The T-cell antigen receptor regulates sustained increases in cytoplasmic free Ca^{2+} through extracellular Ca^{2+} influx and ongoing intracellular Ca^{2+} mobilization

John B. IMBODEN*† and Arthur WEISS†

*Immunology Section, Veterans Administration Medical Center, and Department of Medicine, University of California, San Francisco, CA 94121, and †The Howard Hughes Medical Institute and Department of Medicine, University of California, San Francisco, CA 94143, U.S.A.

Signal transduction by the T-cell antigen receptor involves the turnover of polyphosphoinositides and an increase in the concentration of cytoplasmic free Ca^{2+} ($[\text{Ca}^{2+}]_i$). This increase in $[\text{Ca}^{2+}]_i$ is due initially to the release of Ca^{2+} from intracellular stores, but is sustained by the influx of extracellular Ca^{2+} . To examine the regulation of sustained antigen-receptor-mediated increases in $[\text{Ca}^{2+}]_i$, we studied the relationships between extracellular Ca^{2+} influx, the mobilization of Ca^{2+} from intracellular stores, and the contents of inositol polyphosphates after stimulation of the antigen receptor on a human T-cell line, Jurkat. We demonstrate that sustained antigen-receptor-mediated increases in $[\text{Ca}^{2+}]_i$ are associated with ongoing depletion of intracellular Ca^{2+} stores. When antigen-receptor–ligand interactions are disrupted, $[\text{Ca}^{2+}]_i$ and inositol 1,4,5-trisphosphate return to basal values over 3 min. Under these conditions, intracellular Ca^{2+} stores are repleted if extracellular Ca^{2+} is present. There is a tight temporal relationship between the fall in $[\text{Ca}^{2+}]_i$, the return of inositol 1,4,5-trisphosphate to basal values, and the repletion of intracellular Ca^{2+} stores. Reversal of the increase in $[\text{Ca}^{2+}]_i$ precedes any fall in inositol tetrakisphosphate by 2 min. These studies suggest that sustained antigen-receptor-induced increases in $[\text{Ca}^{2+}]_i$, although dependent on extracellular Ca^{2+} influx, are also regulated by ongoing inositol 1,4,5-trisphosphate-mediated intracellular Ca^{2+} mobilization. In addition, an elevated concentration of inositol tetrakisphosphate in itself is insufficient to sustain an increase in $[\text{Ca}^{2+}]_i$ within Jurkat cells.

INTRODUCTION

Perturbation of the T-cell antigen receptor complex (T3/Ti), by monoclonal antibodies (MAbs), or mitogenic lectins, or specific antigen on the surface of appropriate accessory cells, elicits substantial, sustained increases in inositol trisphosphate (InsP_3) and in the concentration of cytoplasmic free calcium ($[\text{Ca}^{2+}]_i$) within T lymphocytes [see Michell (1982) for review of early work; Tsien *et al.* (1982); Weiss *et al.* (1984); Taylor *et al.* (1984); O'Flynn *et al.* (1985); Imboden & Stobo (1985); Nisbet-Brown *et al.* (1985); Imboden *et al.* (1987)]. This T3/Ti-mediated increase in $[\text{Ca}^{2+}]_i$ is an important intracellular signal for the regulation of lymphokine production by T-cells (Weiss *et al.*, 1984; Truneh *et al.*, 1985). Studies of a malignant human T-cell line, Jurkat, have determined that the initial increase in $[\text{Ca}^{2+}]_i$, which occurs within 60 s of the addition of a T3/Ti MAb, is due to the release of Ca^{2+} from intracellular stores (Imboden & Stobo, 1985). Sustained T3/Ti-induced increases in $[\text{Ca}^{2+}]_i$, however, require extracellular Ca^{2+} influx.

Although a link between $\text{Ins}(1,4,5)\text{P}_3$ and intracellular Ca^{2+} mobilization has been established (for review see Berridge & Irvine, 1984), it is not known whether receptor-mediated polyphosphoinositide turnover also regulates the influx of extracellular Ca^{2+} . More recent studies, however, suggest that inositol tetrakisphosphate

(InsP_4), a derivative of $\text{Ins}(1,4,5)\text{P}_3$ (Irvine *et al.*, 1986), may stimulate the flux of Ca^{2+} across the plasma membrane. The micro-injection of InsP_4 into sea-urchin eggs leads to the raising of a fertilization membrane, an event dependent on the presence of extracellular Ca^{2+} and thought to be triggered by increases in $[\text{Ca}^{2+}]_i$ (Irvine & Moor, 1986). This response to InsP_4 displays an absolute requirement for the co-injection of $\text{Ins}(2,4,5)\text{P}_3$, a compound which can mobilize intracellular Ca^{2+} (Irvine & Moor, 1986). These studies of micro-injected InsP_4 suggest that, if InsP_4 does stimulate the influx of extracellular Ca^{2+} , this Ca^{2+} influx may require prior or concomitant release of Ca^{2+} from intracellular stores. To explore this possibility, we have examined the relationships between extracellular Ca^{2+} influx, the mobilization of Ca^{2+} from intracellular stores and the contents of inositol polyphosphates after perturbation of T3/Ti on Jurkat cells.

MATERIALS AND METHODS

Cells and MAbs

Jurkat E6-1 cells were passaged as described by Weiss *et al.* (1984). C305, an IgM (κ) MAb, recognizes Ti on Jurkat cells (Weiss & Stobo, 1984) and was used in a final dilution of 1:300 from culture supernatant.

Abbreviations used: T3/Ti, human T-cell antigen–receptor complex; MAb, monoclonal antibody; $[\text{Ca}^{2+}]_i$, concentration of cytoplasmic free Ca^{2+} ; InsP_3 , inositol trisphosphate (the designation InsP_3 is used when studies have not resolved the 1,3,4 and 1,4,5 isomers of InsP_3 by h.p.l.c.); InsP_4 , inositol tetrakisphosphate; Con A, concanavalin A.

† To whom reprint requests should be sent.

Reagents

Ionomycin was a gift from Squibb Corp. (Princeton, NJ, U.S.A.). Concanavalin A (ConA) was from Difco (Detroit, MI, U.S.A.). All other chemicals were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.).

Determination of $[Ca^{2+}]_i$

Jurkat cells were loaded by incubation with 5 μ M of the acetoxymethyl ester of quin2 (quin2 AM; Calbiochem-Behring, San Diego, CA, U.S.A.), and the fluorescence of a suspension of quin2-loaded Jurkat cells (5×10^6 cells/ml) was monitored with a Perkin-Elmer 650-40 spectrofluorimeter as described by Weiss *et al.* (1984). Measurements were made in a solution (pH 7.40) containing 125 mM-NaCl, 5 mM-KCl, 1 mM- Na_2HPO_4 , 1 g of glucose/l, 25 mM-Hepes, 0.1% bovine serum albumin, 1 mM- $CaCl_2$ and 0.5 mM- $MgCl_2$ ('saline'). EGTA was added as indicated from a 0.5 M stock solution (pH 7.40). $[Ca^{2+}]_i$ was calculated by the method of Tsien *et al.* (1982). At the conclusion of each experiment, the Ca^{2+} -sensitive fluorescence of a known concentration of quin2 (free acid form; Calbiochem-Behring) was determined. From this and the Ca^{2+} -sensitive quin2 fluorescence measured after the lysis of quin2-loaded cells, the cellular quin2 content was calculated (Tsien *et al.*, 1982). Because quin2 binds Ca^{2+} with 1:1 stoichiometry and with a resulting increase in its fluorescence, the amount of Ca^{2+} that binds to quin2 after stimulation of quin2-loaded cells ($Ca^{2+} \cdot \text{quin2}$) can be calculated from the formula:

$$Ca^{2+} \cdot \text{quin2} = (\% \Delta \text{quin2 saturation}) \times (\text{cellular quin2 content})$$

where % Δ quin2 saturation is the T3/Ti-mediated increase in quin2 fluorescence, expressed as a percentage of the total Ca^{2+} -sensitive quin2 fluorescence (Tsien *et al.*, 1982; Lew *et al.*, 1984). In the absence of extracellular Ca^{2+} , the receptor-stimulated increase in $Ca^{2+} \cdot \text{quin2}$ provides a measure of the amount of Ca^{2+} that is released from intracellular Ca^{2+} stores and not bound by endogenous Ca^{2+} buffers.

Separation of inositol polyphosphates by h.p.l.c.

Jurkat cells, labelled with [3H]inositol as described by Imboden *et al.* (1986), were washed extensively, resuspended at 10^7 cells/ml in saline, and then incubated at 37 °C with additions as specified. At the end of the incubation period, samples containing 10^7 cells were sedimented for 10 s in an Eppendorf 5414 microfuge. After aspiration of the medium, 1 ml of ice-cold 10% (w/v) trichloroacetic acid was added to the cellular pellet. After incubation for 10 min on ice, insoluble material was removed by centrifugation at 900 g for 10 min, and the supernatant was extracted with 6 vol. of diethyl ether and then neutralized. Inositol polyphosphates were separated by a modification of the h.p.l.c. method of Irvine *et al.* (1986), with a Whatman Partisil 10 SAX column and a guard column packed with Whatman pellicular anion exchanger. After sample injection, the column was washed for 10 min with water and then subjected to increasing concentrations of ammonium formate buffer (adjusted to pH 3.7 with H_3PO_4) with a flow rate of 1.2 ml/min. After application of a linear gradient over 30 min to 0.8 M-ammonium formate, $Ins(1,3,4)P_3$ and $Ins(1,4,5)P_3$ were

Fig. 1. The sustained T3/Ti-mediated increase in $[Ca^{2+}]_i$ requires the presence of extracellular Ca^{2+}

The fluorescence of quin2-loaded cells was monitored over time, and the calculated $[Ca^{2+}]_i$ and maximal fluorescence (F_{max}) are displayed on the ordinate. (a) C305, a MAb to T3/Ti, was added in the presence of 1 mM extracellular Ca^{2+} . Extracellular Ca^{2+} was subsequently depleted by the addition of 3 mM-EGTA and then replenished with 3 mM- Ca^{2+} . (b) Extracellular Ca^{2+} was depleted with 3 mM-EGTA before addition of C305. After the C305-induced increase in $[Ca^{2+}]_i$, extracellular Ca^{2+} was replenished by the addition of 3 mM- Ca^{2+} .

separated by isocratic elution in 0.8 M-ammonium formate and collected in 0.5 min fractions. After 18 min at 0.8 M, the concentration of ammonium formate was increased to 1.8 M by a linear gradient over 12 min, and $InsP_4$ was then collected in 1 min fractions. 3H radioactivity was quantified by liquid-scintillation counting in Aquasol (New England Nuclear, Boston, MA, U.S.A.). Under these conditions, peak elution times for $Ins(1,3,4)P_3$, $Ins(1,4,5)P_3$ and $InsP_4$ were 48, 52 and 75 min respectively. $Ins(1,4,5)P_3$ and $InsP_4$ in samples were identified on the basis of identity with the elution time of standards. [3H] $Ins(1,4,5)P_3$ standard was purchased from Amersham (Arlington Heights, IL, U.S.A.). $InsP_4$ standard was prepared by using [3H] $Ins(1,4,5)P_3$ standard and $Ins(1,4,5)P_3$ kinase partially purified from Jurkat cells by the method of Hansen *et al.* (1986).

RESULTS

The addition of C305, a T3/Ti MAb, to Jurkat cells loaded with the Ca^{2+} -sensitive fluor, quin2, elicits a prompt increase in $[Ca^{2+}]_i$ (Imboden *et al.*, 1985). In the presence of 1 mM extracellular Ca^{2+} , this T3/Ti-mediated increase in $[Ca^{2+}]_i$ reaches a peak of 500 nM within 60 s and then falls to a plateau of 200–250 nM, remaining elevated above basal values for at least 30 min. This sustained increase in $[Ca^{2+}]_i$ is abruptly terminated by the

Fig. 2. C305 and ionomycin mobilize Ca^{2+} from the same intracellular Ca^{2+} pool in quin2-loaded Jurkat cells

Jurkat cells were loaded with quin2 and then resuspended in saline containing 1 mM extracellular Ca^{2+} . EGTA (10 mM) was added at the indicated time points. The final concentration of ionomycin was 1 μM .

addition of EGTA and can be restored by the repletion of extracellular Ca^{2+} (Fig. 1a). When extracellular Ca^{2+} is depleted before the addition of C305, the T3/Ti-mediated increase in $[\text{Ca}^{2+}]_i$ is substantial but of less than 3 min duration. The subsequent repletion of extracellular Ca^{2+} leads to a sustained increase in $[\text{Ca}^{2+}]_i$ (Fig. 1b). Intracellular Ca^{2+} mobilization therefore accounts for the initial peak increase in $[\text{Ca}^{2+}]_i$ after the addition of C305 to quin2-loaded Jurkat cells, and Ca^{2+} influx is required for sustained increases in $[\text{Ca}^{2+}]_i$.

The Ca^{2+} ionophore, ionomycin, releases Ca^{2+} from the $\text{Ins}(1,4,5)\text{P}_3$ -sensitive intracellular Ca^{2+} pool in a wide variety of permeabilized cells, and has also been reported to release Ca^{2+} from receptor-sensitive intracellular stores of intact cells (Joseph *et al.*, 1984; Albert & Tashjian, 1984). Ionomycin mobilizes intracellular Ca^{2+} within intact quin2-loaded Jurkat cells (Fig. 2a). When extracellular Ca^{2+} is depleted with EGTA, the ionophore causes a substantial but transient increase in $[\text{Ca}^{2+}]_i$ that is comparable with that induced by C305 (Figs. 2a and 2b). The prior addition of ionomycin completely abolishes the ability of C305 to increase

Fig. 3. Intracellular Ca^{2+} stores are repleted after reversal of Con A-mediated increases in $[\text{Ca}^{2+}]_i$

Quin2-loaded Jurkat cells were stimulated with Con A (10 $\mu\text{g}/\text{ml}$). (a) After stimulation with Con A for 8 min, extracellular Ca^{2+} was depleted with EGTA (10 mM), and intracellular Ca^{2+} stores were then assessed by the addition of ionomycin (1 μM). (b) After 8 min, α -methyl mannoside (αMM ; 0.1 M) was added. When $[\text{Ca}^{2+}]_i$ returned to basal values, extracellular Ca^{2+} was depleted with EGTA (10 mM) to prevent further uptake of Ca^{2+} , and intracellular Ca^{2+} stores were assessed by the addition of ionomycin (1 μM).

$[\text{Ca}^{2+}]_i$ subsequently (Fig. 2a), but does not inhibit C305-induced increases in InsP_3 (results not shown). When the reciprocal experiment is performed, prior T3/Ti-mediated mobilization of intracellular Ca^{2+} markedly diminishes the ability of ionomycin to release Ca^{2+} from intracellular stores (Fig. 2b). In the experiment shown in Figs. 2(a) and 2(b), ionomycin mobilized 28 pmol of $\text{Ca}^{2+}/10^6$ untreated Jurkat cells, but only 5 pmol of $\text{Ca}^{2+}/10^6$ cells after the addition of C305 (see the Materials and methods section for the derivation of these values). The most likely explanation for these findings is that ionomycin and T3/Ti Mab stimulated the release of Ca^{2+} from the same intracellular pool within intact Jurkat cells, although there is a small amount (about 15%) of the ionomycin-mobilized Ca^{2+} which is not C305-sensitive.

Although intracellular stores apparently contribute only to the initial T3/Ti-mediated increase in $[\text{Ca}^{2+}]_i$, the T3/Ti-mediated increase in the Ca^{2+} -mobilizing 1,4,5-isomer of InsP_3 is maintained for > 15 min (Stewart *et al.*, 1986; also see Fig. 5 below). To determine whether this sustained increase in $\text{Ins}(1,4,5)\text{P}_3$ is associated with continued mobilization of intracellular Ca^{2+} , we added C305 to quin2-loaded Jurkat cells in the presence of 1 mM extracellular Ca^{2+} (Fig. 2c). EGTA, added 10 min later, reverses the sustained T3/Ti-mediated increase in $[\text{Ca}^{2+}]_i$. The subsequent addition of ionomycin then mobilizes only 5 pmol of $\text{Ca}^{2+}/10^6$ cells (Fig. 2c), demonstrating that the ionomycin-sensitive intracellular Ca^{2+} pool remains depleted.

Con A, which binds components of T3/Ti (Kanel-

Fig. 4. Time courses of the fall in $[Ca^{2+}]_i$ and the repletion of ionomycin-sensitive intracellular Ca^{2+} stores after addition of α -methyl mannoside to Con A-treated Jurkat cells

Jurkat cells were loaded with quin2 and then stimulated with Con A ($10 \mu\text{g/ml}$). After 8 min, α -methyl mannoside was added as in Fig. 3(b). The increase in $[Ca^{2+}]_i$ over basal values (Δ) was measured at the indicated time points and is expressed as a percentage of the Con A-mediated increase in $[Ca^{2+}]_i$ just before the addition of α -methyl mannoside. The time course of the repletion of intracellular Ca^{2+} stores ($\circ$) was determined by depleting extracellular Ca^{2+} with EGTA 20, 40, 60 and 180 s after the addition of α -methyl mannoside. After the addition of EGTA, the ionomycin-sensitive intracellular Ca^{2+} pool was measured as in Fig. 3(b). The results are expressed as the percentage of the total ionomycin-sensitive pool that is depleted by Con A (i.e. the difference between the amount of Ca^{2+} released by ionomycin in untreated Jurkat cells and the amount released by ionomycin in Jurkat cells treated for 8 min with Con A as in Fig. 3a).

lopoulos *et al.*, 1985), provides a means of reversibly stimulating T3/Ti (Hesketh *et al.*, 1983). The addition of Con A to Jurkat cells results in the generation of InsP_3 , the mobilization of intracellular Ca^{2+} , and a sustained increase in $[Ca^{2+}]_i$, all of which can be reversed by the competitive antagonist α -methyl mannoside (Weiss *et al.*, 1987). The effect of α -methyl mannoside is specific, in that α -methyl mannoside does not prevent C305-induced increases in InsP_3 or $[Ca^{2+}]_i$ (Weiss *et al.*, 1987). Although Con A binds a number of glycoproteins in addition to T3/Ti, Con A-mediated increases in $[Ca^{2+}]_i$ require the cell-surface expression of T3/Ti on Jurkat cells. Con A does not increase $[Ca^{2+}]_i$ in, and does not activate, mutants of Jurkat cells that fail to express T3/Ti (Weiss & Stobo, 1984; Weiss *et al.*, 1987). Re-expression of cell-surface T3/Ti after transfection of one such mutant with the Ti β -chain gene reconstitutes the Con A-mediated increase in $[Ca^{2+}]_i$ and Con A-induced production of interleukin-2 (Weiss *et al.*, 1987).

Fig. 5. α -Methyl mannoside reverses the Con A-induced increases in $\text{Ins}(1,4,5)\text{P}_3$ and InsP_4

Jurkat cells, labelled with ^3H -inositol as described in the Materials and methods section, were incubated either in saline alone ($\square$) or in saline with Con A ($10 \mu\text{g/ml}$) ($\blacksquare$). At 10 min the Con A-treated cells were divided and received either 0.1 M α -methyl mannoside ($\blacktriangle$) or saline ($\blacksquare$). The indicated time points are the interval from the addition of Con A to the lysis of the cells in 10% trichloroacetic acid. ^3H - $\text{Ins}(1,4,5)\text{P}_3$ (a) and ^3H - $\text{Ins}(1,3,4,5)\text{P}_4$ (b) were extracted, separated by h.p.l.c., and quantified as described in the Materials and methods section. Each point represents the total of the designated ^3H -inositol polyphosphate in 10^7 cells.

The addition of Con A to Jurkat cells leads to a sustained increase in $[Ca^{2+}]_i$ (Fig. 3a) that requires the presence of extracellular Ca^{2+} . As is the case after perturbation of T3/Ti by MAb, this sustained Con A-induced increase in $[Ca^{2+}]_i$ is associated with depletion of the ionomycin-sensitive intracellular Ca^{2+} pool. After EGTA-induced reversal of the Con A-mediated increase in $[Ca^{2+}]_i$, ionomycin mobilizes only 5 pmol of $Ca^{2+}/10^6$ cells (Fig. 3a). In four separate experiments, the amount of intracellular Ca^{2+} released by ionomycin from Con A-treated Jurkat cells was only $22 \pm 3\%$ (mean $\pm$ S.E.M.) of that mobilized from untreated cells.

After the addition of α -methyl mannoside, the Con A-induced increase in $[Ca^{2+}]_i$ returns to basal values over 3 min (Fig. 3b). Under these conditions the ionomycin-sensitive intracellular Ca^{2+} pool becomes largely repleted (Fig. 3b). At 3 min after the addition of α -methyl mannoside, the ionomycin-sensitive pool is $81 \pm 4\%$ (mean $\pm$ S.E.M.; $n = 4$) of that of untreated cells. The repletion of intracellular Ca^{2+} pools after the addition of α -methyl mannoside requires the presence of extracellular

Ca^{2+} , and can be prevented by EGTA (results not shown). A comparison of the kinetics of the repletion of the ionomycin-sensitive intracellular Ca^{2+} store and of the α -methyl mannoside-induced fall in $[\text{Ca}^{2+}]_i$ demonstrates a tight temporal relationship between these two events (Fig. 4).

Increases in $\text{Ins}(1,4,5)\text{P}_3$ and InsP_4 are sustained for at least 15 min in Con A-treated Jurkat cells (Fig. 5). The addition of α -methyl mannoside to Con A-treated Jurkat cells leads to a decrease in $\text{Ins}(1,4,5)\text{P}_3$ which is immediate and which coincides with the reversal of the Con A-induced increase in $[\text{Ca}^{2+}]_i$ (Fig. 5a). In contrast, InsP_4 continues to increase for at least 60 s after the addition of α -methyl mannoside, and reversal of the increase in InsP_4 lags 2 min behind the fall in $[\text{Ca}^{2+}]_i$ (Fig. 5b). The continued increase in InsP_4 , despite decreases in $\text{Ins}(1,4,5)\text{P}_3$, may reflect the high affinity of the $\text{Ins}(1,4,5)\text{P}_3$ kinase for $\text{Ins}(1,4,5)\text{P}_3$ (Irvine *et al.*, 1986). $\text{Ins}(1,3,4)\text{P}_3$ decreased between 3 and 5 min after the addition of α -methyl mannoside (results not shown).

DISCUSSION

Although uptake of extracellular Ca^{2+} is required to sustain T3/Ti-mediated increases in $[\text{Ca}^{2+}]_i$ in Jurkat cells, this sustained increase is associated with continued depletion of the intracellular Ca^{2+} store that is released by ionomycin. The failure of this intracellular Ca^{2+} pool to replete is remarkable, in view of the observation that $[\text{Ca}^{2+}]_i$ is elevated at a value several times that in unstimulated cells. In experiments with Con A, repletion of the ionomycin-sensitive intracellular Ca^{2+} store occurs as α -methyl mannoside reverses the Con A-receptor interactions. Repletion of intracellular Ca^{2+} stores under these conditions requires extracellular Ca^{2+} and is temporally associated with the return of $\text{Ins}(1,4,5)\text{P}_3$ and $[\text{Ca}^{2+}]_i$ to basal values. Reversal of the T3/Ti-mediated increase in $[\text{Ca}^{2+}]_i$ precedes any decrease in InsP_4 .

Our findings suggest that the sustained T3/Ti-induced increase in $[\text{Ca}^{2+}]_i$, although dependent on extracellular Ca^{2+} influx, is also regulated by continuing $\text{Ins}(1,4,5)\text{P}_3$ -mediated intracellular Ca^{2+} mobilization. One explanation for our observations is that there is an obligatory link between T3/Ti-induced Ca^{2+} influx and $\text{Ins}(1,4,5)\text{P}_3$ -mediated discharge of Ca^{2+} from intracellular stores. Putney (1986) has proposed a model for receptor-stimulated Ca^{2+} influx along these lines, postulating that Ca^{2+} influx does not occur directly into the cytoplasm, but rather cycles through an $\text{Ins}(1,4,5)\text{P}_3$ -sensitive pool within the endoplasmic reticulum. An alternative, but not mutually exclusive, explanation for our findings is that continued $\text{Ins}(1,4,5)\text{P}_3$ -mediated intracellular Ca^{2+} mobilization serves to regulate finely receptor-induced increases in $[\text{Ca}^{2+}]_i$. Support for this notion stems from the observation that constant infusion of $\text{Ins}(1,4,5)\text{P}_3$ into permeabilized cells results in an increase in $[\text{Ca}^{2+}]_i$ to a new steady state, which can then be regulated by the bidirectional Ca^{2+} -buffering capacity of the endoplasmic reticulum (Prentki *et al.*, 1985).

The mechanism by which perturbation of T3/Ti stimulates Ca^{2+} influx is not known, although this process appears to involve recently identified voltage-independent Ca^{2+} channels (Kuno *et al.*, 1986). The close temporal association of the concentrations of $\text{Ins}(1,4,5)\text{P}_3$ with the sustained T3/Ti-induced increases in $[\text{Ca}^{2+}]_i$ raises the possibility of a direct link between $\text{Ins}(1,4,5)\text{P}_3$

and Ca^{2+} influx. The studies by Irvine & Moor (1986), however, make InsP_4 an attractive candidate for the regulator of Ca^{2+} influx after stimulation of T3/Ti. Our results indicate that, in Jurkat cells, an increase in InsP_4 itself is insufficient to sustain an increase in $[\text{Ca}^{2+}]_i$. This finding, however, is completely consistent with the demonstration that the second-messenger activity of InsP_4 is dependent on the presence of a Ca^{2+} -mobilizing isomer of InsP_3 , and supports the hypothesis (Irvine & Moor, 1986) that depletion of intracellular Ca^{2+} stores is a prerequisite for InsP_4 -induced Ca^{2+} influx.

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