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Mitogen-regulated Ca^{2+} current of T lymphocytes is activated by depletion of intracellular Ca^{2+} stores

(thapsigargin/patch clamp/inositol trisphosphate receptor/calcium signaling/T-cell activation)

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ABSTRACT **Stimulated influx of Ca^{2+} across the plasma membrane of T lymphocytes is an essential triggering signal for T-cell activation by antigen. Regulation of the T-cell Ca^{2+} conductance is not understood; conflicting evidence supports direct activation by inositol 1,4,5-trisphosphate (IP_3) or by a signal generated by the depletion of intracellular Ca^{2+} stores. We have used the perforated-patch recording technique to compare the biophysical properties of Ca^{2+} currents activated by T-cell receptor stimulation and by thapsigargin, a Ca^{2+}-ATPase inhibitor that depletes intracellular stores without generating IP_3. Both currents are blocked by Ni^{2+}, are inwardly rectifying, are highly Ca^{2+}-selective, and exhibit voltage-independent gating with a unitary chord conductance of ≈24 fS in isotonic Ca^{2+}. Fluctuation analysis suggests that the underlying Ca^{2+} transporter is a channel rather than an ion carrier. Thus, in terms of ion permeation, gating, and unitary conductance, the Ca^{2+} current activated by thapsigargin is indistinguishable from that elicited by crosslinking of T-cell receptors. Moreover, the unitary Ca^{2+} conductance is >100-fold smaller than that of previously described IP_3-gated, Ca^{2+}-permeable channels in T cells [Kuno, M. & Gardner, P. (1987) *Nature (London)* 326, 301–304]. These results demonstrate that mitogen-activated Ca^{2+} influx is controlled by the state of intracellular Ca^{2+} stores rather than by the direct action of IP_3 on Ca^{2+} channels in the plasma membrane.**

In many electrically nonexcitable cells, the activation of receptors coupled to phosphatidylinositol metabolism evokes a biphasic rise in the intracellular concentration of free Ca^{2+} ($[Ca^{2+}]_i$), resulting from Ca^{2+} release from intracellular stores followed by Ca^{2+} influx across the plasma membrane (1). In T lymphocytes, a biphasic $[Ca^{2+}]_i$ increase is elicited by the binding of antigen or polyclonal mitogens to the T-cell antigen receptor (TCR) (2–6). The $[Ca^{2+}]_i$ rise constitutes an essential triggering signal for T-cell differentiation and proliferation (7). $[Ca^{2+}]_i$ must remain elevated for several hours to commit T cells to the activation pathway, a period during which Ca^{2+} influx is required (4, 7, 8). The Ca^{2+} signaling cascade is driven by the activation of phospholipase C-γ1 to cleave phosphatidylinositol 4,5-bisphosphate, generating diacylglycerol and inositol 1,4,5-trisphosphate (IP_3) (9). While it is generally accepted that IP_3 releases Ca^{2+} from intracellular stores through IP_3-activated channels (4), the mechanisms that regulate sustained Ca^{2+} entry across the plasma membrane during the precommitment period are controversial. The debate centers on whether Ca^{2+} channels in the plasma membrane are opened by IP_3 (the IP_3 hypothesis) or by a signal generated by the IP_3-triggered depletion of Ca^{2+} stores (the depletion hypothesis).

The IP_3 hypothesis has received its most direct support from observations of Ca^{2+}/Ba^{2+}-permeable single-channel currents in membrane patches from T cells (10–12). Gardner and colleagues reported that these channels were activated in cell-attached patches by bath-applied mitogens such as phytohemagglutinin (PHA) (10) or antibodies to CD2 or CD3 (12), agents that stimulate an increase in intracellular IP_3 concentration ($[IP_3]_i$) (4, 12). Similar channels were activated by IP_3 applied directly to the intracellular surface of excised patches (11). The IP_3 hypothesis has received biochemical support from recent reports of a low-affinity IP_3-binding protein in the plasma membrane of T cells which cocaps with the TCR and colocalizes with the zone of highest cytosolic Ca^{2+} concentration in concanavalin A (Con A)-treated cells (13, 14). The conclusion of these studies is that IP_3 plays a dual role of directly mobilizing Ca^{2+} from both intracellular and extracellular sources.

In contrast, the depletion hypothesis, proposed originally by Putney and coworkers (15, 16), asserts that IP_3 only liberates Ca^{2+} from the endoplasmic reticulum (ER) and that the ensuing depletion of this Ca^{2+} store generates a second, as yet uncharacterized, signal that activates Ca^{2+} influx. This hypothesis is consistent with the finding that, in many cell types, conditions promoting release of Ca^{2+} from the ER can elicit robust Ca^{2+} influx without affecting $[IP_3]_i$ (2, 6, 15–21). Fluorescence and radioactive flux measurements have demonstrated Ca^{2+} or Mn^{2+} entry in T cells and thymocytes following depletion of intracellular stores by ER Ca^{2+}-ATPase inhibitors such as thapsigargin (20, 22) or by prolonged incubation in Ca^{2+}-free medium (2, 6, 17–19). Ca^{2+} influx evoked by ER depletion or TCR stimulation is inhibited by membrane depolarization and by blockers of receptor-mediated Ca^{2+} entry (18). Moreover, TCR stimulation fails to elevate $[Ca^{2+}]_i$ further when applied after a maximally effective dose of thapsigargin (6, 18). Taken together, these results suggest that TCR engagement and depletion of intracellular stores activate the same plasma-membrane Ca^{2+} conductance. Ca^{2+} current (I_{Ca}) activated by PHA has been described in the human leukemic T-cell line Jurkat through the use of whole-cell patch-clamp techniques (23); however, thapsigargin-activated I_{Ca} has not been described for any cell type. A Ca^{2+} current stimulated by depletion of Ca^{2+} stores has been reported in mast cells (21).

To resolve the issue of how Ca^{2+} entry is controlled in T cells, we have compared the biophysical properties of Ca^{2+} currents stimulated by thapsigargin and PHA in Jurkat T cells. Our results indicate that thapsigargin and PHA stimulate apparently identical Ca^{2+} currents whose unitary amplitude is too small to be consistent with previously described IP_3-gated channels. These results provide strong evidence that mitogens regulate Ca^{2+} influx via the depletion of intracellular Ca^{2+} stores rather than through the action of IP_3

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Abbreviations: IP_3, inositol 1,4,5-trisphosphate; TCR, T-cell antigen receptor; PHA, phytohemagglutinin; ER, endoplasmic reticulum; BAPTA, 1,2-bis(2-aminophenoxy)ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid; $[Ca^{2+}]_i$, intracellular concentration of free Ca^{2+}.

on channels in the plasma membrane. A preliminary account of this work has appeared in abstract form (24).

MATERIALS AND METHODS

Cells. The Jurkat NFATZ cell line (25) was maintained in RPMI 1640 with 10% fetal bovine serum, 2 mM glutamine, and 25 mM Hepes in a 6% CO_2 incubator at 37°C. Logarithmic-phase cells (0.2–1.2 × 10^6 per ml) were loaded at a density of 10^6 per ml in culture medium containing 20 μM bis(2-aminophenoxy)ethane-*N*,*N*,*N'*,*N'*-tetraacetic acid (BAPTA) tetrakis(acetoxymethyl) ester (Molecular Probes) for 30 min at 37°C, washed twice, and allowed to settle onto poly(D-lysine)-treated glass coverslip chambers.

Patch-Clamp Recording. Perforated-patch recording (26) was used to circumvent the spontaneous activation of Ca^{2+} current during conventional whole-cell recording (27) from Jurkat T cells (ref. 23; see *Discussion*). Pipettes were pulled from 100-μl capillaries (VWR Scientific), coated with Sylgard near their tips, and fire-polished to a resistance of 2–8 MΩ. The K^+ pipette solution contained 55 mM KCl, 70 mM K_2SO_4, 7 mM $MgCl_2$, 1 mM $CaCl_2$, 5 mM D-glucose, and 10 mM Hepes (pH 7.2 with KOH) with nystatin (Sigma) at 200 μg/ml. To obtain the Ca^{2+} current–voltage relation in the absence of K^+ currents, Cs^+ salts were substituted for K^+ salts. Conventional whole-cell recording was conducted with a pipette solution containing 140 mM cesium aspartate, 2 mM $MgCl_2$, 1 mM $CaCl_2$, 10 mM EGTA, and 5 mM Hepes (pH 7.2 with CsOH). Cells were bathed in Ringer's solution containing 155 mM NaCl, 4.5 mM KCl, 1 mM $MgCl_2$, 2 mM $CaCl_2$, 5 mM D-glucose, and 10 mM Hepes (pH 7.4 with NaOH). Equimolar amounts of $BaCl_2$, $SrCl_2$, or $MgCl_2$ replaced $CaCl_2$ in the ion-substitution experiments. Isotonic Ca^{2+} solution contained 110 mM $CaCl_2$, 4.5 mM KCl, 1 mM $MgCl_2$, and 5 mM Hepes (pH 7.4 with KOH). The patch-clamp output (Axopatch 200; Axon Instruments, Foster City, CA) was filtered at 1.5 kHz with an eight-pole Bessel filter (Frequency Devices, Haverhill, MA). An Apple Macintosh IIci computer driving an ITC-16 interface (Instrutech, Elmont, NY) was used for stimulation and recording; typically, average current over a 200-msec period was determined at the holding potential of −80 mV every 2 sec, immediately prior to a 256-msec voltage ramp from −80 to 0 mV. Experiments were conducted at 22–25°C. PHA-P (Sigma) and thapsigargin (LC Services, Woburn, MA) were applied by perfusing the entire chamber; all other solution changes were made with an extracellular puffer pipette placed within 20 μm of the cell.

Fluctuation Analysis. Data were recorded at 10-kHz bandwidth onto digital audio tape (Sony DTC-700, modified for DC-coupled input). Data replayed from tape were low-pass-filtered at 500 Hz and digitized at 2.5 kHz. The current mean and variance were calculated for 500-point sweeps collected every 2 sec, and unitary current amplitudes were estimated from linear regression fits to plots of mean versus variance. To generate the curves shown in Fig. 3, sweeps were acquired continuously, and the mean and variance were averaged over 10 consecutive sweeps (thapsigargin) or 5 sweeps (PHA). Averaging in this manner did not alter the slopes of the fitted lines. Variance values calculated during several abrupt current transitions prior to induction of the Ca^{2+} current (see Fig. 3*B*) were omitted from the analysis. These sudden transitions of unknown origin did not occur in all experiments. For spectral analysis, data from tape were replayed through a 10-Hz high-pass (Tektronix) and a 1-kHz low-pass Bessel filter in series and digitized at a rate of 5 kHz. After subtraction of a linear-regression fit from each of 64 sweeps (512 points per sweep), power spectra were computed and averaged.

RESULTS

BAPTA Loading Delays the $[Ca^{2+}]_i$ Increase Evoked by Thapsigargin. Thapsigargin (1 μM) elicits a rapid and sustained rise in $[Ca^{2+}]_i$ in Jurkat T cells due to Ca^{2+} release from intracellular stores followed by influx across the plasma membrane (5, 6). In Ca^{2+} imaging experiments using fura-2, we found that the initial $[Ca^{2+}]_i$ increase, which normally commences within 10 sec, is delayed ≈200 sec in cells preloaded with BAPTA, a Ca^{2+} chelator (data not shown). This BAPTA preloading procedure was used in the experiments described below to delay or prevent the activation of Ca^{2+}-dependent K^+ (28) and Cl^- (29) channels and the inhibition of Ca^{2+} current by intracellular Ca^{2+} (12, 23).

Thapsigargin and PHA Stimulate Identical Currents. We used the perforated-patch recording technique to compare the Ca^{2+} currents stimulated by thapsigargin and PHA. As illustrated in Fig. 1, thapsigargin stimulates an inward current closely resembling the PHA-activated Ca^{2+} current (23) in several key characteristics: both currents are selective for Ca^{2+} over monovalent cations, are blocked by 5 mM Ni^{2+}, and display slight inward rectification and a lack of voltage-dependent activation. These features also match those of spontaneously activated Ca^{2+} currents seen in conventional whole-cell recordings from Jurkat cells (23). Moreover, thapsigargin- and PHA-activated currents share a distinctive selectivity sequence for divalent cations, $Ca^{2+} > Ba^{2+} \approx Sr^{2+}$, with both currents carrying Ca^{2+} about twice as well as Ba^{2+} or Sr^{2+} (Fig. 2). These results are summarized in Table 1.

Fluctuation Analysis of Thapsigargin- and PHA-Stimulated Ca^{2+} Currents. The close similarities between thapsigargin- and PHA-activated Ca^{2+} currents led us to reconsider the

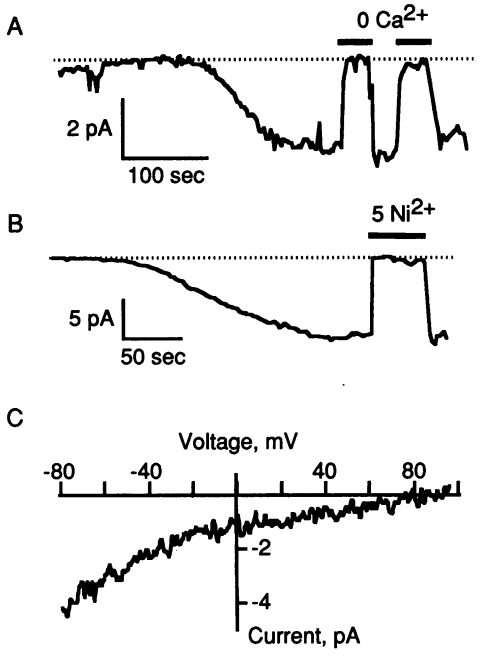

FIG. 1. Activation of Ca^{2+} current by thapsigargin. In *A* and *B*, 1 μM thapsigargin was added 0 sec (*A*) or 70 sec (*B*) before the start of the displayed trace, and current was measured in perforated-patch mode at a holding potential of −80 mV. (*A*) Dependence of thapsigargin-induced current on extracellular Ca^{2+}. Ca^{2+}-free Ringer's solution was applied from a puffer pipette during the periods indicated by bars. (*B*) Reversible blockade of Ca^{2+} current by 5 mM Ni^{2+}. (*C*) Current–voltage relation of the thapsigargin-stimulated Ca^{2+} current. The curve shown was generated by subtracting the average ramp current from three sweeps collected before induction of Ca^{2+} current from the average current after induction. Ramp duration was 256 msec, filtered at 500 Hz.

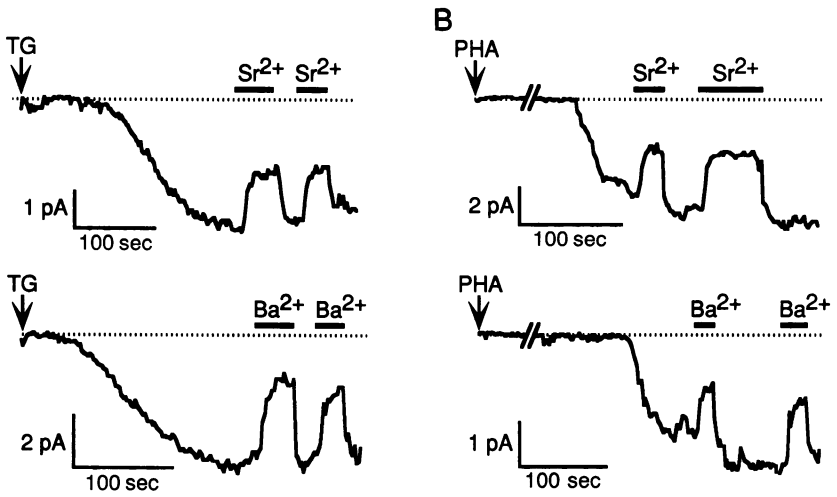

FIG. 2. Divalent-cation selectivity of currents activated by thapsigargin and PHA. (*A*) Effect of Sr^{2+} (*Upper*) and Ba^{2+} (*Lower*) on thapsigargin-stimulated current. Thapsigargin (TG, 1 μM) was added at the times indicated by the arrows. Equimolar substitution of Sr^{2+} and Ba^{2+} for external Ca^{2+} reduced the thapsigargin-stimulated current in these cells by 44% and 54%, respectively. (*B*) Effect of Sr^{2+} and Ba^{2+} on PHA-activated current. PHA (10 μg/ml) was added at the times shown by the arrows. Baseline current was stable over the 200-sec period not shown (slashes). Sr^{2+} and Ba^{2+} substitution for Ca^{2+} reduced the PHA-activated current in these cells by 57% and 55%, respectively. Data were obtained from four different cells.

possible role of IP_3-gated Ca^{2+}-permeable channels (10–12) in mediating mitogenic Ca^{2+} influx. IP_3-gated channels could contribute to the whole-cell current if they were also regulated by the depletion-induced signal (13, 30). To test this possibility, we compared their reported unitary conductance (7 pS in isotonic Ca^{2+}; refs. 10 and 11) with that of channels underlying the macroscopic Ca^{2+} current. The mean ($\bar{I}$) and variance (σ^2) of the macroscopic current were calculated for a series of 200-msec epochs after stimulation with thapsigargin or PHA, and unitary current amplitudes were estimated from the ratio $\sigma^2/\bar{I}$ (Fig. 3) (31). Results are summarized in Table 1. During activation in 2 mM Ca^{2+}, $\sigma^2/\bar{I}$ for both currents maintained a constant average value of -1.4 fA at -80 mV.* After exposure to 110 mM Ca^{2+}, both currents increased rapidly and then decayed slowly; during this decay, $\sigma^2/\bar{I}$ had an approximately constant value of -3.6 fA. Assuming a reversal potential of +80 mV (Fig. 1*C*), these values correspond to a unitary chord conductance of $\approx$9 fS in 2 mM Ca^{2+} and $\approx$24 fS in 110 mM Ca^{2+}. Together with the other results listed in Table 1, the similar unitary conductances provide strong evidence that thapsigargin and PHA activate the same Ca^{2+} conductance and imply that 7-pS IP_3-gated channels do not contribute significantly to Ca^{2+} influx during mitogenic stimulation.

We considered the possibility that access resistance (R_s) through the nystatin-permeabilized patch could cause an underestimate of the fluctuation amplitude. R_s was as high as 100 MΩ in some experiments, which in series with the membrane capacitance of $\approx$8 pF created a single-pole, low-pass filter characteristic above $\approx$200 Hz. To assess this problem, we measured the noise associated with spontaneously activated Ca^{2+} currents in conventional whole-cell recordings with low R_s. This current was shown previously to be indistinguishable from the PHA-stimulated current (23). In four cells with $R_s < 10$ MΩ and a recording bandwidth of 1 kHz, $\sigma^2/\bar{I}$ ratios were similar to those of the perforated-patch experiments. Furthermore, spectral analysis (Fig. 4) showed that the Ca^{2+} current fluctuated primarily at frequencies below 200 Hz. The spectrum was well approximated by a Lorentzian function with a characteristic frequency of 45–50 Hz in four cells. That the excess noise at low frequencies was due specifically to Ca^{2+} current was verified in several ways. First, the noise was reversibly inhibited to the background level by 5 mM Ni^{2+} and was reversibly reduced to an intermediate level by lowering extracellular Ca^{2+} concentration from 110 mM to 2 mM. In addition, the total variance calculated from the background-corrected spectrum (0.014 pA^2 in Fig. 4*B*) agrees well with the variance measured from fluctuations around the mean current in the same experiment (0.016 pA^2). Together, these results indicate that the bandwidth of perforated-patch and whole-cell recordings is sufficient to yield meaningful estimates of the unitary Ca^{2+} current.

*Theory predicts a parabolic relation between mean and variance, given the assumption of a homogeneous population of statistically independent channels with a single conducting state (31). The linear relation of mean to variance that was observed (Fig. 3) implies that the open probability of the Ca^{2+} channels is less than $\approx$0.3.

DISCUSSION

In this report we demonstrate that PHA and thapsigargin activate Ca^{2+} currents that are indistinguishable in terms of

Table 1. Comparison of Ca^{2+} currents activated by PHA and thapsigargin

Property	PHA	Thapsigargin
Activation kinetics*		
t_{10}, sec	272 ± 130 (11)	109 ± 75 (16)
t_{90}–t_{10}, sec	41 ± 14 (11)	131 ± 45 (16)
Ion selectivity†		
I_{Ba}/I_{Ca}	0.45 ± 0.07 (4)	0.51 ± 0.07 (4)
I_{Sr}/I_{Ca}	0.53 ± 0.09 (3)	0.52 ± 0.05 (3)
I_{Mg}/I_{Ca}	0.13 ± 0.02 (6)‡	0.11 ± 0.08 (5)
Ni^{2+} blockade§, %	100‡	100
Unitary current, fA		
In 2 mM Ca^{2+}	−1.5 ± 0.3 (3)	−1.4 ± 0.4 (5)
In 110 mM Ca^{2+}	−3.7 ± 0.9 (3)	−3.5 ± 1.0 (5)

Where indicated, values are mean ± SD, with no. of cells in parentheses.

*t_{10} is the time required for the current to increase to 10% of its maximal value. t_{90}–t_{10} is the additional time for the current to attain 90% of its maximal value.

†Relative current magnitude after equimolar substitution of the indicated ion for 2 mM Ca^{2+}.

‡Data from ref. 23.

§Measured with 5 mM Ni^{2+} added to Ringer's solution.

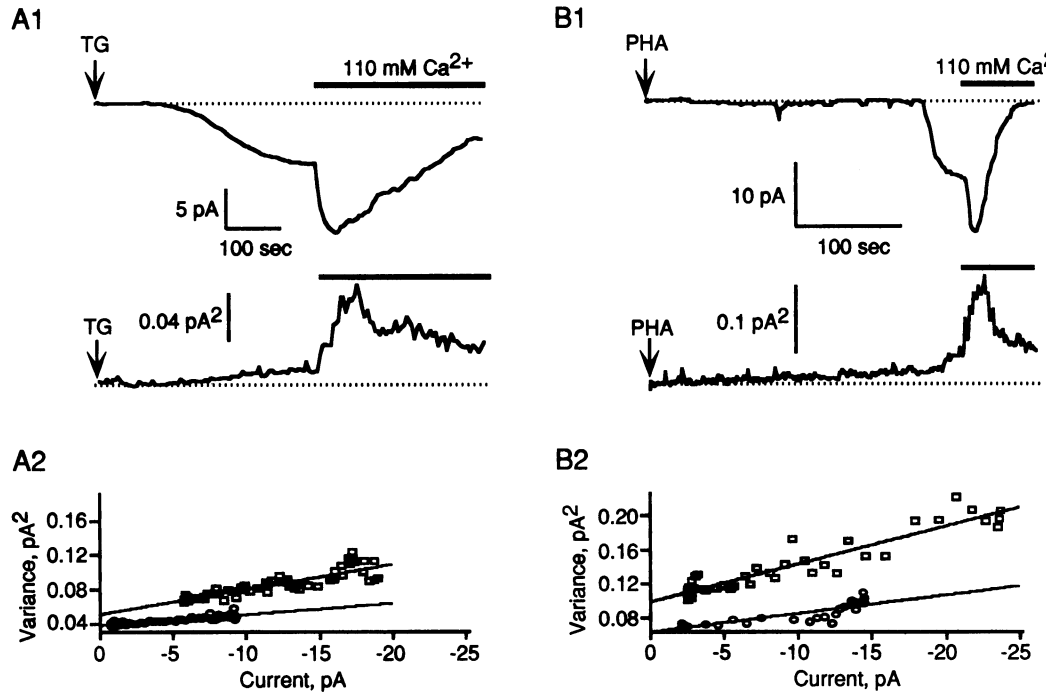

FIG. 3. Fluctuation analysis of currents stimulated by thapsigargin and PHA. (*A1*) Macroscopic Ca^{2+} current (*Upper*) and variance (*Lower*) elicited by 1 μM thapsigargin (TG). (*A2*) Variance plotted against mean current for the data from *A1*. Linear regression fits are superimposed on the data collected in 2 mM Ca^{2+} (○) and 110 mM Ca^{2+} (□). Unitary current amplitudes as indicated by the slopes, are −1.3 fA (2 mM Ca^{2+}) and −2.9 fA (110 mM Ca^{2+}). (*B1*) Macroscopic current (*Upper*) and variance (*Lower*) elicited by PHA (10 μg/ml). (*B2*) Plot of variance against mean current for the data of *B1*. Linear best fits correspond to unitary current amplitudes of −2.1 fA (2 mM Ca^{2+}) and −4.4 fA (110 mM Ca^{2+}).

their divalent cation selectivity, blockade by Ni^{2+}, voltage-independent gating, current–voltage relation, and unitary size. The simplest conclusion consistent with these results is that the Ca^{2+} conductance of T cells is regulated by the state of intracellular Ca^{2+} stores and that TCR stimulation elicits Ca^{2+} current indirectly through the ability of IP_3 to deplete the stores.

The depletion hypothesis predicts that the time course of current activation and deactivation should depend on the rate of store depletion or repletion. In fact, the kinetics of current activation by thapsigargin and PHA (Figs. 2 and 3 and Table 1) parallel the speed with which each agent releases stored Ca^{2+}. The shorter current-onset latency with thapsigargin may reflect the fact that thapsigargin begins to deplete the ER within 10 sec, whereas PHA-triggered release begins only after 200–500 sec at room temperature (data not shown). Once opened, however, IP_3 receptors release Ca^{2+} from the ER much more efficiently than the leak conductance unmasked by thapsigargin, consistent with the more rapid increase of the PHA-induced Ca^{2+} current. The decline of the whole-cell current during exposure to 110 mM Ca^{2+} agrees with previous evidence that elevation of extracellular Ca^{2+} concentration only transiently increases Ca^{2+} current in Jurkat cells (23). This effect may result from direct inhibition of the channels by increased $[Ca^{2+}]_i$ (21) and/or from deactivation consequent to refilling the ER Ca^{2+} store. The latter mechanism may explain why thapsigargin-induced current declines more slowly than PHA-activated current, since inhibition of Ca^{2+}-ATPases is expected to slow or prevent Ca^{2+} reuptake into the stores.

The unitary Ca^{2+} current amplitude in isotonic Ca^{2+} corresponds to a flux of ≈11,000 Ca^{2+} ions per second. While

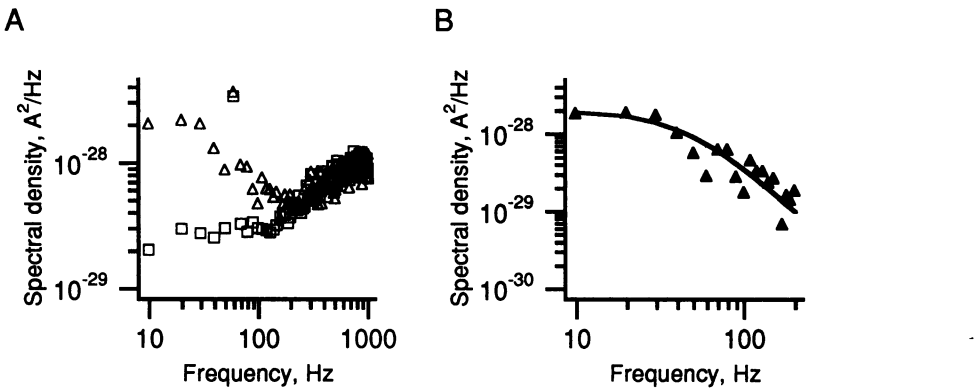

FIG. 4. Spectral analysis of the Ca^{2+} current. (*A*) Noise spectra collected before (□) and after (△) spontaneous induction of Ca^{2+} current during whole-cell recording. The background spectrum is accounted for by the sum of thermal noise from the patch-clamp headstage and from the combination of series resistance (29 MΩ) and membrane capacitance (4.5 pF). The spectrum after induction of Ca^{2+} current was collected in the presence of 110 mM Ca^{2+}. (*B*) Difference spectrum of the Ca^{2+} current from the data in *A*. A Lorentzian function of the form $S(f) = S(0)/(1 + (f/f_c)^2)$ has been visually fitted to the data, with $S(0) = 2.0 \times 10^{-28}$ A^2/Hz and $f_c = 45$ Hz. Total variance = $S(0) \cdot \pi f_c/2 = 0.014$ pA^2.

this flux is small enough to be produced by an ion carrier, the Lorentzian shape of the noise spectrum (Fig. 4*B*) indicates that the transporter is an ion channel rather than a carrier (32, 33). From the size of the unitary and whole-cell currents, we estimate that each cell expresses >10,000 Ca^{2+} channels. These estimates appear reasonable, since the frequency components of the fluctuations are well contained within the recording bandwidth (Fig. 4), and recording conditions were chosen to avoid activation of other types of channels.

We find that fluctuations of the thapsigargin- and PHA-stimulated Ca^{2+} currents are too small by a factor of >100 to be generated by IP_3-gated, Ca^{2+}-permeable channels (10, 11), implying that IP_3-gated channels do not contribute significantly to Ca^{2+} influx. This conclusion appears to be at odds with a report (13) showing that Con A caps IP_3-receptor immunoreactivity in the T-cell plasma membrane and that the cap coincides spatially with the zone of highest $[Ca^{2+}]_i$. Two possibilities may reconcile these data with ours. First, because Con A binds to a large variety of membrane glycoproteins, it may also cap the depletion-activated Ca^{2+} channel. Alternatively, the immunoreactivity may reflect a novel type of IP_3 receptor that can be activated by the depletion of intracellular stores. However, this type of IP_3 receptor would be constrained to display the characteristics we have described, in particular a low unitary conductance and selectivity for Ca^{2+} over Sr^{2+} and Ba^{2+}, and therefore would be functionally distinct from both the microsomal IP_3 receptor (34) and the IP_3-gated channels described by Kuno and Gardner (10, 11).

Activation of Ca^{2+} current by the depletion of intracellular Ca^{2+} stores can explain previous observations of a spontaneously activating Ca^{2+} current in whole-cell recordings from Jurkat cells (23). This current, which appears to be identical to the PHA- and thapsigargin-activated Ca^{2+} current, is induced reliably by internal dialysis with Ca^{2+}-free solutions containing 1–10 mM EGTA, conditions that are likely to cause depletion of stores. In a subset of cells in the previous study, PHA elicited an oscillatory Ca^{2+} current that was temporally linked to $[Ca^{2+}]_i$ oscillations (23). We attribute the absence of oscillatory currents in the present study to the fact that the cells contained twice the previous amount of BAPTA, which impedes oscillations, and that our chances of observing oscillations may have been further reduced by the comparatively small number of cells we stimulated with PHA.

The mitogen- and depletion-activated Ca^{2+} channel of T cells is clearly distinguished from voltage-gated Ca^{2+} channels of excitable cells in terms of its gating, unitary conductance, and ionic selectivity (35). However, numerous reports of depletion-activated Ca^{2+} influx in nonexcitable cells suggest that this class of channel is widespread (16). Patch-clamp studies have demonstrated that the agonist-stimulated Ca^{2+} current of mast cells is also activated by stimuli that deplete intracellular stores, such as intracellular EGTA, ionomycin, or IP_3 (21, 36). This current, termed I_{CRAC} (for "Ca^{2+}-release-activated Ca^{2+} current"), closely resembles the Ca^{2+} current in T cells in terms of inward rectification, voltage-independent gating, and low conductance to Ba^{2+} and Sr^{2+} (21).

The signal that couples depletion of stores to activation of influx cannot be Ca^{2+} itself, as Ca^{2+} current develops in these BAPTA-loaded cells before any increase in $[Ca^{2+}]_i$. In principle, the activating signal may involve a diffusible messenger or contact between molecules in the ER and plasma membranes (15, 30). The nature of the signal remains to be defined.

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