimulation or to supply a Ca^{2+} source for the refilling of intracellular stores and, therefore, to enable repetitive $[Ca^{2+}]_i$ spiking. The general significance of the capacitative Ca^{2+} entry (CCE) pathway can be appreciated in certain forms of immunodeficiency associated with the absence of antigen-triggered Ca^{2+} influx and defective T cell proliferation (Partiseti *et al.*, 1994).

The transient receptor potential (trp) gene product from *Drosophila* has been implicated to function as a Ca^{2+} permeable channel in invertebrate phototransduction (Hardie and Minke, 1992; Niemeyer *et al.*, 1996) with biophysical properties assigned to CCE, when expressed in Sf9 cells (Vaca *et al.*, 1994) and *Xenopus* oocytes (Petersen *et al.*, 1995). Recently, partial and full-length cDNA sequences homologous to trp have been published (Petersen *et al.*, 1995; Wes *et al.*, 1995; Zhu *et al.*, 1995, 1996), pointing to the existence of a new gene family involved in vertebrate Ca^{2+} fluxes (Zhu *et al.*, 1996; Zitt *et al.*, 1996). We have now isolated complementary DNAs from bovine tissues encoding a protein CCE, which shows significant homology to the dipterian trp (Montell and Rubin, 1989) and trp-like (trpl, Phillips *et al.*, 1992) gene products. Transient expression of CCE in human embryonic kidney cells was sufficient to induce currents that were activated upon depletion of intracellular Ca^{2+} stores by cell dialysis with $InsP_3$. CCE currents showed strong inward rectification and the relative ion permeabilities were $P_{Ba} \geq P_{Ca} > P_{Na} \approx P_{Cs}$. GTPγS also activated CCE currents. More interestingly, thapsigargin, which passively depletes intracellular Ca^{2+} stores, mimicked the action of $InsP_3$.

Results and discussion

Isolation of cDNAs encoding bCCE

We amplified a 134 bp cDNA fragment (Figure 1A) from bovine retina using degenerate oligonucleotides derived from the dipterian TRP (Montell and Rubin, 1989) and TRPL (Phillips *et al.*, 1992). By screening bovine cDNA libraries, full-length cDNAs were obtained from retina and adrenal gland, encoding identical proteins. The translation initiation site was assigned to the first ATG triplet that appears downstream of nonsense codons found in-frame. The amino acid sequence of the bovine CCE (bCCE) protein is composed of 981 amino acids and has a relative molecular mass calculated as 112 528 (Figure 1B). The size of the putative polypeptide was confirmed by *in vitro* translation of bCCE cRNA, yielding an ~110 kDa product (Figure 2A). Hydropathy analysis (Kyte and Doolittle, 1982) reveals a hydrophobic core in the bCCE protein with six peaks likely to represent membrane-spanning helices (Figure 1, S1, S2, S3, S4, S5 and S6). The hydrophobic core is flanked by long presumptive cytoplasmic domains at the N- and C-termini. A similar topology has been suggested for dipterian TRP and TRPL that share ~ 40% sequence identity to the bCCE protein. The C-terminal regions revealed no significant similarities and mainly account for the different overall length of the three polypeptides ranging from 981 (bCCE) to 1275 (TRP) amino acids. Like its dipterian homologues, the bCCE channel contains a region (Figure 1B), which is related to the pore forming regions of cyclic nucleotide gated ion channels (Heginbotham *et al.*, 1992). Within

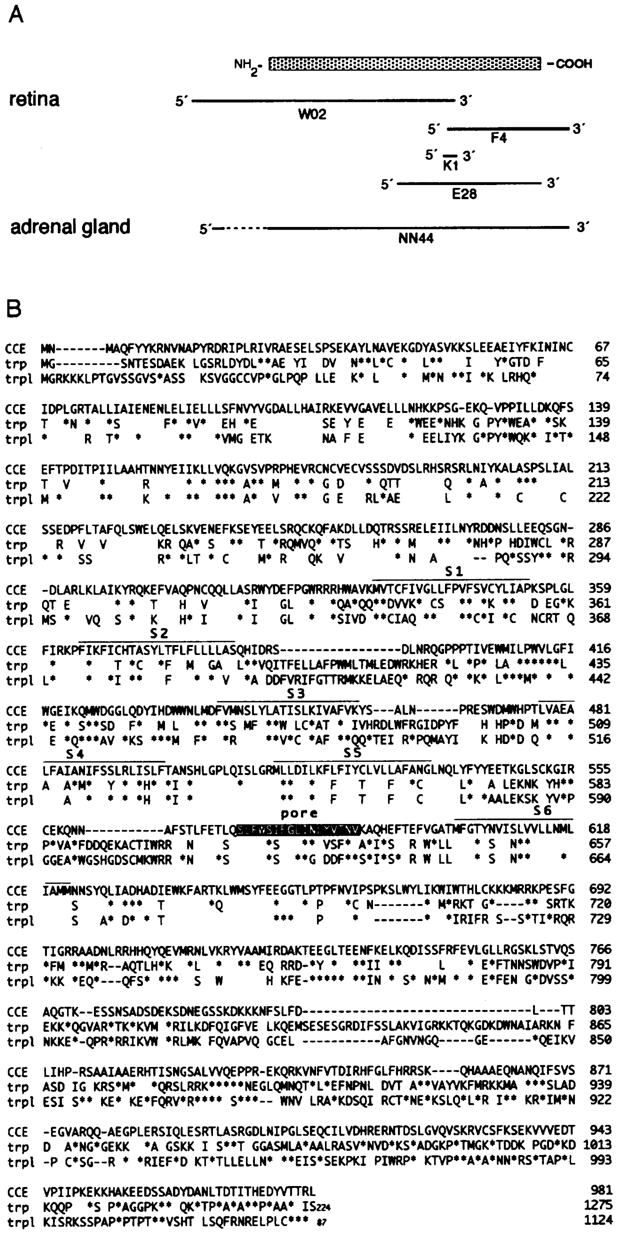

Fig. 1. Primary structure of the bovine capacitative Ca^{2+} entry (CCE) channel. (**A**) Cloning strategy. Bar represents CCE protein, lines indicate isolated cDNA clones from retina and adrenal gland; dashed line in 5′ non-coding region of adrenal CCE represents a spliced sequence present in retinal CCE cDNA. (**B**) The deduced amino acid sequence of CCE is shown in alignment with *Drosophila* TRP (Montell and Rubin, 1989) and TRPL (Phillips *et al.*, 1992). Numbers of amino acid residues are given on the right (the last 224 and 87 residues of TRP and TRPL are not shown). Identical residues with respect to the bovine channel sequence are represented by a space; conserved residues by stars; gaps by dashes. The putative transmembrane sequences S1–S6 (segments of at least 19 residues with an average hydropathy index >1.6) and the putative pore region are shown.

this stretch of 17 amino acids, six are identical and three conserved. No further similarities between bCCE and cyclic nucleotide gated ion channels were found. The topology of the bCCE protein is consistent with one potential cyclic AMP dependent phosphorylation site (Ser124) located on the cytoplasmic side. The ankyrin motif present in TRP (Montell and Rubin, 1989) and TRPL (Phillips *et al.*, 1992) is mainly conserved (amino

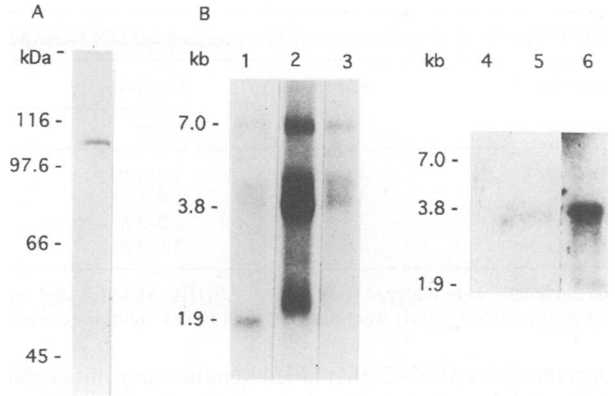

Fig. 2. Bovine CCE cDNA encodes a 110 kDa protein and its mRNA is abundantly expressed in adrenal gland and testis. (**A**) *In vitro* translation (TNT™-system, Promega) of the bCCE cRNA yields a [^{35}S]methionine-labelled protein that migrates at ~110 kDa. The position of molecular mass standards is indicated. (**B**) Expression of bCCE mRNA in bovine tissues. The figure shows a Northern blot of polyadenylated RNAs derived from heart, adrenal gland, retina, cerebrum, cerebellum and testis (lanes 1–6, respectively) probed with a bCCE cDNA encompassing most of the protein coding region. The identity of the 1.9 kb mRNA (heart and testis) is not known.

acids 143–177) but not the calmodulin binding sites present in TRPL (Warr and Kelly, 1996).

Tissue specific expression of the bCCE gene

By Northern analysis (Figure 2B) we found that bCCE mRNA is less abundant in heart and retina than in adrenal gland, where $InsP_3$ formation and CCE are stimulated by several transmitters including angiotensin II and participate in the formation of aldosterone (Burnay *et al.*, 1994). The cloning of several cDNAs from retina and adrenal gland, which differ in their 5′-untranslated sequences, indicate that the 4.8 and 7.0 transcripts may result from alternative RNA processing. The 2.1 kb mRNA species (adrenal gland) corresponds to cDNA clones derived from adrenal gland representing the 3′-terminal region of bCCE. Transcripts of bCCE are also expressed in testis (~3.8 kb) and apparently at very low levels in cerebellum. Correspondingly, the existence of CCE in brain was shown by PCR amplification of a 415 bp cDNA fragment (Petersen *et al.*, 1995).

The bCCE protein is a capacitative calcium entry channel

The ion currents associated with CCE have been extensively studied in various non-excitable cells including mast cells, lymphocytes and epidermal cells (Hoth and Penner, 1992; Zweifach and Lewis, 1993; Lückhoff and Clapham, 1994). The common feature of these ion currents is their activation upon depletion of intracellular Ca^{2+} stores by $InsP_3$, inhibitors of endoplasmic reticulum pumps such as thapsigargin, Ca^{2+} ionophores or removal of extracellular Ca^{2+}. However, differences have been found in unitary conductance, ion permeability and inactivation, supporting the idea that ion channels associated with CCE may be encoded by a number of related genes. Since the bCCE gene may belong to this family, we assayed the functional viability of the protein encoded by the bCCE clone after overexpression in human embryonic kidney (HEK) cells. Cell dialysis with solutions containing various free Ca^{2+}

Table I. Density of inward currents (CD) in control and bCCE-transfected (bCCE) cells

Treatment	CD (pA/pF)	Control		CD (pA/pF)	bCCE	
		range	*n*		range	*n*
None	4.6 ± 1.2	1.2–10.8	22	5.2 ± 0.6	1.7–11.8	25
GTP-γ-S	5.1 ± 0.6	1.9–12.3	21	34.4 ± 9.1	15.1–88.7	8
$InsP_3$	3.5 ± 0.2	2.0–5.8	17	31.7 ± 8.0	13.5–67.5	5
Thapsigargin	4.6 ± 0.9	2.1–7.5	8	50.7 ± 12.2	26.3–118.9	8

Untreated cells were dialyzed with EGTA or EDTA (Materials and methods). Cells treated with GTP-γ-S (200–300 μM), $InsP_3$ (5–10 μM) or thapsigargin (0.5–1.5 μM) were dialyzed with EDTA solutions containing ~500 nM Ca^{2+}. Data are given as mean ± SE.

concentrations (0.05–2 μM) failed to induce any noticeable change of the whole-cell currents scanned with voltage-clamp ramps from −150 mV to +100 mV (Table I). However, in the presence of 500 nM free Ca^{2+}, inclusion of 5–10 μM $InsP_3$ in the dialysate or application of 0.5–1.5 μM thapsigargin dramatically changed the current–voltage profile of bCCE-transfected cells (Figure 3A and B, left panels). The increase of ion currents was more accentuated at negative than at positive potentials (Figure 3A and B, right panels), indicating that the whole-cell currents developed an inward-going rectification upon store depletion. Similarly, native currents activated by depletion of Ca^{2+} stores in various non-excitable cells are mainly characterized by a prominent inward rectification (Hoth, 1995). In our hands, GTP-γ-S was also effective in inducing inward rectifying currents in bCCE-transfected cells (Table I). Considering that GTP-γ-S potentially activates a number of G proteins, the latter result does not rule out the possibility that selective activation of G proteins may have inhibitory effects (Xu *et al.*, 1995). All in all, no matter at which level the intracellular pathway in bCCE-transfected HEK cells was activated, depletion of intracellular Ca^{2+} stores induced inward currents with densities up to 118 pA/pF (Table I). Similar manoeuvres had no detectable effect on current densities of control cells (Table I). However, we observed a minute increase of inward currents upon store depletion (Figure 3A and B; left panels, insets), indicating that HEK cells also possess an endogenous capacitative entry, supported by channels that might be functionally related to bCCE. In fact, transcripts homologous to trp from *Drosophila* (Zhu *et al.*, 1995; 1996; Wes *et al.*, 1995) but no bCCE specific transcripts (data not shown) were detected in poly(A)$^+$ RNA from HEK cells. Endogenous and bCCE currents differ mainly in reversal potential (Figure 3A and B) and ion selectivity (see below) and can be easily distinguished from each other according to current densities (Table I).

In bCCE-transfected cells, we obtained an average current density of 39.6 ± 5.9 pA/pF (mean ± SE, n = 21) at −145 mV, which correspond to inward currents of ~200 pA amplitude at a resting potential of −50 mV (Figure 3A and B, left panels). However, despite this relative large density of whole-cell currents, we could not detect unitary single channel currents in cell-attached patches (n = 14) formed on bCCE-transfected cells that were treated with 1 μM thapsigargin. One possibility is that the unitary conductance is too small to allow detectable currents when the membrane patches face 10 mM extracellular Ca^{2+}. Alternatively, bCCE channels may inactivate rapidly if cytosolic Ca^{2+} is not appropriately buffered or calcium overload may compromise channel activation. In fact, bCCE currents could not be activated by $InsP_3$ and thapsigargin using pipette solutions containing 1–2 μM intracellular calcium (n = 8). We required high concentrations of Ca^{2+} chelators to record stable whole-cell currents (Figure 3A and B), even when continuous inward currents were prevented by clamping the membrane potential at 0 mV between the depolarizing ramps. A straightforward implication of these observations is that a large proportion of the inward currents must be carried by Ca^{2+} ions in bCCE-transfected cells. Future experiments will state more precisely the contribution of bCCE currents during changes of the $[Ca^{2+}]_i$ upon store depletion. Nevertheless, as in previous studies of CCE in native cells (see Putney and Bird, 1993), we assessed whether Ca^{2+} ions support the inward currents in bCCE-transfected cells by removing Ca^{2+} form the extracellular solution. Almost no inward current was observed in nominal zero extracellular Ca^{2+}. However, substitution of Ca^{2+} with Ba^{2+} led to comparable currents (Figure 3C, left panel), supporting our proposal that bCCE currents are carried by divalent ions. Accordingly, we observed indeed that the reversal potential of bCCE currents shifted to a potential above 0 mV simultaneously with the appearance of inward rectifying currents in 10 mM extracellular Ca^{2+} (Figure 3A and B, right panels). Since whole-cell currents of control cells reversed around 0 mV (Figure 3A and B, left panels), the former observation suggested that bCCE forms Ca^{2+} selective channels. Measurements of the reversal potential from whole-cell currents carried by Ca^{2+} and Ba^{2+} in bCCE-transfected cells revealed a distinct profile in the dependence of the reversal potential on the extracellular cation concentration (Figure 3C), even though leak and probably residual Cl^- currents may bias the results (see Materials and methods). According to the constant-field theory (Hille, 1992), the reversal potential of control and bCCE currents can be accounted by a permeability ratio $P_{Cs}/P_{Na} \approx 0.9$ at nominal extracellular free Ca^{2+}. Extrapolating this ratio to higher divalent ion concentrations, the lower limits for the relative permeabilities $P_{Cs}:P_{Na}:P_{Ca}:P_{Ba}$ of the bCCE channel was 1:1.1:7.7:12.3. Since inward currents were recorded after replacement of extracellular Ca^{2+} with Ba^{2+} (Figure 3, left panel), these results suggests that bCCE channels allow primarily the entry of divalent cations. Due to the inward rectifying characteristic of whole-cell currents, bCCE channels are endowed, therefore, to support Ca^{2+} entry primarily in the range of the cell resting potential.

In conclusion, we show that bCCE is an ion channel from vertebrates that is activated by Ca^{2+} store depletion and, therefore, responsible for CCE. Since the actual

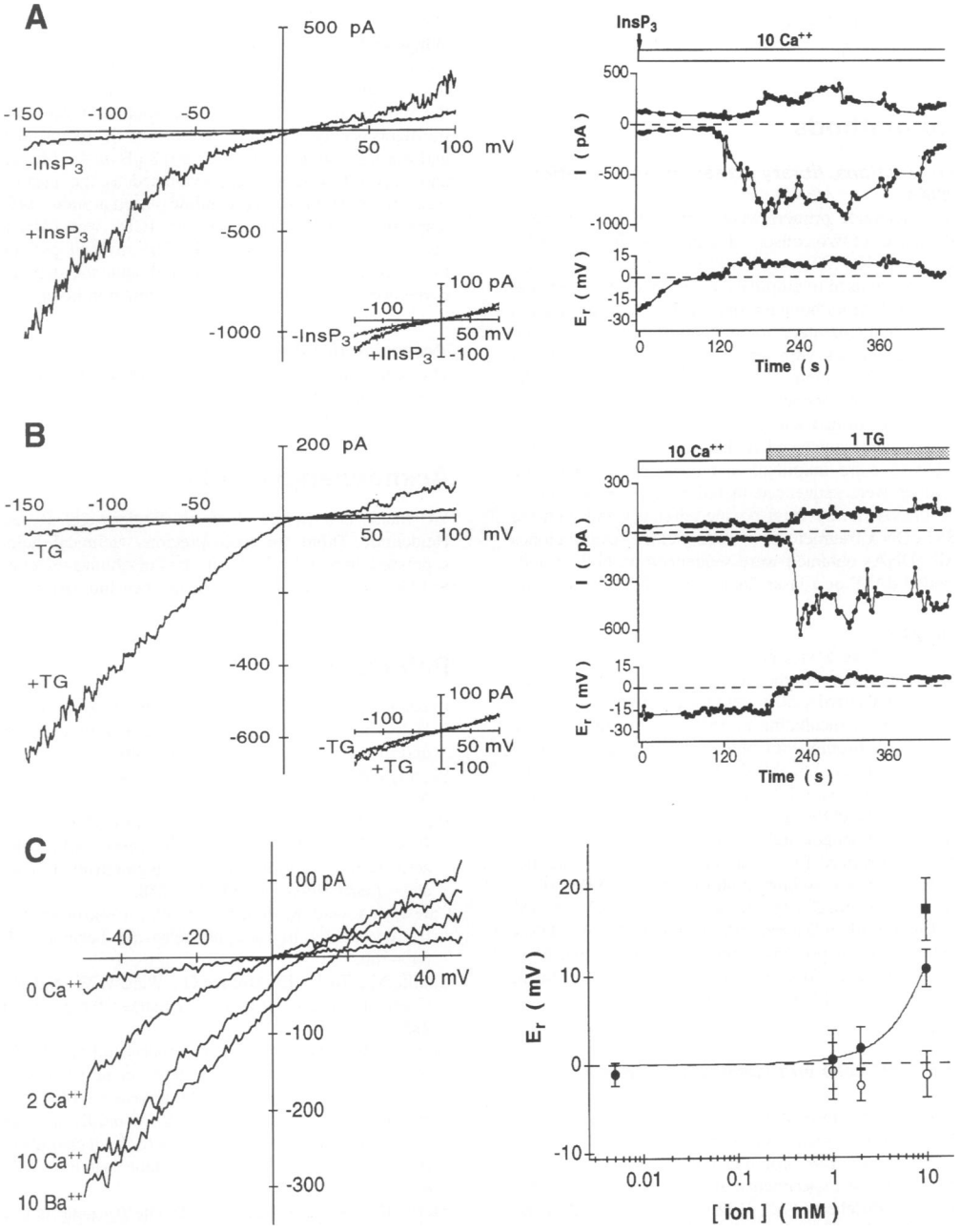

Fig. 3. Ion currents through bCCE channels expressed in HEK cells. Cell dialysis with 10 μM $InsP_3$ (**A**) or external application of 1 μM thapsigargin (**B**) resulted in a predominant increase of inward currents. The representative current traces (A and B; left panels) show the current–voltage relationship of bCCE-transfected cells before (–$InsP_3$, 120 s; –TG, 75 s) and after maximal current increase (+$InsP_3$, 189 s; +TG, 234 s). C_m: 19.2 pF (A), 16 pF (B). Control cells displayed minor current changes after prolonged dialysis of $InsP_3$ (A, left panel inset; +$InsP_3$, 210 s; 16.3 pF) or thapsigargin exposure (B, left panel inset; +TG, 150 s; 19 pF). Additionally to the increase of inward and outward currents (A and B; right panels) measured at +95 mV (upper traces) and –145 mV (lower traces) in bCCE-transfected cells, respectively, the reversal potential (E_r) shifted into the positive potential range. Bars indicate the superfusion of 10 mM external Ca^{2+}, beginning of cell dialysis with 10 μM $InsP_3$ and exposure to 1 μM thapsigargin. The delay of bCCE current activation was in a similar time range after application of thapsigargin (30–120 s) and $InsP_3$ dialysis (90–210 s). Depending on the external concentration of divalent cations, activated bCCE currents reversed between 0 and 20 mV (**C**). The original traces illustrate current recordings from bCCE-transfected cells (15–21 pF) obtained at the indicated concentrations (mM) of Ca^{2+} and Ba^{2+} (C, left panel). E_r was measured in the presence of Ca^{2+}, (control, ○; bCCE, ●) and Ba^{2+} (bCCE, ▲) in 2-21 cells (C, right panel). Symbols represent mean ± SE.

subunit arrangement of capacitative calcium entry channels is unknown, we cannot rule out the possibility that bCCE may be only one component of the channel structure and that endogenous proteins may also contribute to the new currents expressed in the transfected cells. The cloning and expression of bCCE will facilitate efforts to identify signals that link depleted stores to Ca^{2+} entry. Since CCE can be stimulated by growth factors via receptor tyrosine kinases, it will be also interesting to see whether diseases resulting, at least in part, from the release of growth factors such as some forms of human cancer, many immune and non-immune proliferative diseases will respond to compounds that interact with CCE channels. In fact, the antimycotic drug clotrimazol blocks CCE and inhibits

cell proliferation *in vitro* as well as metastases *in vivo* (Benzaquem *et al.*, 1995).

Materials and methods

Polymerase chain reactions, library screening and isolation of full-length cDNAs

Two degenerate oligonucleotide primers were synthesized on the basis of the amino acid sequences of two conserved regions in TRP and TRPL (W^{644}GLLMFG and E^{682}WKFART, numbering of amino acid residues corresponding to TRPL) and used to amplify the 134 bp cDNA fragment K1 (nucleotides 1792–1925) from bovine retina by RT–PCR. Elongation of a synthetic primer complementary to nucleotides 1861–1882 and screening of the resulting clones with an oligonucleotide covering nt 1813–1842 yielded 22 positive clones including W02. To isolate the cDNA containing the 3′-terminal sequence, rapid amplification of cDNA 3′ ends (3′RACE) was performed using nested primer combinations. Six independent clones were sequenced including F4. Additional F4–W02-overlapping cDNAs were amplified using specific primers and three independent clones were sequenced including E28. Screening of an oligo(dT)-primed cDNA library from bovine adrenal gland with the nucleotides 713–1882 cDNA fragment from W02 yielded several clones including NN44. All cDNAs obtained were sequenced on both strands using either ^{35}S-labelled dATP or a laser fluorescence DNA sequencer.

Northern blot analysis

For Northern blot analysis 10 μg bovine poly(A)$^+$ RNA was resuspended in 20 mM 4-morpholinepropanesulfonic acid, 5 mM sodium acetate, 1 mM EDTA, 5.92% formaldehyde, 50% deionized formamide and 40 ng/μl ethidium bromide. After incubating at 55°C for 15 min, samples were put on ice and Ficoll, bromphenol blue and xylene cyanol were added at final concentrations of 2.5, 0.042 and 0.042%, respectively. The RNA was electrophoresed on a 1.2% agarose gel containing 1.1% formaldehyde, transferred thereafter to Hybond N nylon membranes (Amersham) by diffusion overnight and UV crosslinked to the filters. Northern blots were prehybridized for 2 h at 42°C in 50% formamide, 50 mM Tris–HCl, pH 7.5, 0.1% sodium diphosphate, 1% SDS, 0.2% polyvinylpyrolidone, 0.2% Ficoll, 5 mM EDTA, 750 mM NaCl, 75 mM sodium citrate and 150 μg/ml denatured salmon sperm DNA. cDNA probes were labelled by random priming using [α-^{32}P]dCTP and blots were hybridized for 20 h at 42°C. After a final wash with 30 mM NaCl, 3 mM sodium citrate, 0.1% SDS filters were exposed to X-ray films for 3 days.

Transfection of HEK 293 cells and electrophysiological recordings

Lipofections were carried out with recombinant plasmids containing the cDNA of bCCE (pCCE) and the green fluorescent protein (pGFP; Chalfie *et al.*, 1994) under cytomegalovirus promoter control in a ratio of 3:1 or with pGFP alone in control experiments. As expected for transient expressions in HEK cells (Pritchett *et al.*, 1988), about one third of the cells were transfected under the present experimental conditions. Isolated HEK cells were voltage-clamped in the whole-cell mode (Hamill *et al.*, 1981) using a patch clamp amplifier EPC-9 (HEKA) 30–72 h after transfection. The pipettes (2–5 MΩ) were filled with a solution containing 7 mM $CaCl_2$, 42 mM H–EDTA, 10 mM HEPES. The pH was adjusted to 7.3–7.4 with 45 mM NaOH plus 80 mM CsOH. The calculated free Ca^{2+} concentration was ~500 nM. In some experiments, EGTA was used instead of EDTA at a similar free Ca^{2+} concentration and the pipette solution contained 135 mM CsCl, 7 mM $CaCl_2$, 3 mM $MgCl_2$, 10 mM H–EGTA, 10 mM HEPES, pH (CsOH) 7.3–7.4. To obtain free Ca^{2+} concentrations in the range of 1–2 μM, the latter solution was modified by increasing the $CaCl_2$ concentration to 9–10 mM. Equivalent current recordings were obtained in control and bCCE-transfected cells with EGTA (10 mM) or EDTA (42 mM) in the pipette solutions. However, current recordings never lasted longer than 50–70 s with the Cs/EGTA based solution and, therefore, the Cs/Na/EDTA solution was preferred for the present experiments. The bath solution contained 115 mM NaCl, 5 mM KCl, 2 mM $MgCl_2$, 10 mM HEPES, pH (NaOH) 7.3–7.4. As required, the bath solution was supplemented with $CaCl_2$ (1, 2 or 10 mM) or $BaCl_2$ (10 mM). Since dialysis with pipette solutions containing at least 500 nM Ca^{2+} induced typical Cl^- currents, the latter were minimized with DIDS at concentrations (100–200 μM) which did not distort current recordings in HEK cells. Whole-cell currents were elicited by 250 ms voltage-clamp ramps from –150 mV to + 100 mV every 3 s, otherwise the membrane potential was clamped at 0 mV. Whole-cell currents are displayed without any leak subtraction and membrane potentials have been corrected for junction potentials according to Neher (1992). The membrane capacitance (C_m) was read from the settings provided by the amplifier after automatic cancellation of transients occurring in the whole-cell mode. Independent of cell treatment and transfection, C_m was 20.1 ± 0.8 pF (n = 114; mean ± SE). Inward and outward currents were evaluated as the mean current amplitude measured within a 10 mV window placed around –145 mV and +95 mV, respectively. The current density (CD) was calculated from inward currents at –145 mV (Table I). The reversal potential (E_r) represents the zero-current potential measured with the depolarizing ramps after correction of series resistances and junction potentials.

Accession number

The sequence of the bCCE cDNA has been deposited in GenBank under accession number X99792.

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References

Benzaquen,L.R., Brugnara,C., Byers,R., Gattoni-Celli,S. and Halperin,J.A. (1995) Clotrimazole inhibits cell proliferation *in vitro* and *in vivo*. *Nature Med.*, **1**, 534–540.

Berridge,M.J. (1993) Inositol trisphosphate and calcium signalling. *Nature*, **361**, 315–325.

Burnay,M.M., Python,C.P., Vallotton,M.B., Capponi,A.M. and Rossier,M.F. (1994) Role of the capacitative calcium influx in the activation of steroidogenesis by angiotensin-II in adrenal glomerulosa cells. *Endocrinology*, **135**, 751–758.

Carew,M.A. and Mason,W.T. (1995) Control of Ca^{2+} entry into rat lactotrophs by thyrotrophin-releasing hormone. *J. Physiol.*, **486.2**, 349–360.

Chalfie,M., Tu,Y., Euskirchen,G., Ward,W.W. and Prasher,D.C. (1994) Green fluorescent protein as a marker for gene expression. *Science*, **263**, 802–805.

Gomez,T.M., Snow,D.M. and Letourneau,P.C. (1995) Characterization of spontaneous calcium transients in nerve growth cones and their effect on growth cone migration. *Neuron*, **14**, 1233–1246.

Hamill,O.P., Marty,A., Neher,E., Sakmann,B. and Sigworth,F.J. (1981) Improved patch-clamp techniques for high resolution current recording from cells and cell-free membrane patches. *Pflügers Arch.*, **391**, 85–100.

Hardie,R.C. and Minke,B. (1992) The *trp* gene is essential for a light-activated Ca^{2+} channel in *Drosophila* photoreceptors. *Neuron*, **8**, 643–651.

Heginbotham,L., Abramson,T. and MacKinnon,R. (1992) A functional connection between the pores of distantly related ion channels as revealed by mutant K^+ channels. *Science*, **258**, 1152–1155.

Hille,B. (1992) *Ionic Channels of Excitable Membranes*. Sinauer Associates Inc., Sunderland, MA, USA, pp. 226–248.

Hoth,M. (1995) Calcium and barium permeation through calcium release-activated calcium (CRAC) channels. *Pflügers Arch.*, **430**, 315–322.

Hoth,M. and Penner,R. (1992) Depletion of intracellular calcium stores activates a calcium current in mast cells. *Nature*, **355**, 353–356.

Kasai,H. and Augustine,G.J. (1990) Cytosolic Ca^{2+} gradients triggering unidirectional fluid secretion from exocrine pancreas. *Nature*, **348**, 735–738.

Kyte,J. and Doolittle,R.F. (1982) A simple method for displaying the hydropathic character of a protein. *J. Mol. Biol.*, **157**, 105–132.

Lückhoff,A. and Clapham,D.E. (1994) Calcium channels activated by depletion of internal calcium stores in A431 cells. *Biophys. J.*, **67**, 177–182.

Montell,C. and Rubin,G.M. (1989) Molecular characterization of the *Drosophila trp* locus: A putative integral membrane protein required for phototransduction. *Neuron*, **2**, 1313–1323.

Neher,E. (1992) Correction for liquid junction potentials in patch clamp experiments. *Methods Enzymol.* **207**, 123–131.

Niemeyer,B.A., Suzuki,E., Scott,K., Jalink,K. and Zuker,C.S. (1996) The *Drosophila* light-activated conductance is composed of the two channels TRP and TRPL. *Cell*, **85**, 651–659.

Partiseti,M., Le Deist,F., Hivroz,C., Fischer,A., Korn,H. and Choquet,D. (1994) The calcium current activated by T cell receptor and store depletion in human lymphocytes is absent in a primary immunodefeciency. *J. Biol. Chem.*, **269**, 32327–32335.

Petersen,C.C.H., Berridge,M.J., Borgese,M.F. and Bennett,D.L. (1995) Putative capacitative calcium entry channels: expression of *Drosophila trp* and evidence for the existence of vertebrate homologues. *Biochem. J.*, **311**, 41–44.

Phillips,A.M., Bull,A. and Kelly,L.E. Identification of a *Drosophila* gene encoding a calmodulin-binding protein with homology to the *trp* phototransduction gene. (1992) *Neuron*, **8**, 631–642.

Pritchett,D.B., Sontheimer,H., Gorman,C.M., Kettenmann,H., Seeburg,P. and Schofield,P.R. (1988) Transient expression shows ligand gating and allosteric potentiation of $GABA_A$ receptor subunits. Science, **242**, 1306–1308.

Putney,J.W.,Jr (1990) Capacitative calcium entry revisited. *Cell Calcium*, **11**, 611–624.

Putney,J.W.,Jr and Bird,G.St.J. (1993) The inositol phosphate-calcium signaling system in nonexcitable cells. *Endocrine Rev.*, **14**, 610–630.

Vaca,L., Sinkins,W.G., Hu,Y., Kunze,D.L. and Schilling,W.P. (1994) Activation of recombinant *trp* by thapsigargin in SF9 insect cells. *Am. J. Physiol.*, **267**, C1501–C1505.

Warr,C.G. and Kelly,L.E. (1996) Identification and characterisation of two distinct calmodulin-binding sites in the trpl ion-channel protein of *Drosophila melanogaster. Biochem. J.*, **314**, 497–503.

Wes,P.D., Chevesich.J., Jeromin,A., Rosenberg,C., Stetten,G. and Montell,C. (1995) TRPC1, a human homolog of a *Drosophila* store-operated channel. *Proc. Natl Acad. Sci. USA*, **92**, 9652–9656.

Xu,X., Kitamura,K., Lau,K.S., Muallem,S. and Miller,R.T. (1995) Differential regulation of Ca^{2+} release-activated Ca^{2+} influx by heterotrimeric G proteins. *J. Biol. Chem.*, **270**, 29169–29175.

Zhu,X., Chu,P.B., Peyton,M. and Birnbaumer,L. (1995) Molecular cloning of a widely expressed human homologue for the *Drosophila trp* gene. *FEBS Lett.*, **373**, 193–198.

Zhu,X., Jiang,M., Peyton,M., Boulay,G., Hurst,R., Stefani,E. and Birnbaumer,L. (1996) *trp*, a novel mammalian gene family essential for agonist-activated capacitative Ca^{2+} entry. *Cell*, **85**, 661–671.

Zitt,C., Zobel,A., Obukhov,A.G., Harteneck,C., Kalkbrenner,F., Lückhoff,A. and Schultz,G. (1996) Cloning and functional expression of a human Ca^{2+}-permeable cation channel activated by calcium store depletion. *Neuron*, **16**, 1189–1196.

Zweifach,A. and Lewis,R.S. (1993) Mitogen-regulated Ca^{2+} current of T lymphocytes is activated by depletion of intracellular Ca^{2+} stores. *Proc. Natl Acad. Sci. USA*, **90**, 6295–6299.

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