The activation mechanism of the recently cloned human transient receptor potential vanilloid type 6 (TRPV6) channel, originally termed Ca$^{2+}$ transporter-like protein and Ca$^{2+}$ transporter type 1, was investigated in whole-cell patch-clamp experiments using transiently transfected human embryonic kidney and rat basophilic leukemia cells. The TRPV6-mediated currents are highly Ca$^{2+}$-selective, show a strong inward rectification, and reverse at positive potentials, which is similar to store-operated Ca$^{2+}$ entry in electrically non-excitable cells. The gating of TRPV6 channels is strongly dependent on the cytosolic free Ca$^{2+}$ concentration; lowering the intracellular free Ca$^{2+}$ concentration results in Ca$^{2+}$ influx, and current amplitude correlates with the intracellular EGTA or BAPTA concentration. This is also the case for TRPV6-mediated currents in the absence of extracellular divalent cations; compared with endogenous currents in nontransfected rat basophilic leukemia cells, these TRPV6-mediated monovalent currents reveal differences in reversal potential, inward rectification, and slope at very negative potentials. Release of stored Ca$^{2+}$ by inositol 1,4,5-trisphosphate and/or the sarco/endoplasmic reticulum Ca$^{2+}$-ATPase inhibitor thapsigargin appears not to be involved in TRPV6 channel gating in both cell lines but, in rat basophilic leukemia cells, readily activates the endogenous Ca$^{2+}$ release-activated Ca$^{2+}$ current. In conclusion, TRPV6, expressed in human embryonic kidney cells and in rat basophilic leukemia cells, functions as a Ca$^{2+}$-sensing Ca$^{2+}$ channel independently of procedures known to deplete Ca$^{2+}$ stores.

Calcium is involved in a multitude of intracellular signal transduction mechanisms ranging from contraction to secretion. To achieve a high bandwidth of signal transmission with sometimes even opposing effects, cells need to control the spatio-temporal resolution of their Ca$^{2+}$ signals. In nonexcitable cells the rise in [Ca$^{2+}$]$_i$, during stimulation of G-protein-coupled receptors or receptor tyrosine kinases is regulated in a complex fashion by Ca$^{2+}$ release from endogenous IP$_3$-sensitive stores followed by store-operated Ca$^{2+}$ influx across the plasma membrane. One of the best characterized store-operated Ca$^{2+}$ entry pathways is the Ca$^{2+}$ release-activated Ca$^{2+}$ current, termed I$_{CRAC}$ (2, 3).

The molecular identity of store-operated channels is a matter of debate. Several members of the TRP family, a group of Ca$^{2+}$ permeable channels related to the Drosophila melanogaster TRP gene product, have been implicated in store-dependent cation influx (4, 5). However, so far, none of the trp genes has been shown to encode channels with the ion-permeation properties and the sensitivity to store depleting agents of CRAC channels.

The family of the trp genes in vertebrates can be divided into three subfamilies, based on similarities in the structures of the encoded proteins (6), the TRPC, the TRPM, and the TRPV subfamilies. Members of the TRPV subfamily appear to be regulated by physical or chemical stimuli such as heat, osmotic, or mechanical stress. The recently cloned human TRPV6 gene product (GenBank$^\text{TM}$ accession no. CAC20417), formerly called CaT-L or TRPV6 (7, 8) or CaT1 (9), belongs into this group. Human TRPV6 channels (7), like rat TRPV6 (formerly called rat CaT1; Ref. 10) and rabbit TRPV5 channels (former ECaC for epithelial Ca$^{2+}$ channel; Ref. 11), show a high Ca$^{2+}$ selectivity, Ca$^{2+}$ and Na$^+$ permeation properties indicative of an anomalous mole fraction behavior, and current-voltage relationships similar to CRAC channels.

Recently these common features between TRPV6 and CRAC channels were demonstrated by two groups (12, 13). In addition Voets et al. (13) also revealed several differences between I$_{CRAC}$ in RBL cells and TRPV6-mediated Ca$^{2+}$ currents when expressed in HEK cells, including insensitivity of TRPV6 channels to store depletion and the effect of intracellular Mg$^{2+}$, which causes voltage-dependent block of TRPV6, but not of CRAC channels. In contrast, Yue et al. (12) demonstrated activation of TRPV6 channels in CHO cells by depletion of calcium stores with IP$_3$ and thapsigargin. This gating mechanism could be accomplished when recordings were performed 8–12 h but not 24 h after transfection (12). The authors assumed that, after 24 h, TRPV6 overexpression is not matched by the signal transduction apparatus presumed to be endogenously present in CHO cells and responsible for sensing store depletion. Subsequently, Yue et al. (12) suggested that the TRPV6 protein comprises all or a part of the CRAC pore.

In the present study, two different cell lines, RBL and HEK cells, were used to study the gating of human TRPV6 channels. RBL cells are a common model system to study I$_{CRAC}$, whereas in HEK cells ionic conductances associated with store depletion

family M (melastatin type); TRPV, transient receptor potential subfamily V (vanilloid-receptor type); wt, wild-type.
are not prominent (14–16). Accordingly, one could assume a higher or different expression level of the signal transduction machinery that leads to I_{CRAC} activation, in RBL than in HEK cells. We therefore expressed the TRPV6 cDNA in both cells and studied whether there is a cell-specific modulation of TRPV6 channel function. Under conditions of high intracellular Ca^{2+} buffering, TRPV6-mediated Ca^{2+} currents develop to such an extent even in RBL cells that it swamps the current through CRAC channels. However, at low intracellular Ca^{2+} buffering, store depletion activates I_{CRAC} but not TRPV6 channels, suggesting that the TRPV6 protein is not sensitive to store depletion under these conditions. Instead, TRPV6 channels function as Ca^{2+}-sensing Ca^{2+} pores and their current amplitudes are inversely correlated with the [Ca^{2+}]_{i}.

**Experimental Procedures**

**Cell Culture, Transfected cDNA, and Transfection—**HEK-293 (ATCC, 1573-CRL) and RBL-1 cells (ATCC, 1378-CRL) were from the American Type Culture Collection (Manassas, VA). Minimum essential medium with Earle’s salts and l-glutamine was used for HEK cells and Dulbecco’s modified Eagle’s medium including sodium pyruvate, and 1000 mg/liter t-glucose for RBL cells. Both culture media were supplemented with 10% fetal calf serum (Invitrogen, Paisley, UK).

Electrophysiologic Recordings and Solutions—For experiments, coverslips with cells were transferred 24–32 h after transfection to the recording chamber and kept in a modified Ringer solution (in mM): 145 NaCl, 10 CaCl_{2}, 10 CsCl, 2.8 KCl, 11 glucose, 10 HEPES, adjusted to pH 7.2 with NaOH. The divalent-free solution was contained (in mM): 145 cesium glutamate, 10 HEPES, 8 NaCl, 1 MgCl_{2}, and 2.8 KCl. This solution was used in the patch-clamp experiments and in the pipette solution. No currents were observed in the divalent-free conditions and amplitudes was measured 1 min after establishing the whole-cell configuration, because TRPV6 channels fully activate usually within this time. The global [Ca^{2+}]_{i} and [Mg^{2+}]_{i} were calculated by custom software (0.58, 1.16, 3.49, 11.66, 23.31, 34.97, 69.91, and 348.59 pmol Ca^{2+} and 0.64, 0.79, 0.95, 1.02, 1.03, 1.04, and 1.05 mM Mg^{2+}) at 60, 30, 10, 3, 1.5, 1, 0.5, and 0.1 mM EGTA, respectively. Throughout, average data are given as means ± S.E. for n cells. Student’s paired t test was used for comparison of means.

**Drugs—**All chemicals were purchased from Sigma, except BAPTA (Molecular Probes).

**RESULTS**

Current-Voltage Relationship from TRPV6-expressing HEK Cells and Nontransfected RBL Cells—TRPV6-expressing HEK cells, a large current was elicited by voltage ramps when [Ca^{2+}]_{i} was buffered by 10 mM EGTA in the patch pipette (Fig. 1). The inwardly rectifying current reversed at positive potentials (≥30 mV, n = 5). At potentials more negative to −40 mV the current showed a fast inactivation (time constant of 7.6 ± 1.3 ms at −100 mV, n = 5). No such current was recorded in the absence of external Ca^{2+} from TRPV6-transfected HEK cells (data not shown, n = 5). Mock and nontransfected cells exhibited a small background current with a linear I-V relationship (data not shown, n = 5 each).

The biophysical properties of heterologously expressed TRPV6 channels are similar to those of endogenous store-operated channels in RBL cells (Fig. 1B). Both currents show a strong inward rectification with a positive reversal potential, indicating a high Ca^{2+} selectivity.

**Store Depletion at High Intracellular Ca^{2+} Buffering in TRPV6-expressing HEK and RBL Cells in Comparison to Untransfected Cells—**It has been argued for the TRPV6 protein from rat that this channel is only store-operated when its expression level stands in a special relation to the native signal transduction machinery of CHO cells (12). This idea was tested by expressing the human clone in RBL cells, which show a much more pronounced I_{CRAC}, than CHO and HEK cells. Subsequently, RBL cells might offer a more native environment for store-operated channels than HEK cells. Whether this affects the gating of TRPV6 channels was tested in the following experiments.

I_{CRAC} is gated by the filling state of IP_{3}-sensitive Ca^{2+} stores. It can be activated by store depletion obtained by intracellular perfusion of IP_{3}, the sarco/endo-plasmatic reticulum Ca^{2+}-ATPase inhibitor thapsigargin, and/or EGTA. Using IP_{3} (30 μM) and EGTA (10 mM) in the internal solution resulted in rapid activation of I_{CRAC} (Fig. 2). TRPV6-expressing HEK cells...
showed a rapid current activation followed by a slow inactivation until a steady-state level was reached (Fig. 2). This was also the case for TRPV6-expressing RBL cells; immediately after obtaining the whole-cell configuration, the Ca²⁺ current developed, reached a maximum, and subsequently decayed to a lower level (Fig. 2).

Very similar results were obtained with thapsigargin (2 μM) instead of IP₃ or both substances (Table I); I_Crac activated rapidly in RBL cells, no prominent current was detected in HEK cells, and a large inward current was measured in TRPV6-expressing HEK and RBL cells such as shown in Figs. 1 and 2. No significant differences between both cell types were detected with respect to the peak Ca²⁺ current, activation, and inactivation kinetics.

**FIG. 1.** Current-voltage relationship from TRPV6-expressing HEK and nontransfected RBL cells. Whole-cell patch-clamp experiments were performed on TRPV6-transfected HEK cells and RBL wild-type cells as described under “Experimental Procedures.” Currents were measured after maximum activation under identical conditions. The cells were dialyzed with a cesium glutamate-based internal solution containing 10 mM EGTA. A modified Ringer’s solution with 10 mM Ca²⁺ was used extracellularly. The holding potential was −10 mV. A shows the I-V relationship (mean ± S.E.) measured from a 4-ms average at the start (4 ms after the beginning of the voltage step) and the end of the pulse (4 ms prior to the end of the voltage step) as indicated by the arrows (n = 5). The inset in A illustrates the voltage protocol from −110 mV to 10 mV for 200-s pulse duration and with 1 s between steps. Typical current traces are shown after maximal activation. The dashed line in the lower figure of the inset represents zero current. B illustrates the voltage protocol from −110 mV to 90 mV in 50 ms. Representative current traces for a TRPV6-expressing HEK cell (black) and a nontransfected RBL cell (gray) are shown. Both current traces were leak-subtracted as described under “Experimental Procedures” and scaled to their maximum amplitude. The dashed line represents zero current.

**FIG. 2.** Effects of store depletion at high intracellular Ca²⁺ buffering in TRPV6-expressing HEK and RBL cells in comparison to untransfected RBL cells. Development of Ca²⁺ influx in TRPV6-transfected HEK and RBL cells and nontransfected RBL cells following dialysis with the internal solution containing EGTA (10 mM) or EGTA (10 mM) and IP₃ (30 μM). The current was measured after correction for initial conductance at −80 mV of the voltage ramps, which were applied every 2 s. Note the different scaling of the time axis for RBL wild-type cells perfused with 10 mM EGTA. Averaged data with double-sided S.E. are illustrated (10 mM EGTA and 30 μM IP₃: n = 4 for HEK-TRPV6, n = 5 for RBL-TRPV6; 10 mM EGTA: n = 7 for HEK-TRPV6, n = 3 for RBL-TRPV6, n = 6 for RBL-wt).
Ca^{2+} currents were recorded from TRPV6-transfected HEK and RBL cells. The patch pipette was filled with the standard cesium glutamate-based solution supplemented with EGTA (10 mM), thapsigargin (2 μM), and IP3 (30 μM) as indicated. The bath solution contained 10 mM Ca^{2+}. Cells were clamped at −10 mV, and maximal inward currents were measured at −80 mV during repetitive voltage ramps after background current subtraction as described under “Experimental Procedures.” Mean data ± S.E. (n) are given.

| HEK-TRPV6 | RBL-TRPV6 |
|-----------|-----------|
| EGTA + thapsigargin + IP3 | −17.0 ± 2.9 (6) | −25.6 ± 4.6 (3) |
| EGTA + thapsigargin | −23.8 ± 4.2 (3) | −23.2 ± 3.9 (3) |
| EGTA + IP3 | −22.2 ± 5.7 (4) | −26.3 ± 4.2 (5) |
| EGTA | −18.2 ± 2.7 (7) | −19.1 ± 1.2 (3) |

The activation of I_{CRAC} by high concentrations of the Ca^{2+} buffer EGTA (10 mM) in the intracellular solution is much slower than in the recordings with IP3 and/or thapsigargin (Fig. 2). Strikingly, the activation kinetic of TRPV6-mediated Ca^{2+} currents was not changed in both expression systems tested. Similar results were obtained with BAPTA instead of EGTA (data not shown). Taken together, these data suggest that the presence of store-depleting agents such as thapsigargin and IP3 does not influence TRPV6 channel activation.

**Store Depletion at Low Intracellular Ca^{2+} Buffering in TRPV6-expressing HEK and RBL Cells in Comparison to Untransfected Cells**—It is possible to record I_{CRAC} under conditions of moderate cytoplasmic Ca^{2+} buffering (20). With low concentrations of EGTA (1.5 mM) and the addition of IP3 (30 μM) in the pipette solution, peak current amplitude in RBL wild-type cells was similar to the recordings presented before (Fig. 2). However, current densities were dramatically decreased in TRPV6-transfected cells (Fig. 3) when compared with the recordings with higher EGTA concentrations (10 mM, Fig. 2).

This difference became more obvious when I_{CRAC} was activated with thapsigargin (2 μM) and IP3 (30 μM) under more physiological conditions of low intracellular Ca^{2+} buffering (0.1 mM EGTA). Prominent I_{CRAC} activation was recorded in RBL wild-type cells (Fig. 3), which was very similar to the recordings with higher EGTA concentrations (10 mM) under otherwise identical conditions (data not shown, n = 5). Although store depletion was achieved as proved by the activation of I_{CRAC} in RBL wild-type cells, no macroscopic current was detected in TRPV6-expressing HEK cells (Fig. 3). However, using 10 mM EGTA with thapsigargin and IP3 in the intracellular solution induced a large Ca^{2+} influx, as described earlier (Table 1). Therefore, it is likely that the Ca^{2+} current seen in TRPV6-transfected RBL cells was I_{CRAC} and that TRPV6 channels are not activated by thapsigargin and IP3.

**TRPV6 Channel Activity Depends on the Intracellular Ca^{2+} Chelator Concentration**—Because TRPV6 channels activated independently to store-depleting agents, it is possible that the intracellular EGTA concentration is crucial for current activation. Various intracellular EGTA concentrations were tested on their effect to change TRPV6-mediated Ca^{2+} entry (Fig. 4A). At low intracellular Ca^{2+} buffering with 0.1 mM EGTA in the pipette solution, no current was activated. Increasing the EGTA concentration slightly up to 0.5 mM resulted in Ca^{2+} current activation with a small current density. Higher concentrations of the chelator caused a further increase in current size. The relationship between Ca^{2+} current amplitude and EGTA concentration in the intracellular solution is plotted as a dose-response curve in Fig. 4B. Interestingly, no saturation of the current amplitude was detected with 60 mM EGTA in comparison to the current densities recorded with 30 mM EGTA. No such behavior was detected in nontransfected HEK cells.

Immediately after establishing the whole-cell configuration, the current, measured from the first ramp at −80 mV, was not significantly different between TRPV6-expressing and nontransfected HEK cells, arguing against a maximally activated channel under resting conditions (Table II).

**TRPV6 Channels Function as a Ca^{2+} Sensor**—The chelator EGTA is characterized by selectivity more than 5 orders of magnitude higher in chelating Ca^{2+} over Mg^{2+} (21). Therefore, mainly the [Ca^{2+}], and to a lesser extent the [Mg^{2+}], are decreased by EGTA. Thus, it is necessary to find out which ion is responsible for the EGTA-induced augmentation of TRPV6-mediated Ca^{2+} currents. In the previous experiments, the standard internal solution contained 1 mM MgCl₂ and 2 mM Mg-ATP, which in the presence of 0.1 mM EGTA translates to 1.05 mM Mg^{2+} and is reduced to 0.64 mM in the presence of 60 mM EGTA (see “Experimental Procedures”). With the latter solution a prominent Ca^{2+} current was detected, as shown in Figs. 4 and 5. Using the same pipette solution but elevating the [Mg^{2+}], to 1.05 mM resulted in very similar Ca^{2+} entry with respect to the activation kinetic and peak current amplitude (Fig. 5). However, no current activation was detected with an internal solution containing 1.05 mM [Mg^{2+}], and 100 mM [Ca^{2+}], (Fig. 5). Because chelators can induce effects unrelated to their Ca^{2+} buffering properties (22), it was necessary to use a pipette solution with the same [Mg^{2+}] and [Ca^{2+}], but 60 mM EGTA instead of 10 mM. Again, no current developed, which makes it unlikely that EGTA activates TRPV6 channels by pharmacological means (data not shown, n = 3). Furthermore, no inhibition of peak current amplitude was detected in the presence of 4.2 mM [Mg^{2+}], in comparison to the recordings at more physiological [Mg^{2+}], (1.05 and 0.64 mM) under otherwise identical conditions (60 mM EGTA, −33.3 ± 10.4 pA/pF, n = 4). These results suggest that the EGTA-induced Ca^{2+} current activation was the result of a fall of [Ca^{2+}].

**TRPV6-mediated Ca^{2+} Currents in the Presence of High Intracellular Concentrations of Either EGTA or BAPTA**—Prominent TRPV6-mediated Ca^{2+} entry was detected at a calculated global [Ca^{2+}], in the picomolar range. Because the physiological [Ca^{2+}], is not that low, it is tempting to speculate that higher levels were achieved within a microdomain of elevated Ca^{2+} near the channel. This idea was tested by using intracellular BAPTA instead of EGTA. BAPTA has a faster Ca^{2+} binding kinetic than EGTA (20), resulting in more effective competition with the slow endogenous Ca^{2+} buffers. High chelator concentrations (30 or 60 mM) were diazylated into the cells to avoid buffer saturation on channel opening in the presumed microdomain. Under these conditions Ca^{2+} currents were larger and showed less slow inactivation with BAPTA in comparison to EGTA, despite the fact that Ca^{2+} entry was elicited by short voltage-ramps of 50-ms duration, which were applied only once every 2 s (Fig. 6). These differences in the time courses of TRPV6-mediated Ca^{2+} influx was also seen with chelator concentrations of 60 mM instead of 30 mM (data not shown; n = 7 for 60 mM BAPTA and n = 10 for 60 mM EGTA). Hence, it is likely that local Ca^{2+} gradients control TRPV6 channels rather than the overall [Ca^{2+}].

**TRPV6-mediated Monovalent Ca^{2+} Currents When Divalent Cations Are Removed from the Extracellular Solution**—The presumable built-up of subplasmalemmal Ca^{2+} gradients can be abolished by recording monovalent currents in the absence of extracellular divalent cations. Fig. 7 illustrates the current-voltage relationship under these conditions where TRPV6 channels were activated with 10 mM intracellular EGTA. The I-V curves showed a pronounced inward rectification of a large
current with a slightly positive reversal potential. During hyperpolarizing steps the current activated slowly, which results in a negative slope at potentials roughly below \(-80\) mV during the ramp protocol, apparently because of a time-dependent removal of an intracellular Mg\(^{2+}\) block (data not shown, \(n = 8\); Ref. 13). However, no such behavior was observed for monovalent currents, which reversed at more positive potentials in nontransfected RBL cells after ICRAC activation by IP\(_3\) (30 \(\mu\)M) and EGTA (10 mM; Fig. 7B).

Dialysis of 10 mM EGTA into a TRPV6-expressing HEK cell resulted in rapid activation of Ca\(^{2+}\) currents followed by inactivation (Fig. 8). When the external Ringer’s solution was substituted by a divalent-free saline, inward currents were initially blocked in an anomalous fashion before large monovalent currents gradually developed and remained quite stable with time. Thus, the time course of monovalent current activation and inactivation was relatively slow in comparison to the fast development and run-down of Ca\(^{2+}\) currents. When switching back to the normal bath solution, monovalent currents rapidly decayed in opposition to their slow development under divalent-free conditions. The negative correlation of \([\text{Ca}^{2+}]_i\) and TRPV6 channel activity was shown for Ca\(^{2+}\) currents and also remained valid for monovalent currents; under divalent-free conditions, currents increased in parallel to an increase of intracellular EGTA concentrations (\(-97 \pm 27\) pA/pF; \(n = 6\) for 1 mM EGTA; \(-195 \pm 84\) pA/pF, \(n = 6\) for 10 mM EGTA;...
Averaged current traces are shown without correction for the initial background conductance (30 mM EGTA).

The intracellular solution contained various EGTA concentrations to study the effect of divalent cations on TRPV6 channel activity. The standard pipette solution supplemented with 60 mM EGTA had calculated [Ca\(^{2+}\)]/[H\(^{9262}\)] was 1.5 mM EGTA and [Mg\(^{2+}\)\_i] was adjusted in the third intracellular solution (1.08 mM MgCl\(_2\) and 2 mM Mg-ATP in total) to the solution increased [Mg\(^{2+}\)]/[H\(^{11006}\)] to 1.05 mM, whereas the [Ca\(^{2+}\)]/[H\(^{11001}\)] was unaffected (n = 11). The same [Mg\(^{2+}\)]/[H\(^{11001}\)] was adjusted in the third intracellular solution (1.08 mM MgCl\(_2\) and 2 mM Mg-ATP), and the [Ca\(^{2+}\)]/[H\(^{11001}\)] was clamped at 100 nM with 10 mM EGTA and 3.6 mM CaCl\(_2\) (n = 5). Averaged current traces are shown without correction for the initial current.

Voltage-activated EAG K\(^+\) channels undergo Ca\(^{2+}\)-mediated inhibition in situations where K\(_{Ca}\) channels start to become activated (23). The ubiquitous Ca\(^{2+}\)-binding protein CaM was identified as the Ca\(^{2+}\) sensor protein that activates K\(_{Ca}\) channels in response to rising [Ca\(^{2+}\)], but closes EAG channels. This reverse action is regulated by CaM binding to the C terminus of hEAG1 (24) and to the \(\alpha\)-subunit of small conductance K\(_{Ca}\) channels (25). Interestingly, the TRPV6 protein also binds CaM in a Ca\(^{2+}\)-dependent manner (8), which makes it an attractive candidate for the Ca\(^{2+}\) sensor.

It has been postulated that a decrease in [Ca\(^{2+}\)], near the plasma membrane in response to store depletion leads to activation of I\(_{CRAC}\) (26). Indeed it could be shown for RBL cells that low [Ca\(^{2+}\)], in the range of 30–50 nM activates I\(_{CRAC}\) spontaneously and independently of global Ca\(^{2+}\) store depletion, whereas elevation of [Ca\(^{2+}\)], up to 100 nM resulted in store-depleted gating of I\(_{CRAC}\) (27). Cell dialysis with low [Ca\(^{2+}\)] might passively deplete subtypes of Ca\(^{2+}\) stores, which are presumably responsible for I\(_{CRAC}\) activation. Unfortunately it was not possible to resolve these CRAC stores with mag fura-2. The activation properties of recombinant TRPV6 channels presented here were similar to those reported by Krause et al. (27) for I\(_{CRAC}\) with respect to the spontaneous activation at very low [Ca\(^{2+}\)]. However, no Ca\(^{2+}\) current increase was detected for TRPV6 channels if stores were depleted with thapsigargin and/or IP\(_3\) at 100 nM free Ca\(^{2+}\) in the pipette solution (data not shown).

The recombinant TRPV6 channel from rat (12) shows electrophysiological properties similar to those reported here and previously for the human channel (7) e.g. the high Ca\(^{2+}\) selectivity, anomalous mole fraction effect, block by inorganic cations, and loss of selectivity in the absence of divalents. The activation kinetics of rat TRPV6 channels were unaltered if store-depleting agents such as IP\(_3\) and thapsigargin were added to the EGTA (10 mM) containing pipette solution. Furthermore, no current activation was detected under conditions of weak intracellular Ca\(^{2+}\) buffering (0.05 and 0.5 mM EGTA). Very similar results were recorded for human TRPV6 channels in

**TABLE II**

| HEK-TRPV6 | HEK-wt |
|-----------|--------|
| 0.1 mM EGTA | -2.0 ± 0.3 (12) | -2.4 ± 0.4 (6) |
| 0.5 mM EGTA | -3.9 ± 0.9 (5) | -2.5 ± 0.3 (6) |
| 1.5 mM EGTA | -3.0 ± 0.6 (5) | -2.0 ± 0.5 (5) |

**DISCUSSION**

Ca\(^{2+}\) currents mediated by recombinant TRPV6 and endogenous CRAC channels had similar biophysical properties and could not clearly be discriminated by their current-voltage relationship. The data presented here allow one to distinguish both currents by their current-voltage relationship in divalent-free saline and by their activation mechanism; TRPV6-mediated Ca\(^{2+}\) currents were strongly dependent on [Ca\(^{2+}\)], and were significantly augmented by decreasing [Ca\(^{2+}\)]. However, I\(_{CRAC}\) activated by store depletion, which was without effect on the gating of TRPV6 channels. Interestingly, current amplitudes of TRPV6-expressing cells did not saturate but continuously increased if very high concentrations of Ca\(^{2+}\) chelators were used in the intracellular solution (30 and 60 mM EGTA or BAPTA). The calculated global [Ca\(^{2+}\)] was several magnitudes below physiological values (see “Experimental Procedures”). Therefore, it is likely that TRPV6 channels sense the local intracellular Ca\(^{2+}\) concentration in the close proximity of the pore.

Northern Blot Analysis for HEK and RBL Cells—TRPV6-mediated Ca\(^{2+}\) currents behaved very similarly if expressed either in HEK or RBL cells, although store-operated Ca\(^{2+}\) influx is much more prominent in RBL than in HEK cells. Northern blot experiments were, therefore, performed to reveal TRPV6 transcript expression. Using a human TRPV6 cDNA as a probe, no transcripts could be detected in nontransfected HEK cells (data not shown). With the rat cDNA as probe, the 3.0-kb transcript of rat TRPV6 (10) was readily detected in poly(A\(^{+}\)) from rat duodenum but not in poly(A\(^{+}\)) from RBL cells (Fig. 9), indicating no expression of TRPV6 in these cells.
the present study (Table II). On the other hand, it has been suggested that TRPV6 and the closely related TRPV5 protein form constitutively active Ca\textsuperscript{2+} channels (7, 11). This conclusion is based on recordings where intracellular Ca\textsuperscript{2+} buffering capacities were relatively high (11) or only considered in part (7).

When IP\textsubscript{3} and thapsigargin were included under conditions of low Ca\textsuperscript{2+} buffering, rat TRPV6 channels were activated in CHO cells transfected 8–12 h previously. One possible explanation is a mismatch between the protein expression levels and the still unknown signal transduction apparatus that senses store depletion (12). This possibility was tested for human TRPV6 channels by using different expression systems. RBL cells have a pronounced store-operated Ca\textsuperscript{2+} entry and are therefore extensively used to study I\textsubscript{CRAC}. HEK cells represent a common model system to express foreign cDNAs, and it has recently been suggested that store depletion also activates I\textsubscript{CRAC} in these cells (14–16). However, current densities were extremely small (−0.2 pA/pF at 0 mV with 20 mM external
Ca\(^{2+}\)) and only 20\% of that measured in Jurkat T-lymphocytes, where \(I_{\text{CRAC}}\) reaches peak amplitudes comparable with those in RBL cells. Accordingly, one can assume that the signal transduction machinery that leads to \(I_{\text{CRAC}}\) activation is expressed to a higher degree in RBL than HEK cells. However, no cell-specific modulation of TRPV6 channel function was found 24–32 h after transfection in both HEK and RBL cells.

The kinetic of human TRPV6 channel activation remained unaltered if Ca\(^{2+}\) stores were either actively or passively depleted (Fig. 2). Peak current densities were slightly larger in RBL than HEK cells when store depletion was achieved by IP\(_3\) and/or thapsigargin (Table I). These values were measured \(\sim\)1 min after establishing the whole-cell configuration and reflect Ca\(^{2+}\) currents from TRPV6 and CRAC channels, which activate on a similar time scale. The small difference in current density might, therefore, be the result of larger \(I_{\text{CRAC}}\) in RBL cells. Using only EGTA in the pipette solution resulted in rapid activation of TRPV6-mediated Ca\(^{2+}\) currents and slow activation of \(I_{\text{CRAC}}\) (Fig. 2). The maximum Ca\(^{2+}\) entry was determined by TRPV6 channels so that the peak current was measured early in the experiments and without contamination of \(I_{\text{CRAC}}\). Subsequently the difference in current size was smaller between RBL and HEK cells in comparison with the data with thapsigargin and/or IP\(_3\) (Table I). However, no significant differences were detected as a result of the broad fluctuations of these Ca\(^{2+}\) currents, which is at least partially a consequence of variations in the expression levels.

Yue et al. (12) concluded that TRPV6 comprises all or part of the CRAC pore, whereas Voets et al. (13) reported that the TRPV6 protein and CRAC channels are not identical. In the latter study, no differences were detected between TRPV6 channel activation in dependence to Ca\(^{2+}\) current densities. In these experiments, currents were measured within 12 h after transfection and selected for HEK cells with small current densities (below 100 pA/pF at \(-100\) mV with 10 mM external Ca\(^{2+}\) and 10 mM EGTA in the internal solution). Interestingly, these authors recorded a large inward current with 100 mM free Ca\(^{2+}\) in the intracellular solution. The amplitude was largest immediately after establishing the whole-cell mode and rapidly decreased until a steady-state level was reached. No such activation kinetic was seen for human TRPV6 channels in HEK cells at the same Ca\(^{2+}\) concentration, despite the fact that voltage ramps were applied immediately after establishing the whole-cell mode. It remains to be determined whether structural differences among TRPV6 channels from different species are responsible for the heterogeneous electrophysiological finding.
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