The contribution of endogenous and recombinant transient receptor potential vanilloid type 6 (TRPV6) channels to \( \text{Ca}^{2+} \) entry across the plasma membrane was studied in the human lymph node prostate cancer cell line (LNCaP). LNCaP cells do express the TRPV6 gene, and \( \text{Ca}^{2+} \) entry currents in these cells were detected after active and passive \( \text{Ca}^{2+} \) store depletion by intracellular application of inositol 1,4,5-trisphosphate, \( \text{Ca}^{2+} \) chelators, and the sarcoplasmic/endoplasmic reticulum \( \text{Ca}^{2+}\text{-ATPase} \) inhibitor thapsigargin. This store-operated \( \text{Ca}^{2+} \) current (\( I_{\text{SOC}} \)) had biophysical properties similar to those of the \( \text{Ca}^{2+} \) release-activated \( \text{Ca}^{2+} \) current (\( I_{\text{CRAC}} \)) in rat basophilic leukemia cells such as the activation mechanism, inward rectification, and \( \text{Ca}^{2+} \) selectivity. These properties are also shared by the \( \text{Ca}^{2+}\text{-sensing} \) \( \text{Ca}^{2+} \) current (\( I_{\text{TRPV6}} \)) recorded after heterologous expression of TRPV6 cDNA in human embryonic kidney (HEK) cells and rat basophilic leukemia cells (Bödding, M., Wissenbach, U., Flockerzi, V. (2002) J. Biol. Chem. 277, 36656–36664). TRPV6 cDNA transfection of LNCaP cells restored recombinant \( I_{\text{TRPV6}} \) which can be distinguished from \( I_{\text{SOC}} \) by the mechanism of activation, the voltage dependence of monovalent currents in the absence of external divalent cations, and the changes in \( \text{Ca}^{2+} \) current densities due to different membrane potentials. In addition, \( I_{\text{SOC}} \) was not affected by antiandrogen or 1,25-dihydroxyvitamin D\(_3\) treatment of LNCaP cells, which up-regulates TRPV6 gene expression, or by androgen treatment, which has the opposite effect. Therefore, native channels responsible for \( I_{\text{SOC}} \) are different from those for recombinant \( I_{\text{TRPV6}} \) and do not appear to be affected if one of their assumed subunits, TRPV6, is up- or down-regulated, suggesting a rather rigid subunit composition in vivo.

Calcium is necessary for several intracellular signal transduction mechanisms such as muscle contraction and secretion of neurotransmitters. A wide variety of physiological and pathological processes are regulated by the intracellular free \( \text{Ca}^{2+} \) concentration ([\( \text{Ca}^{2+} \)],\(^1\)) which is tightly controlled with respect to its spatiotemporal resolution (2). The rise in [\( \text{Ca}^{2+} \)], during receptor stimulation is regulated by both \( \text{Ca}^{2+} \) release from intracellular inositol 1,4,5-trisphosphate (IP\(_3\))-sensitive stores and \( \text{Ca}^{2+} \) entry across the plasma membrane. In many cases, the underlying plasma membrane ion channels require phospholipase C for activity, and stimulation of phospholipase C could be linked to channel gating via production of IP\(_3\) and/or diacylglycerol. According to one mechanism referred to as store-operated \( \text{Ca}^{2+} \) entry, transient release of \( \text{Ca}^{2+} \) from internal stores induces sustained \( \text{Ca}^{2+} \) influx. Of particular interest is a highly \( \text{Ca}^{2+} \)-selective store-operated channel referred to as the \( \text{Ca}^{2+} \) release-activated \( \text{Ca}^{2+} \) (CRAC) channel, which has been implicated to be crucial in T-cell activation and mast cell functions (3, 4). The molecular structure of this channel is still elusive. However, the mammalian transient receptor potential proteins (5), structurally related to the transient receptor potential cation channel underlying transduction in \textit{Drosophila} melanogaster photoreceptors, have been proposed to participate in phospholipase C- and/or phosphoinositide-mediated \( \text{Ca}^{2+} \) entry, including store-operated \( \text{Ca}^{2+} \) influx (6, 7).

Recently, a member of the vanilloid type transient receptor potential subfamily, TRPV6, was reported to display electrophysiological properties resembling those of the CRAC channel, such as high \( \text{Ca}^{2+} \) selectivity and the current-voltage (I-V) signature under physiological conditions (8). However, there were also differences between both channels, including the protocols necessary to activate \( I_{\text{CRAC}} \) in rat basophilic leukemia (RBL) cells and to activate recombinant \( I_{\text{TRPV6}} \) recorded after overexpressing its cDNA in RBL cells (1) or human embryonic kidney (HEK) cells (1, 9). Both currents, \( I_{\text{CRAC}} \) and \( I_{\text{TRPV6}} \), were activated under conditions of high intracellular \( \text{Ca}^{2+} \) buffering in TRPV6-transfected RBL cells, whereas only \( I_{\text{CRAC}} \) was activated at low intracellular \( \text{Ca}^{2+} \) buffering after \( \text{Ca}^{2+} \) store depletion (1). It remains possible that TRPV6 channels may also be activated by procedures known to deplete cellular \( \text{Ca}^{2+} \) stores and that differences between \( I_{\text{TRPV6}} \) and \( I_{\text{CRAC}} \) are due to aberrant TRPV6 expression levels in the heterologous expression systems or may be a consequences of heteromultimerization between TRPV6 and other channel subunits.

The \( \text{TRPV6} \) gene is primarily expressed in pancreatic acinar cells and placental syncytiotrophoblasts and trophoblasts (10) and in various intestinal epithelial cells (11). However, to our knowledge, no data are available demonstrating activation of endogenously expressed TRPV6 channels using the various protocols sufficient to activate recombinant \( I_{\text{TRPV6}} \) RBL cells, which we used in our previous study (1), and the human Jurkat T-lymphocytes are common model systems to record \( I_{\text{CRAC}} \) and therefore should contain the essential components necessary for channel activation by store-operated mechanisms. However, \( \text{TRPV6} \) transcripts could not be detected in RBL cells by Northern blot analysis (1, 8) or in Jurkat T-cells (12). We therefore performed this study on the androgen-sensitive human lymph node prostate cancer cell line LNCaP, which, in

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\(\) The abbreviations used are: [\( \text{Ca}^{2+} \)], intracellular free \( \text{Ca}^{2+} \) concentration; IP\(_3\), inositol 1,4,5-trisphosphate; CRAC, \( \text{Ca}^{2+} \) release-activated \( \text{Ca}^{2+} \); RBL, rat basophilic leukemia; HEK, human embryonic kidney; SOC, store-operated \( \text{Ca}^{2+} \); BAPEA, 1,2-bis(2-aminoephosphonyl-ethane-N,N,N'-tetraacetic acid; pH, pfluoridase; TRP, transient receptor potential; TRPV, TRP subfamily (vanilloid receptor type); LNCaP, lymph node cancer prostate.
contrast to RBL cells, endogenously expresses the TRPV6 gene (see Fig. 1). Moreover, store-operated currents have been suggested to be present in these cells (13–17). Therefore, LNCaP cells are an appropriate system to study Ca2+ entry currents with respect to store-operated and TRPV6 channels.

In this study, we describe a store-operated Ca2+ current (\(I_{\text{SOC}}\)) in LNCaP cells that closely resembles \(I_{\text{IRAC}}\) in RBL cells with respect to its activation by protocols known to deplete intracellular Ca2+ stores, inward rectification, and high Ca2+ selectivity. LNCaP cells endogenously express the TRPV6 gene; but unlike recombinant \(I_{\text{TRPv6}}\) in HEK or RBL cells, \(I_{\text{SOC}}\) is not increased under elevated Ca2+ buffering conditions, and the activation kinetics correlate with the expected time course of active or passive store depletion, which is not the case for recombinant \(I_{\text{TRPv6}}\). TRPV6 cDNA transfection of LNCaP cells restores \(I_{\text{TRPv6}}\) with its typical responses due to changes in the membrane potential and [Ca2+]i, and in the absence of external divalent cations. Endogenous TRPV6 mRNA expression in LNCaP cells is suppressed by androgen treatment (18) and is induced by a specific androgen receptor antagonist (18) and by 1,25-dihydroxyvitamin D3 (19, 20). However, \(I_{\text{SOC}}\) was not affected by any of these treatments, indicating that TRPV6 alone or passive store depletion, which is not the case for recombinant TRPV6.

**EXPERIMENTAL PROCEDURES**

**Cell Culture and Transfection—**LNCaP cells (CRL-10995, lot 1216242) were from American Type Culture Collection (Manassas, VA) and from a second, independent source (ACC256, Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany). Cells were cultured in RPMI 1640 medium with l-glutamine, 10% fetal bovine serum, 50,000 IU/liter penicillin G (sodium), and 50 μg/ml streptomycin sulfate (Invitrogen, Karlsruhe, Germany). Cells were grown in 250- and 750-ml flasks (BD Biosciences) under 5% CO2 at 37 °C, induced by a specific androgen receptor antagonist (18) and by 1,25-dihydroxyvitamin D3-treated cells.

**Northern Blot Analysis—**For Northern blot experiments, LNCaP cells were cultured in the presence of dihydrotestosterone (1 μM) for 24 h, bicalutamide (1 μM) for 48 h, 1,25-dihydroxyvitamin D3 (1 μM) for 24 h, and MeSO (0.01%) for 48 h. LNCaP cells from three 750-ml (175-mm) flasks for each treatment were harvested by trypsinization. Cell pellets were prepared in a Potter-Elvehjem homogenizer, and RNA was purified using GOLD RNApureTM (peqlab Biotechnologie GmbH, Erlangen, Germany). Total RNA was extracted with chloroform, precipitated with isopropl alcohol, washed with 70% ethanol, and dissolved in diethyl pyrocarbonate-treated water. Isolation of mRNA was carried out using the poly(A) SpinTM mRNA isolation kit (New England Biolabs GmbH, Frankfurt, Germany) by capillary transfer overnight. The RNA was fixed on the nylon membranes by UV light, followed by heating at 80 °C for 2 h. The blots were then prehybridized at 65 °C for 2 days and then incubated at 42 °C for 1 h in 50% (v/v) formamide, 50 mM Tris-HCl (pH 7.5), 0.1% SDS, 2× 125-I]Na2P-O4, 1% SDS, 0.2% (v/v) polyvinylpyrrolidone 4000, 0.2% (v/v) Ficol 400, 5 mM EDTA (pH 8.0), 750 mM NaCl, 75 mM sodium citrate, and 0.15 mM freshly denatured salmon sperm DNA. Hybridizations were performed at 42 °C for 18–20 h in the presence of the prehybridization solution including the radioactive probe at 3&times;105 cpm/ml. The probe was the 571-bp PstI cDNA fragment of human TRPV6 encoding amino acid residues 40–228 labeled by random priming with [α-32P]dCTP (RediprimeII) with random primer labeling system, Amersham Biosciences) and isolated from non-incorporated nucleotides by gel filtration (Sephadex G-50 NickTM columns, Amersham Biosciences). Hybridizations were performed for 2 days and developed on a FujiFilm BAS-2500 phosphor imager (Fuji Photo Film, Kanagawa, Japan). Individual background subtraction was performed for each lane. Glyceraldehyde-3-phosphate dehydrogenase was used as an internal control and the housekeeping gene 18S. The ratio of signal intensity of the target RNA to the internal control was calculated for each lane. The expression levels were normalized to the expression levels of the housekeeping gene 18S.
Ca\(^{2+}\) concentration to 10 mM. The intracellular solution usually contained 10 mM EGTA to decrease the [Ca\(^{2+}\)] and thereby prevent the activation of Ca\(^{2+}\)-dependent channels. Contamination by K\(^+\) currents was eliminated by internal and external Cs\(^-\) and external tetraethylammonium. Osmolarities of the pipette and bath solutions were carefully adjusted and kept within the physiological range to minimize contributions of volume-sensitive channels.

Diazoating IP\(_3\) (30 \(\mu\)M) and the sarcoplasmic/endoplasmic reticulum Ca\(^{2+}\)-ATPase inhibitor thapsigargin (2 \(\mu\)M) into the cell has been shown to deplete intracellular Ca\(^{2+}\) stores rapidly (3). Shortly after establishing the whole-cell configuration, a small inward current activated (\(\tau = 27 \pm 3\) s, \(n = 4; I_{\text{max}} = -1.8 \pm 0.4\) pA/pF, \(n = 5\) (Fig. 2A). Similar results were obtained with either IP\(_3\) (\(\tau = 22 \pm 1\) s, \(n = 15\); \(I_{\text{max}} = -2.4 \pm 0.1\) pA/pF, \(n = 18\) (Fig. 2B) or thapsigargin (\(\tau = 24 \pm 3\) s, \(n = 5\); \(I_{\text{max}} = -2.0 \pm 0.1\) pA/pF, \(n = 10\) (Fig. 2C)) alone in the pipette solution. In the absence of IP\(_3\) and thapsigargin, the same small inward current was activated, but with a much slower time course. After a delay of 1 min, the current slowly developed and reached a similar peak current amplitude as in the recordings with IP\(_3\) and/or thapsigargin (\(I_{\text{max}} = -1.8 \pm 0.2\) pA/pF, \(n = 3\) (Fig. 2D). Because the cell was diazoyed by a solution containing 10 mM EGTA, it is likely that this chelator prevented re-uptake of the Ca\(^{2+}\) that had leaked out back into the stores. Such store depletion is known to be considerably slower than depletion caused by inhibition of Ca\(^{2+}\) re-uptake by thapsigargin or stimulation of Ca\(^{2+}\) release by IP\(_3\) and might explain the different activation kinetics of the inward current under the various conditions. Similar results were obtained with the Ca\(^{2+}\) chelator BAPTA (10 mM; \(n = 5\)) (data not shown) instead of EGTA.

Current-Voltage Relationship of \(I_{\text{SOC}}\)—The current-voltage relationship of \(I_{\text{SOC}}\) was the same in all experiments (Fig. 3): the current showed a strong inward rectification and reversed at positive potentials (>30 mV). During voltage steps, there was a biphasic current inactivation at potentials more negative than ~50 mV, similar to the inactivation behavior of \(I_{\text{CRAC}}\) in RBL cells (data not shown).

Cytoplasm-permeable Store-operated Channel in LNCaP Cells—The Ca\(^{2+}\)-permeability of the store-operated channel in LNCaP cells was tested by replacing Ca\(^{2+}\) with Mg\(^{2+}\) in the standard bath solution. During application of this Ca\(^{2+}\)-free solution, the inward current was abruptly abolished (Fig. 4). The block was reversible because the current could be restored in the presence of the Ca\(^{2+}\)-containing bath solution. The loss of inward current in the absence of extracellular Ca\(^{2+}\) and the positive reversal potentials in the presence of extracellular Ca\(^{2+}\) indicate that the store-operated inward current is carried by Ca\(^{2+}\).

Activation of \(I_{\text{SOC}}\) by Store Depletion at Low Intracellular Ca\(^{2+}\) Buffering—It is possible to activate \(I_{\text{CRAC}}\) under conditions of low intracellular Ca\(^{2+}\) buffering if sarcoplasmic/endoplasmic reticulum Ca\(^{2+}\)-ATPases are inhibited; under these conditions, \(I_{\text{TRPV6}}\) is not activated (1). In the presence of 2 \(\mu\)M thapsigargin, 40 \(\mu\)M IP\(_3\), and 0.5 mM EGTA (instead of the 10 mM EGTA used before), an inward current was activated in LNCaP cells (\(\tau = 26 \pm 4\) s, \(n = 5\); \(I_{\text{max}} = -3.1 \pm 0.3\) pA/pF, \(n = 6\)) (Fig. 5), which was identical to \(I_{\text{SOC}}\) measured under conditions of high intracellular Ca\(^{2+}\) before in terms of its IV relationship and Ca\(^{2+}\) selectivity. The same current developed if the identical internal solution was used, but with only 0.1 mM EGTA instead of 0.5 mM (\(n = 3\)). However, no current developed if the pipette solution contained only low concentrations of EGTA, but no store-depleting agents such as thapsigargin and IP\(_3\) (\(n = 3\) for 0.1 mM and \(n = 4\) for 0.5 mM EGTA).

Fig. 1. Expression of TRPV6 mRNA (A) and protein (B) in LNCaP cells. A, shown is an autoradiogram from blot hybridization analysis using poly(A\(^+\)) RNA (8.9 \(\mu\)g) isolated from LNCaP cells probed with the 571-\(\beta\) Psxl cDNA fragment of human TRPV6 at the top and the \(\beta\)-actin control at the bottom. B, proteins from non-transfected HEK-293 cells (lane 1), TRPV6-expressing HEK-293 cells (250 \(\mu\)g; lane 2), and LNCaP cells (250 \(\mu\)g; lane 4) and proteins immunoprecipitated from LNCaP microsomal proteins (7.5 mg) by antibody 429 (lanes 3 and 4) were separated on 8% SDS-polyacrylamide gels. The proteins were immunoblotted with monoclonal antibody 20C6. The antibody recognizes the TRPV6 proteins in TRPV6-expressing HEK-293 cells and LNCaP cells (arrowheads), but not in non-transfected HEK-293 cells. The various sizes of the proteins recognized by the antibody might be due to varying degrees of glycosylation, as suggested previously (25, 26).
Recombinant I_{TRPV6} in LNCaP Cells Resembles, but Is Not Identical to, I_{SOC}—The data presented so far argue in favor of a Ca^{2+}-selective store-operated current in LNCaP cells similar to I_{CRAC} in RBL and Jurkat cells. However, unlike recombinant I_{TRPV6} in HEK and RBL cells, I_{SOC} in LNCaP cells was not increased by raising the intracellular EGTA concentration to 30 mM (I_{max} = -2.1 ± 0.3 pA/pF, n = 6; compare with Fig. 2B). These data suggest that I_{SOC} was already maximally activated if Ca^{2+} stores were depleted under conditions of lower Ca^{2+} buffering (Figs. 2 and 5). In addition, the activation kinetics of I_{SOC} were slow when cells were dialyzed with EGTA or BAPTA at high concentrations in the absence of IP_{3} and thapsigargin, which contrasts with the rapid activation of recombinant I_{TRPV6}. To examine whether TRPV6 might be regulated differentially in LNCaP cells or whether current activation depends on TRPV6 expression levels, we transfected LNCaP cells with the TRPV6 cDNA. Dialyzing these cells with 10 mM EGTA resulted in rapid activation of a large inward current (Fig. 6A) very similar to the recombinant I_{TRPV6} previously recorded in TRPV6-transfected HEK and RBL cells (1). Peak amplitudes were reached within 1 min of whole-cell recording; and subsequently, current densities decreased until a steady-state level was reached (Fig. 6A). Like I_{SOC}, I_{TRPV6} is an inward rectifying current with a positive reversal potential (Fig. 6B). However, its activation precedes the depletion of intracellular Ca^{2+} stores caused by intracellular perfusion with high concentra-
potential augments recombinant I_{SOC}.

Previously, it has been shown that increasing the holding potential of LNCaP cells (Fig. 2D) and of I_{CRAC} in RBL cells strongly depends on the kinetics of Ca^{2+} release from intracellular stores. Ca^{2+}-selective channels like voltage-gated Ca^{2+} channels (27) and CRAC channels (3) become permeable for monovalent ions if divalent cations are removed from the extracellular solution. Similar properties of recombinant I_{TRPV6} in HEK cells have been described (1, 9, 10, 28). Under these conditions, I_{TRPV6} showed a typical voltage-dependent Mg^{2+}-block (9, 1), resulting in a negative slope at strong hyperpolarization, which was also observed in TRPV6-overexpressed LNCaP cells (Fig. 6D), but not in non-transfected LNCaP cells (n = 5) (data not shown).

Modulation of I_{SOC} and I_{TRPV6} by the Membrane Potential—Previously, it has been shown that increasing the holding potential augments recombinant I_{TRPV6} most probably by reducing the driving force for Ca^{2+} to enter the cell in between pulses and subsequently the Ca^{2+}-induced negative feedback (10). However, these typical properties of recombinant I_{TRPV6} were not detected in non-transfected LNCaP cells using various activation protocols (10 mM EGTA, 30 μM IP_{3}, and 2 μM thapsigargin (n = 5); 0.5 mM EGTA, 30 μM IP_{3}, and 2 μM thapsigargin, (n = 6); and 10 mM EGTA and 30 μM IP_{3} (n = 4)), whereas Ca^{2+} currents in TRPV6-overexpressed LNCaP cells reversibly increased during membrane depolarization (Fig. 7).

I_{SOC} in Androgen-, Antiandrogen-, and 1,25-Dihydroxyvitamin D_{3}-treated LNCaP Cells—So far, the data demonstrate that LNCaP cells contain store-operated channels similar to CRAC channels in RBL and Jurkat cells, whereas recombinant TRPV6 can be recorded only if the TRPV6 cDNA is overexpressed in these cells. Apparently, TRPV6 at low expression levels might constitute a subunit of the store-operated channel intrinsic to LNCaP and contribute to I_{SOC}. At high expression levels as obtained after TRPV6 cDNA transfection, TRPV6 protein levels may not be matched by protein levels of the other channel subunits and/or of molecules of the signal transduction mechanism that sense store depletion, and the resulting homomeric TRPV6 channel gives rise to I_{TRPV6}. Interestingly, it has been previously shown that androgens and antiandrogens (18) and 1,25-dihydroxyvitamin D_{3} (19, 20) change TRPV6 expression levels. The TRPV6 mRNA is up-regulated in the presence of antiandrogens like bicalutamide and 1,25-dihydroxyvitamin D_{3} and down-regulated in the presence of dihydrotestosterone. We examined whether changes in endogenous TRPV6 expression levels might influence the properties of I_{SOC} and therefore incubated LNCaP cells in the presence of 1 μM dihydrotestosterone (24 h), 100 nM 1,25-dihydroxyvitamin D_{3} (48 h), or 1 μM bicalutamide (48 h) prior to analysis of TRPV6 mRNA expres-

ion and \( I_{\text{SOC}} \). Control cells were incubated in the presence of vehicle alone (0.01% MeSO). \( TRPV6 \) mRNA expression increased by 1.3- and 2-fold in the presence of bicalutamide
\( (n = 2) \) and 1,25-dihydroxyvitamin D\(_3\) \( (n = 2) \), respectively, or was reduced by \( \sim 62-79\% \) in the presence of dihydrotestosterone \( (n = 2) \). However, no significant differences were found in paired recordings with respect to the peak current amplitude (dihydrotestosterone: \( 2.0 \pm 0.2 \, \text{pA/pF}, n = 13 \); control: \( 2.4 \pm 0.2 \, \text{pA/pF}, n = 9 \); 1,25-dihydroxyvitamin D\(_3\): \( 1.4 \pm 0.2 \, \text{pA/pF}, n = 11 \); bicalutamide: \( 1.7 \pm 0.2 \, \text{pA/pF}, n = 11 \); bicalutamide: \( 1.9 \pm 0.2 \, \text{pA/pF}, n = 9 \); control: \( 2.3 \pm 0.2 \, \text{pA/pF}, n = 14 \)), activation kinetics (dihydrotestosterone: \( \tau = 23 \pm 2 \, s, n = 11 \); control: \( \tau = 22 \pm 2 \, s, n = 6 \); 1,25-dihydroxyvitamin D\(_3\): \( \tau = 20 \pm 1 \, s, n = 11 \); bicalutamide: \( \tau = 23 \pm 1 \, s, n = 10 \); control: \( \tau = 21 \pm 2 \, s, n = 5 \)), and inactivation kinetics (Fig. 8). Similar results were obtained when cells were incubated for a longer period of time (dihydrotestosterone for 48 h \( (n = 10) \) and bicalutamide for 72 h \( (n = 13) \)). As shown before, depolarization resulted in \( I_{\text{TRPV6}} \) augmentation, whereas hyperpolarization induced a reduction of \( I_{\text{TRPV6}} \) (Fig. 7A). Although \( TRPV6 \) expression was increased by bicalutamide and 1,25-dihydroxyvitamin D\(_3\), no increase in \( I_{\text{SOC}} \) could be detected when the plasma membrane was depolarized \((n = 6)\) for bicalutamide-treated cells and \( n = 10 \) for 1,25-dihydroxyvitamin D\(_3\)-treated cells). In agreement, no difference in fura-2 fluorescence was recorded between control and 1,25-dihydroxyvitamin D\(_3\)-treated cells using a Ca\(^{2+}\) re-addition protocol with ionomycin (data not shown).

Although less physiological than Ca\(^{2+}\) currents, monovalent currents should increase the signal-to-noise ratio. This will allow a more accurate determination of whether the various treatments affect \( I_{\text{SOC}} \). To rule out potential variations between different batches of LNCaP cells, we used another source of cells (see “Experimental Procedures”) for these experiments. No differences were detected under external divalent-free conditions with respect to current densities (dihydrotestosterone: \( -5.7 \pm 1.0 \, \text{pA/pF}, n = 8 \); bicalutamide: \( -6.1 \pm 1.2 \, \text{pA/pF}, n = 5 \); 1,25-dihydroxyvitamin D\(_3\): \( -5.7 \pm 0.7 \, \text{pA/pF}, n = 7 \); control: \( -6.3 \pm 0.8 \, \text{pA/pF}, n = 9 \)), activation kinetics (dihydrotestosterone: \( \tau = 17 \pm 2 \, s, n = 8 \); bicalutamide: \( \tau = 15 \pm 3 \, s, n = 5 \); 1,25-dihydroxyvitamin D\(_3\): \( \tau = 19 \pm 2 \, s, n = 6 \); control: \( \tau = 16 \pm 1 \, s, n = 9 \)), and rundown behavior (Fig. 9). Thus, it was not possible to resolve changes in \( I_{\text{SOC}} \) despite the up- and down-regulation of \( TRPV6 \) expression in LNCaP cells.

![Fig. 7. Modulation of \( I_{\text{TRPV6}} \) but not of \( I_{\text{SOC}} \), by changes in the holding potential. Shown is the development of Ca\(^{2+}\) influx in either a typical \( TRPV6 \)-transfected LNCaP cell \((n = 6)\) (A) or a non-transfected wild-type cell \((n = 4)\) (B) following dialysis with the cesium glutamate-based internal solution containing 10 mM EGTA for the experiment shown in A and 10 mM EGTA and 30 \( \mu \text{M} \) IP\(_3\) for the experiment shown in B. The membrane potential was changed from \( -10 \) to \( 50 \, \text{mV} \) as indicated. The time course of current development was monitored at \( -80 \, \text{mV} \) after subtraction of the initial current. Norm., normalized.](Image 318x340 to 562x737)

![Fig. 8. Store-operated Ca\(^{2+}\) entry in LNCaP cells incubated with androgen, antiandrogen, and 1,25-dihydroxyvitamin D\(_3\). LNCaP cells were incubated in medium containing dihydrotestosterone \((1 \, \mu \text{M})\) for 24 h prior to whole-cell patch-clamp recording \((n = 13)\) (A), bicalutamide \((1 \, \mu \text{M})\) for 48 h prior to whole-cell patch-clamp recording \((n = 9)\) (B), and 1,25-dihydroxyvitamin D\(_3\) \((100 \, \text{nM})\) for 24 h prior to whole-cell patch-clamp recording \((n = 11)\) (C). Paired control recordings were performed on the same day using cells that were cultured in the presence of vehicle under otherwise identical conditions \((A, n = 9; B, n = 14; C, n = 11)\). The intracellular solution contained IP\(_3\) \((30 \, \mu \text{M})\) and EGTA \((10 \, \text{mM})\). Averaged data with double-sided S.E. are shown. Norm., normalized.](Image 74x429 to 290x737)
Store-operated Ca\(^{2+}\) Current and TRPV6 Channels in LNCaP Cells

![Fig. 9. Store-dependent monovalent current in androgen-, antiandrogen-, and 1,25-dihydroxyvitamin D\(_3\)-treated LNCaP cells. Cells were incubated, and patch-clamp experiments were performed as described for Fig. 8. The bath solution was divalent-free. Mean data with two-sided S.E. are shown for dihydrotestosterone-treated (n = 8) (A), bicalutamide-treated (n = 5) (B), and 1,25-dihydroxyvitamin D\(_3\)-treated (n = 7) (C) cells and control cells (n = 9). Norm., normalized.](image)

**DISCUSSION**

In this study, we have demonstrated the endogenous expression of the TRPV6 gene in the human lymph node prostate cancer cell line LNCaP. We have characterized a store-operated inward current (I\(_{\text{SOC}}\)) in these cells, which closely resembles I\(_{\text{CRAC}}\) with respect to its activation mechanism, inward rectification, and Ca\(^{2+}\) selectivity. These biophysical properties are also typical for recombinant I\(_{\text{TRPV6}}\). It was, however, possible to discriminate between I\(_{\text{SOC}}\) and I\(_{\text{TRPV6}}\) in three independent ways: the activation mechanism, the behavior in divalent-free external solution, and the response due to changes in the membrane potential were different. In addition, cell culturing in the presence of androgen, antiandrogen, or 1,25-dihydroxyvitamin D\(_3\), each of which is known to change TRPV6 mRNA expression, did not affect I\(_{\text{SOC}}\) with respect to its peak current amplitude and activation and inactivation kinetics.

I\(_{\text{SOC}}\) in LNCaP cells could be activated by three independent protocols that lead to depletion of intracellular Ca\(^{2+}\) stores. 1) Ca\(^{2+}\) release from endogenous stores can be elicited using IP\(_3\) in the pipette solution. 2) The sarcoplasmic/endoplasmic reticulum Ca\(^{2+}\)-ATPase inhibitor thapsigargin leads to store depletion because Ca\(^{2+}\) pumps are blocked and no longer able to replenish the pools. 3) Dialyzing high Ca\(^{2+}\) chelator concentrations into the cell is believed to prevent the re-uptake of Ca\(^{2+}\) that has leaked out from the stores. Subsequently, the Ca\(^{2+}\) pools become empty, and store-operated Ca\(^{2+}\) influx is activated. Depletion of Ca\(^{2+}\) stores is thought to be rapid with IP\(_3\) and thapsigargin, but slow with EGTA or BAPTA alone. The kinetics of I\(_{\text{SOC}}\) activation in LNCaP cells correlate well with the presumed time courses of store depletion due to the various protocols.

It has previously been reported that the I-V relationship of recombinant I\(_{\text{TRPV6}}\) is similar to the prototype of the store-operated current I\(_{\text{CRAC}}\). Borderline differences in the I-V relationship might be a more physiological procedure than overexpression of TRPV6, it is also possible to separate both currents by their I-V relationship in the absence of extracellular divalent cations. Under these conditions, monovalent currents through recombinant TRPV6 channels are characterized by a positive slope at very negative potentials in TRPV6-overexpressing cells. This behavior is not seen if intracellular Mg\(^{2+}\) is omitted, indicating a voltage-dependent block and unblock of the TRPV6 channel by Mg\(^{2+}\), as shown previously by Voets et al. (9, 28). However, no such voltage-dependent Mg\(^{2+}\)-gated block of I\(_{\text{SOC}}\) was detected in non-transfected LNCaP cells.

Another typical property of TRPV6 channels is the modulation of Ca\(^{2+}\) current amplitudes by the membrane potential. Depolarization resulted in I\(_{\text{TRPV6}}\) augmentation, whereas hyperpolarization induced a reduction of I\(_{\text{TRPV6}}\). In contrast, changes in the holding potential did not change the amplitude of I\(_{\text{SOC}}\).

Previously, it has been shown that the levels of endogenously expressed TRPV6 can be changed by treating cells with androgens, antiandrogens, and 1,25-dihydroxyvitamin D\(_3\) (18–20). Changing expression levels of endogenous TRPV6 by hormones might be a more physiological procedure than overexpression of its cDNA. Accordingly, we found that TRPV6 transcript expression levels increased by ~1.3- and 2-fold after treating LNCaP cells with bicalutamide and 1,25-dihydroxyvitamin D\(_3\), respectively, but were reduced by ~62–79% after incubation with dihydrotestosterone. However, no corresponding changes in I\(_{\text{SOC}}\) were detectable. These data, together with the recordings demonstrating differences in the activation mechanism in the behavior under divalent-free conditions as well as the diverse properties due to changes in the membrane potential, suggest that the TRPV6 protein alone cannot form store-operated Ca\(^{2+}\) channels in LNCaP cells. These results were confirmed using LNCaP cells from a second, independent source. As in RBL cells (1), overexpression of the TRPV6 cDNA resulted in the appearance of I\(_{\text{TRPV6}}\) rather than an augmentation of endogenous I\(_{\text{SOC}}\), indicating that under conditions of overexpressed protein, TRPV6 is a Ca\(^{2+}\)-sensing Mg\(^{2+}\)-gated channel.

It is, however, also possible to speculate that TRPV6 is one potential component of the protein complex forming store-operated channels. One could consider that TRPV6 is tightly coupled to its signal transduction machinery. Moreover, this...
coupling cannot be disturbed by overexpression of TRPV6 or by manipulation of its endogenous expression levels as shown in this study. In fact, TRPV6 has been suggested to be a component of store-operated channels in RBL cells (8, 29), Jurkat T-lymphocytes (30), and LNCaP cells (16, 17), although TRPV6 transcripts are not detectable by Northern blotting in RBL cells (1, 8) and Jurkat cells (12). Apparently, the TRPV6-related functions elicited by physiological stimuli have to be identified (1, 8) and Jurkat cells (12). We thank Heidi Lühr and Christine Jung for excellent technical help.

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