TRPV4 exhibits a functional role in cell-volume regulation

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Summary

Tight regulation of the cell volume is important for the maintenance of cellular homeostasis. In a hypotonic environment, cells swell owing to osmosis. With many vertebrate cells, swelling is followed by an active reduction of volume, a process called regulatory volume decrease (RVD). A possible participant in RVD is the non-selective cation channel TRPV4, a member of the TRP superfamily that has been shown to react to hypotonic stimuli with a conductance for Ca^{2+}. As a model for cell-volume regulation, we used a human keratinocyte cell line (HaCaT) that produces TRPV4 endogenously. When HaCaT cells were exposed to a hypotonic solution (200 mOsm) maximal swelling was followed by RVD. During swelling and volume regulation, a strong Ca^{2+} influx was measured. Gd^{3+}, an inhibitor of TRPV4, blocked RVD of HaCaT cells and the accompanying rise of cytosolic Ca^{2+}. To define the role of TRPV4 in volume regulation, a TRPV4-EGFP fusion protein was produced in CHO cells. CHO cells are unable to undergo RVD under hypotonic conditions and do not produce TRPV4 endogenously. Fluorescence imaging revealed that recombinant TRPV4 was localized to the cell membrane. Production of TRPV4 enabled CHO cells to undergo typical RVD after hypo-osmolarity-induced cell swelling. RVD of *TRPV4*-transfected CHO cells was significantly reduced by Gd^{3+} treatment or in Ca^{2+}-free solution. Taken together, these results show a direct participation of TRPV4 in RVD.

Key words: TRPV4, Cell-volume regulation, RVD, Cytosolic Ca^{2+} concentration

Introduction

The ability of cells to regulate their volume if exposed to an anisotonic environment is a fundamental physiological function. Under hypotonic conditions, the influx of water along its osmotic gradient leads to cell swelling. In most vertebrate cells, and even some unicellular organisms, swelling is followed by a process called regulatory volume decrease (RVD), which enables the cell to regain its former volume even though the cell remains in a hypotonic environment. Loss of ions, mainly K^+ and Cl^-, followed by loss of water provides the mechanism of volume reduction (for review, see Okada et al., 2001). The ion-transport processes during RVD have been well characterized in several cell types, but the underlying volume-sensing mechanisms that enable a cell to undergo RVD and to limit this process until a certain volume is reached are still poorly understood.

Following hypo-osmolarity-induced cell swelling, a rise in the cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$) has been observed in different volume-regulating cell types, including human bronchial epithelial cells (HBEs), bovine articular chondrocytes (BACs) and TALH cells, and also in *Trypanosoma cruzi* during all three life-cycle stages (Fernandez-Fernandez et al., 2002; Rohloff et al., 2003; Tinel et al., 2002; Yellowly et al., 2002). Furthermore, RVD of TALH cells and human cervical-cancer cells was abolished in Ca^{2+}-free medium (Shen et al., 2001; Tinel et al., 2002). Two cation channels, TRPV2 and TRPV4, members of the vanilloid subfamily of transient-receptor-potential channels (TRP), might play a role in volume regulation. When produced in a heterologous system, TRPV2 and TRPV4 production led to a rise in $[Ca^{2+}]_i$ in response to hypotonic conditions and *trpv4*$^{-/-}$ mice showed impaired osmoregulation (Liedtke et al., 2000; Liedtke et al., 2003; Muraki et al., 2003; Jendrach et al., 2004). In addition to hypotonicity, TRPV4 is activated by moderate heat, synthetic ligands like 4α-phorbol-12,13-didecanoate (4α-PDD) and also endogenous agonists like endocannabinoid anandamide and its metabolite arachidonic acid (Güler et al., 2002; Watanabe et al., 2002a; Watanabe et al., 2002b; Watanabe et al., 2003). *TRPV4* shows a wide expression range and was detected in a range of tissues including skin, kidney, lung epithelium, vascular endothelium, neurons and brain (Liedtke et al., 2000; Wissenbach et al., 2000; Strotmann et al., 2000; Watanabe et al., 2002b).

We investigated RVD in a human keratinocyte cell line (HaCaT) (Boukamp et al., 1988) that shows strong expression of *TRPV4*. HaCaT cells reacted to hypo-osmotic stimuli by swelling, followed by rapid RVD. In parallel, a rise in $[Ca^{2+}]_i$ was observed that could be blocked by Gd^{3+}. CHO cells do not endogenously produce TRPV4 and showed neither RVD nor an increase in $[Ca^{2+}]_i$ in response to decreased osmolarity. CHO cells expressing a TRPV4-EGFP fusion protein, however, reacted with RVD and a transient rise in $[Ca^{2+}]_i$ to a hypotonic shock. Taken together, these results show that TRPV4 is an important player in RVD.

Materials and Methods

Cell culture

HaCaT cells between passages 30 and 80 were used, and were kept in keratinocyte culture medium (Gibco, Glasgow, UK) supplemented with 10% foetal calf serum (FCS; Gibco, Glasgow, UK), 10 mM HEPES buffer (Gibco, Glasgow, UK). Cells were split twice a week at a 1:6 ratio. For experiments, cells were grown on cover glasses (24 mm diameter) for 24-48 hours. Full medium was replaced by serum-free medium 12 hours before the experiment.

CHO-K1 (ATCC CCL-61) cells were used between passages 25 and 50. They were cultured in Ham's F12 medium (Gibco) with 10% FCS and 10 mM HEPES buffer, and split twice a week at 1:10. 2 days before experiments, cells were seeded on coverslips and, 4 hours before the start of an experiment, growth medium was replaced by serum-free F12 medium. Primary human proximal and distal renal tubule cells were a generous gift from P. Baer (Department of Nephrology, University Clinics, Frankfurt, Germany) (Baer et al., 1997).

Cloning of *TRPV4*, semiquantitative RT-PCR and transfection

Total RNA was isolated from 3×10^6 HaCaT keratinocytes, primary human proximal and distal tubule cells, and CHO-K1 cells with Trizol (Invitrogen) according to the manufacturers instructions. For reverse-transcription PCR (RT-PCR), 2 μg total RNA were used for first-strand synthesis with oligo(dT) and random hexamer primers. cDNA synthesis was performed with the SuperScript First-Strand Synthesis System (Invitrogen). The *TRPV4* ORF was amplified by PCR with gene-specific primers and cloned into the *Bgl*II and *Bam*H1 sites of the vector pEGFPN3 (Clontech) in frame with the GFP coding sequence. For the screening, a 400 bp fragment in the *TRPV4* ORF was amplified with primers 5′-ACTACGGCACCTATCGTCACCAC-3′ (sense) and 5′-ACGCTCAATGGCGATGTGC-3′ (antisense). For normalization, the gene encoding glyceraldehyde-3-phosphate dehydrogenase was used with primers 5′-CCACCCATGGCAAAT-TCCATGGCA-3′ (sense) and 5′-TCTAGACGGCAGGTCAGGTC-CACC-3′ (antisense). CHO cells were transiently transfected with Polyfect transfection reagent (Qiagen) according to the manufacturer's instructions. Experiments were performed 48-72 hours after transfection.

Ca^{2+}-imaging experiments and membrane staining

Cells were stained with 5 μM Fluo-4 AM (Molecular Probes) in serum-free medium at room temperature for 45 minutes. The staining solution was removed by washing three times with serum-free medium. Cells on cover glasses were transferred to custom-built observation chambers and allowed to accommodate for 20 minutes.

$GdCl_3$ (Sigma) was used at a final concentration of 100 μM prepared in Hank's balanced salt solution (138 mM NaCl, 5 mM KCl, 0.5 mM $MgCl_2$, 1.3 mM Ca_2Cl, 5 mM glucose, 10 mM HEPES, pH 7.2). For Ca^{2+}-free experiments, modified Hank's balanced salt solution was used (138 mM NaCl, 5 mM KCl, 0.5 mM $MgCl_2$, 5 mM glucose, 1 mM EGTA, 10 mM HEPES, pH 7.2). Hypotonicity was achieved by the addition of distilled water, lowering the osmolarity from 300 mOsm to 200 mOsm.

Microscopical observations were performed using a Leica TCS 4D confocal laser-scanning microscope fitted with the appropriate filters and PL Fluotar objective (100×, 1.3 NA) that was controlled by SCAN Ware 5.10 software (Leica, Wetzlar, Germany). Live-cell experiments were performed at 37°C and 5% CO_2 in a humidified chamber. Observation started 20-40 minutes after Fluo-4 staining. The *xz* cross-sectional area through the centre of each cell was used as a measure of cell volume. The measurements lasted for 20 minutes, with images taken every 10 seconds.

Membrane localization of EGFP-TRPV4 was shown using a Zeiss LSM 510 laser-scanning microscope (Zeiss, Oberkochen, Germany) with the appropriate filters and Plan Apochromat objective (63×, 1.4 NA). Pictures were processed using Imaris software version 4.0.5 (Bitplane).

Quantitative image analysis

From the time-lapse sequence, the cross-sectional area of the HaCaT and untransfected CHO cells, and the total Fluo-4 fluorescence intensity were measured using ImageJ (version 1.30; W. Rasband, NIH). The cross-sectional area was assumed to be proportional to the cell volume and was expressed as a relative value, normalized to control conditions (t<0 seconds).

In *TRPV4*-transfected CHO cells, Ca^{2+} transients revealed by Fluo-4 fluorescence have been determined measuring emission in the cell-body region. This limitation was necessary to reduce the contribution of membrane located EGFP fluorescence with similar spectral properties.

Statistics

Results are expressed as means±s.e.m. of n observations. To compare sets of data, we used analysis of variance (ANOVA). Differences were considered to be statistically significant when P<0.01.

Results

Volume-regulating cells produced TRPV4

Following the hypothesis that TRPV4 is involved in osmoregulation, the production of this channel in volume-regulating cells (HaCaT keratinocytes and primary human kidney-tubule cells) has been investigated (Fig. 1). HaCaT cells reacted to hypotonic conditions by swelling followed by RVD (Fig. 3A). Kidney-tubule cells must cope with large changes in tonicity and have been shown to have the capacity to regulate their volume by RVD (Tinel et al., 2000; Millar et al., 2004). Therefore, total RNA from HaCaT keratinocytes and proximal and distal primary human renal-tubule cells was probed by RT-PCR. HaCaT and renal-tubule cells strongly expressed *TRPV4* mRNA (Fig. 1). Identity of the PCR product was verified by sequencing. Semiquantitative RT-PCR revealed that proximal and distal tubule cells expressed the same amount of *TRPV4* mRNA. CHO cells, by contrast, were unable to reduce their volume after hypotonicity-induced swelling (Fig. 3B). No amplification product could be detected for CHO cells,

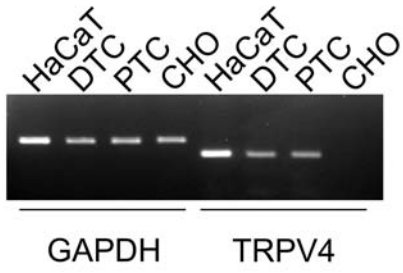

Fig. 1. *TRPV4* mRNA is expressed in volume-regulating cells. Total RNA was isolated from HaCaT keratinocytes, distal renal tubule cells (DTC), proximal renal tubule cells (PTC) and CHO cells. A region in the *TRPV4* ORF and the glyceraldehyde-3-phosphate-dehydrogenase (GAPDH) ORF were amplified by RT-PCR with gene-specific primers and separated on a 1.5% agarose gel. No PCR product could be detected in CHO cells.

indicating the lack of endogenous TRPV4 in this cell type, corresponding to their inability to undergo RVD (Fig. 1).

TRPV4 is essential for RVD

To evaluate the role of TRPV4, the *TRPV4* ORF was amplified from HaCaT mRNA and cloned in frame with and adjacent to a gene encoding EGFP. CHO cells were used for expression studies because they do not express *TRPV4* endogenously (Fig. 1) (Liedtke et al., 2000; Wissenbach et al., 2000; Suzuki et al., 2003). Transiently transfected CHO cells showed TRPV4-EGFP production primarily in the perinuclear region, probably Golgi apparatus and in the cell membrane, after 24-48 hours. After 48-72 hours, TRPV4-EGFP was localized mainly to the plasma membrane (Fig. 2). Production of TRPV4-EGFP was also confirmed by western blotting using an anti-GFP antibody (data not shown).

Reaction to hypotonic swelling of subconfluent HaCaT keratinocytes and *TRPV4*-transfected CHO cells was investigated. Cell swelling was induced by addition of distilled water to the culture medium, decreasing its osmolarity from 300 mOsm to 200 mOsm, and volume changes were recorded. HaCaT keratinocytes reached a maximum volume of 25-35% above control cells in isotonic medium (Fig. 3A). Decrease of cell volume by RVD started 20 seconds after exposure to hypotonicity. After 300 seconds, 70% of the observed cells had completed RVD. Most of them regained their original volume although the volume of some cells was still slightly elevated, as shown in representative individual traces of cell volume changes (Fig. 5A-C). 30% of the HaCaT keratinocytes in our cultures showed no volume regulation at all. In those cells, however, the swelling phase was considerably delayed (data not shown).

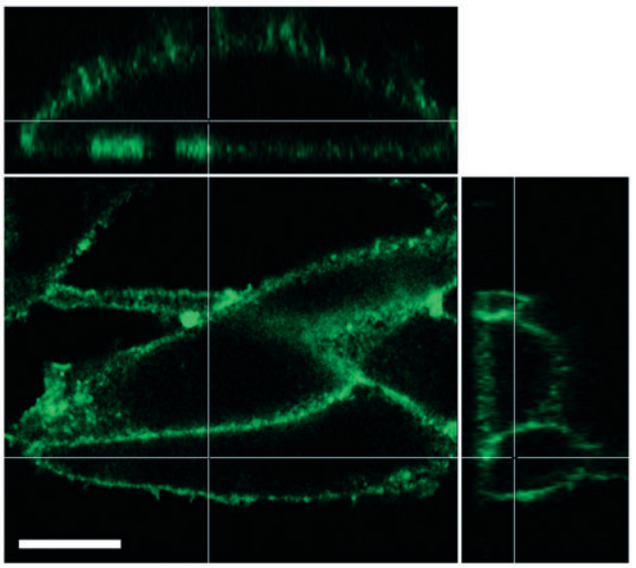

Fig. 2. Membrane localization of EGFP-TRPV4 in transiently transfected CHO cells. CHO cells were transiently transfected with *EGFP-TRPV4* and checked for expression by confocal laser-scanning microscopy. The image shows a single *xy* plane taken slightly above the basal membrane (centre) and the cross-section in *xz* and *yz* planes, respectively (top and right). EGFP-TRPV4 is clearly localized to the plasma membrane 72 hours after transfection. Bar, 10 μm.

Untransfected CHO cells reacted to hypotonicity by a volume increase that finally reached an equilibrium state (Fig. 3B). At the end of recording (900 seconds), the volume of 80% of the untransfected cells was 30±2% higher than that of cells in isotonic medium, whereas 20% of the untransfected cells showed a slight decrease of the volume. *TRPV4*-transfected CHO cells showed a similar cell swelling after hypotonic stimulus. However, after 120 seconds, their volume started to decrease significantly, indicating that *TRPV4*-transfected CHO cells were undergoing RVD. At the end of recording, the final volume was 11±2% larger than the original volume (Fig. 3B).

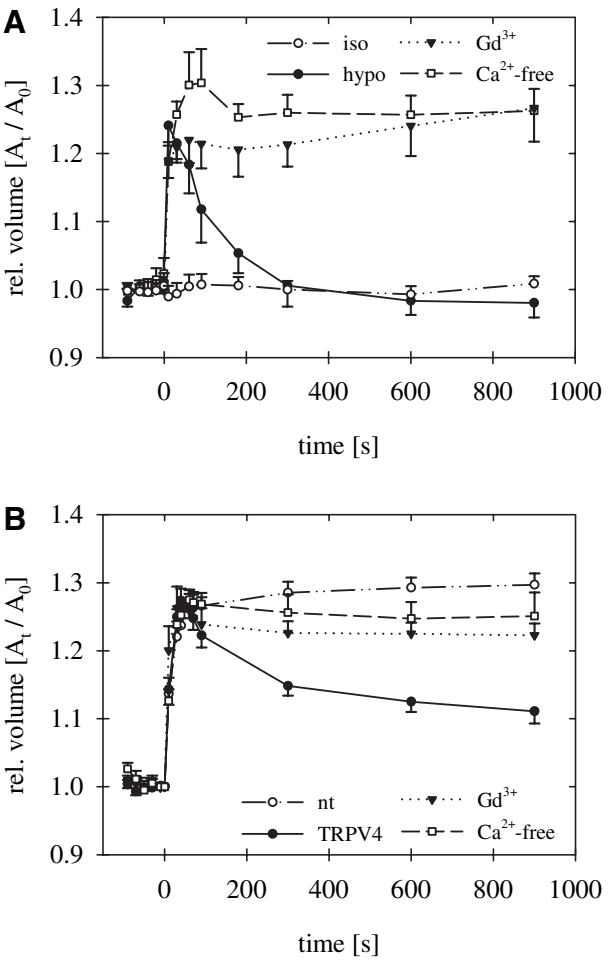

Fig. 3. TRPV4 is necessary for regulatory volume decrease. Relative volume changes of HaCaT cells and CHO cells exposed to hypotonic or isotonic medium at t=0 seconds. (A) Reduction of extracellular osmolarity led to a rapid increase in cell volume of untreated HaCaT cells (hypo, filled circles, n=18) followed by RVD. Replacement of the medium alone did not affect cell volume (iso, open circles, n=12). Treatment with 100 μM Gd^{3+} (filled triangles, n=8) or Ca^{2+}-free solution (open squares, n=8) abolished the RVD response and cell volume stayed elevated. (B) Untransfected CHO cells (nt, open circles, n=22) showed no RVD, whereas *TRPV4*-transfected CHO cells (TRPV4, filled circles, n=25) were able to undergo RVD. Under Ca^{2+}-free conditions (open squares, n=8), no RVD occurred after the initial swelling in transfected cells. Gd^{3+}-pretreated transfected cells also showed no RVD in comparison with untreated cells (filled triangles, n=14). Cell volume was estimated from the central cross-sectional area and normalized to control ($t \leqslant 0$ seconds) values. Data are means±s.e.m.

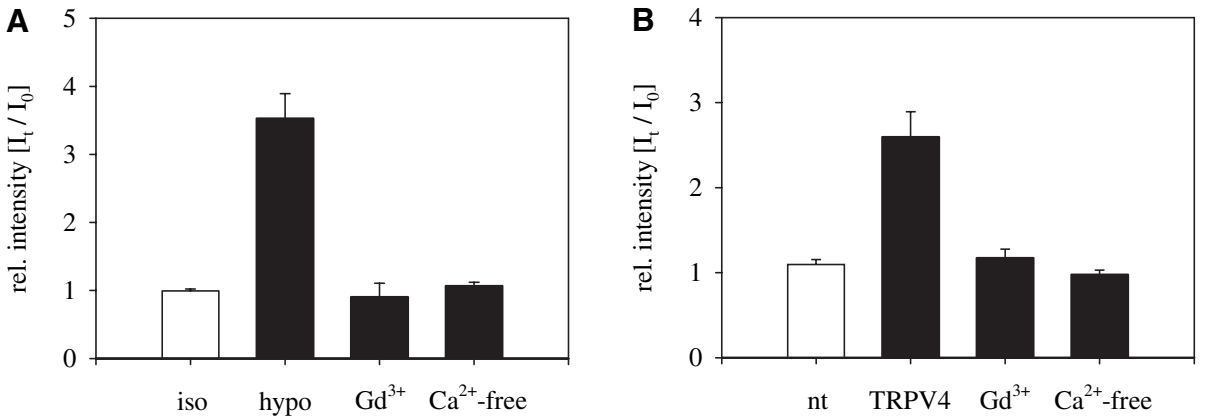

Fig. 4. Changes in $[Ca^{2+}]_i$ after hypotonic treatment. HaCaT and CHO cells were loaded with the Ca^{2+} indicator Fluo-4. Peak fluorescence intensities within 90 seconds after hypotonic treatment are plotted as ratios to control conditions ($t \leqslant 0$ seconds). (A) HaCaT cells exposed to hypotonic (hypo) conditions showed a significant increase in $[Ca^{2+}]_i$ compared with isotonic (iso) conditions ($P<0.01$, ANOVA). By contrast, hypotonically treated cells that were preincubated with 100 μM Gd^{3+} or kept in Ca^{2+}-free solution showed no significant increase in $[Ca^{2+}]_i$. Data are means±s.e.m. (isotonic, $n=12$; hypotonic, $n=18$; Gd^{3+}, $n=8$; Ca^{2+}-free, $n=8$). (B) *EGFP-TRPV4*-transfected CHO cells (TRPV4) exposed to hypotonic conditions showed a significant increase in $[Ca^{2+}]_i$ compared with untransfected cells (nt, $P<0.01$, ANOVA). This increase in $[Ca^{2+}]_i$ was absent from transfected cells pretreated with Gd^{3+} or kept in Ca^{2+}-free solution. Data are means±s.e.m. (untransfected, $n=22$; EGFP-TRPV4, $n=16$; Gd^{3+}, $n=14$; Ca^{2+}-free, $n=8$).

HaCaT keratinocytes were pretreated with 100 μM Gd^{3+}, which is known to inhibit cation-selective stretch-activated ion channels (SACs) (Caldwell et al., 1998). Furthermore, incubation with 100 μM Gd^{3+} has been shown to reduce currents through TRPV4 after hypotonic shock by 70% in a recombinant system (Strotmann et al., 2000; Patapoutian et al., 2003). Gd^{3+}-treated HaCaT cells did not undergo RVD after initial swelling but remained swollen in accordance with the hypothesis of a TRPV4 contribution to RVD (Fig. 3A) The same was true for HaCaT cells treated with 10 μM ruthenium red, which has been shown to inhibit TRPV4 activity (Güler et al., 2002) (data not shown). Also *TRPV4*-transfected and untransfected CHO cells were preincubated with Gd^{3+}; the behaviour of untransfected cells did not change (data not shown) but *TRPV4*-transfected cells failed to show RVD and their final volume at the end of recording was significantly higher than that of untreated transfected cells (Fig. 3B). In transfected cells, treatment with ruthenium red impaired the volume decrease, although not significantly (data not shown).

Extracellular Ca^{2+} has been shown to be a requirement for RVD in some cell types. Therefore, HaCaT keratinocytes and *TRPV4*-transfected CHO cells were kept in Ca^{2+}-free solution. In response to hypotonicity, both cell types increased their volume but showed no RVD (Fig. 3A,B), confirming the relevance of Ca^{2+} influx for RVD.

Role of Ca^{2+} in RVD

TRPV4 had shown a Ca^{2+} conductance in response to hypotonicity (Liedtke et al., 2000) and so we investigated $[Ca^{2+}]_i$ during swelling and RVD in HaCaT cells and *TRPV4*-transfected CHO cells. After exposure to hypotonicity, a significant increase was observed in $[Ca^{2+}]_i$ in HaCaT cells compared with cells in isotonic medium (Fig. 4A). Also, *TRPV4*-transfected CHO cells showed a significant increase in $[Ca^{2+}]_i$ compared with untransfected cells after hypotonic stress (Fig. 4B).

HaCaT and *TRPV4*-transfected CHO cells pretreated with Gd^{3+} or in Ca^{2+}-free solution showed no significant elevation of $[Ca^{2+}]_i$ after a hypotonic stimulus compared with untreated HaCaT cells and untransfected CHO cells, respectively (Fig. 4A,B). Pretreatment of HaCaT cells and *TRPV4*-transfected CHO cells with 10 μM ruthenium red also reduced the increase in $[Ca^{2+}]_i$ (data not shown). These data stress again that influx of Ca^{2+} is necessary for the hypotonicity-induced Ca^{2+} response.

Dynamics of volume changes and elevation of $[Ca^{2+}]_i$ after hypotonic shock

The dynamics of the relationship between volume changes and elevation of $[Ca^{2+}]_i$ are presented in detail in Fig. 5. Representative individual traces of cell-volume changes and relative $[Ca^{2+}]_i$ of HaCaT cells (Fig. 5A-C) and *TRPV4*-transfected CHO cells (Fig. 5D-F) showed that, in both in the native and the reconstructed systems, $[Ca^{2+}]_i$ increased after cell swelling started. The timing of the $[Ca^{2+}]_i$ elevation differed slightly from cell to cell, but only in a few cells did swelling and rise in $[Ca^{2+}]_i$ seem to occur in parallel. The duration of elevated $[Ca^{2+}]_i$ differed between HaCaT cells and transfected CHO cells, in which the $[Ca^{2+}]_i$ peak was much shorter. This could be a possible explanation for the fact that most transfected CHO cells did not regain their original volume after RVD (Fig. 5D-F).

Discussion

We have shown that HaCaT keratinocytes are able to regulate their volume. When placed in a hypotonic environment, they reacted by swelling and an increase in $[Ca^{2+}]_i$, followed by RVD until most of them reached their previous volume. Maximal swelling was reached 10-40 seconds after addition of water, whereas the exact time differed slightly between the individual cells. This variation in the speed of swelling is

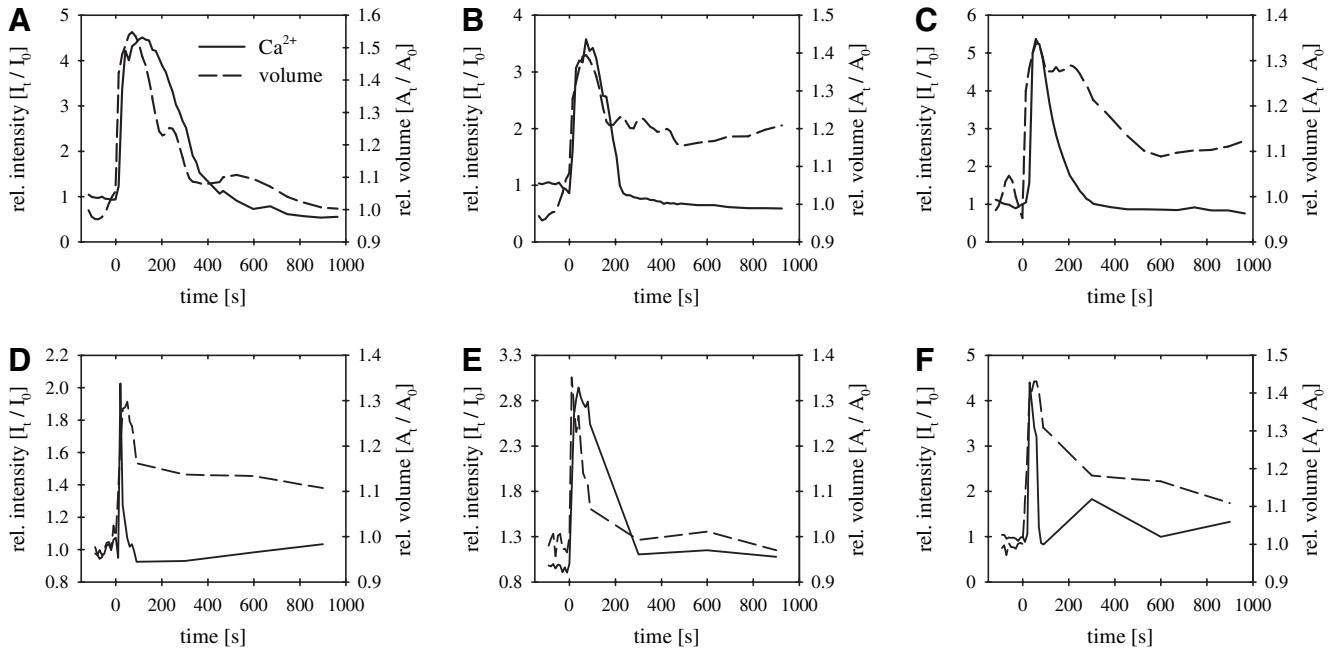

Fig. 5. Dynamics of $[Ca^{2+}]_i$ and cell volume. Individual traces of the relative volume (dashed) and Fluo-4 fluorescence intensity (solid) of single cells exposed to hypotonic conditions at t=0 seconds. Relative values are expressed as ratios to control conditions ($t \leqslant 0$ seconds). (A-C) HaCaT cells. (D-F) *EGFP-TRPV4*-transfected CHO cells. Notice that elevation of $[Ca^{2+}]_i$ follows cell swelling in both cell models. The duration of the $[Ca^{2+}]_i$ peak is in general much shorter in transfected CHO cells than in HaCaT cells, in which $[Ca^{2+}]_i$ is at least elevated throughout the maximum swelling phase.

responsible for the mean volume peak being slightly lower than in the single-cell recordings (Fig. 3A, Fig. 5A-C) and the high standard error in the mean volume regulation curve (Fig. 3A). Both the rise in $[Ca^{2+}]_i$ and RVD depended on the availability of extracellular Ca^{2+} and a Gd^{3+}-dependent Ca^{2+} conductance. Because Gd^{3+} was described as an inhibitor of stretch-activated channels as well as TRPV4 (Caldwell et al., 1998; Strotmann et al., 2000; Patapoutian et al., 2003), TRPV4 would be a likely candidate for mediating RVD.

We revealed expression of *TRPV4* mRNA in HaCaT keratinocytes and in primary renal tubule cells as well. The ability to undergo RVD has been established for all three cell types (Tinel et al., 2002; Millar et al., 2004) (this report). Another volume-regulating cell type, HBE cells, has been shown to contain *TRPV4* transcript as well (Fernandez-Fernandez et al., 2002). By contrast, CHO cells (lacking endogenous TRPV4) were unable to perform RVD and did not increase $[Ca^{2+}]_i$ in response to hypotonic stress, as has been shown previously by other groups (Liedtke et al., 2000; Wissenbach et al., 2000; Suzuki et al., 2003).

Since the discovery of TRPV4 in 2000, several functions have been proposed for this channel. Our experiments extend the function of TRPV4 to an involvement in RVD in a hypotonic environment (Jendrach et al., 2004) (this report). Production of a recombinant TRPV4-EGFP fusion protein in CHO cells was sufficient to initiate $[Ca^{2+}]_i$ rise and RVD in response to hypotonic stress. Preincubation with Gd^{3+} abolished the $[Ca^{2+}]_i$ signal and reduced RVD significantly. In contrast to HaCaT cells, *TRPV4*-transfected CHO cells did not return completely to their former volume but stayed slightly bigger. This can be observed with volume-regulating cells in general: some cell types reach their previous volume after completion of RVD, whereas, with other cell types like murine tracheal epithelial cells or HBE cells, the volume stays slightly elevated (Lock et al., 2000; Fernandez-Fernandez et al., 2002). By contrast, CHO cells are perhaps missing additional factors that allow them to fine tune their volume.

The observed reactions can be summarized in the following scenario. After the hypotonic stimulus, cells start to swell owing to osmosis. By swelling, the tension in the cell membrane and the underlying cortical fibrillar layer is increased and transmitted directly or indirectly to TRPV4, thereby activating it. TRPV4 then mediates the influx of Ca^{2+} from the extracellular space, which activates signalling cascades leading to RVD. This hypothetical regime is supported by the observation that neither HaCaT keratinocytes nor *TRPV4*-transfected CHO cells showed any signs of $[Ca^{2+}]_i$ increase or RVD in Ca^{2+}-free solution. Furthermore, in TALH cells or human cervical cancer cells, Ca^{2+} influx was also necessary for RVD (Shen et al., 2001; Tinel et al., 2002). While this paper was under revision, Arniges et al. (Arniges et al., 2004) showed that downregulation of *TRPV4* using antisense oligonucleotides abolished RVD and Ca^{2+} entry in a volume-regulating tracheal epithelial cell line. This complementary approach strongly reinforces our hypothesis that TRPV4 is essential for Ca^{2+} influx and onset of RVD.

Depending on the cell type and the agonists, TRPV4 can play a role in nociception and can also participate in sensing thermal, mechanical or osmotic stimuli (Liedtke et al., 2003; Mizuno et al., 2003; Alessandri-Haber et al., 2004; Todaka et al., 2004). This heterogeneity is supported by the fact that different agonists of TRPV4 use different signal-transduction pathways (Vriens et al., 2004). Although there was speculation about a role for TRPV4 in volume regulation, we can present

for the first time evidence in a recombinant system that TRPV4 is necessary and, in CHO cells, sufficient for RVD, enabling cells that must deal with varying osmolarities (such as epithelial cells or renal tubule cells) to control their volume.

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