D-*myo*-Inositol 1,3,4,5-tetrakisphosphate releases Ca^{2+} from crude microsomes and enriched vesicular plasma membranes, but not from intracellular stores of permeabilized T-lymphocytes and monocytes

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In the human T-lymphocyte cell lines Jurkat and HPB.ALL and the human monocytoïd cell line U937, $\text{Ins}(1,3,4,5)\text{P}_4$ triggers a dose-dependent release of Ca^{2+} from crude microsomal preparations, with a half-maximal effective concentration (EC_{50}) of 1.2–2.3 μM . Similar results were obtained with enriched vesicular plasma membranes from U937 cells. However, in permeabilized preparations of the same cell types only $\text{Ins}(1,4,5)\text{P}_3$ was able to release Ca^{2+} from intracellular stores, with EC_{50} values in the range 0.11–0.84 μM . In crude microsomes the effects of $\text{Ins}(1,3,4,5)\text{P}_4$ and $\text{Ins}(2,4,5)\text{P}_3$, a non-metabolizable InsP_3 isomer, occurred independently of each other, indicating subpopulations of $\text{Ins}(1,3,4,5)\text{P}_4$ - and $\text{Ins}(1,4,5)\text{P}_3$ -sensitive vesicles. The $\text{Ins}(1,3,4,5)\text{P}_4$ preparation used for the Ca^{2+} -release experiments contains neither Ca^{2+} nor contaminating $\text{Ins}(1,4,5)\text{P}_3$ and was not metabolized to $\text{Ins}(1,4,5)\text{P}_3$ during the Ca^{2+} -release experiments. We conclude that $\text{Ins}(1,3,4,5)\text{P}_4$ independently of $\text{Ins}(1,4,5)\text{P}_3$ induces a Ca^{2+} flux via a membrane compartment, most likely the plasma membrane, that is functionally destroyed during the permeabilization of the cells.

INTRODUCTION

Transmembrane signalling via numerous cell-surface receptors is accompanied by a rapid activation of phosphoinositidase C (for reviews see Berridge, 1987; Berridge & Irvine, 1989). The cleavage of membrane phosphoinositides, in particular $\text{PtdIns}(4,5)\text{P}_2$, generates the two second messengers $\text{Ins}(1,4,5)\text{P}_3$ and *sn*-1,2-diacylglycerol. $\text{Ins}(1,4,5)\text{P}_3$ was shown to induce release of Ca^{2+} from intracellular stores (Streb *et al.*, 1983), whereas Ca^{2+} and *sn*-1,2-diacylglycerol activate protein kinase C (Nishizuka, 1988).

In human peripheral-blood T-lymphocytes, as well as in human T-lymphocyte cell lines, the stimulation of the T-cell receptor/CD3 complex is followed by a rapid rise in both $\text{Ins}(1,4,5)\text{P}_3$ and free cytosolic Ca^{2+} concentration ($[\text{Ca}^{2+}]_i$) (Weiss *et al.*, 1984; Imboden *et al.*, 1987; Goldsmith & Weiss, 1988; Guse & Emmrich, 1992). It is generally accepted that the rise in $[\text{Ca}^{2+}]_i$ is due both to release from intracellular stores and to influx from the extracellular space (Bonvini *et al.*, 1989; Ng *et al.*, 1990; Gelfand & Cheung, 1990). Since T-lymphocytes do not express voltage-gated Ca^{2+} channels, the existence of receptor-operated Ca^{2+} channels in the plasma membrane has been considered (Zschauer *et al.*, 1988). In accordance with this view, a ligand-operated Ca^{2+} -permeable channel was demonstrated in the plasma membrane of T-lymphocytes (Kuno *et al.*, 1986). In excised inside-out patches of plasma membranes, this channel responded to $\text{Ins}(1,4,5)\text{P}_3$ in micromolar quantities, which led to the conclusion that $\text{Ins}(1,4,5)\text{P}_3$ regulates the Ca^{2+} release from intracellular stores as well as the Ca^{2+} influx (Gardner, 1989).

In several non-lymphoid cells it has been shown that $\text{Ins}(1,3,4,5)\text{P}_4$ is also involved in the regulation of $[\text{Ca}^{2+}]_i$, by enhancing the $\text{Ins}(1,4,5)\text{P}_3$ -induced signal, as revealed in sea-urchin eggs (Irvine & Moore, 1986) and in mouse lacrimal cells (Morris *et al.*, 1987), or by inducing sequestration of cytosolic

Ca^{2+} in neoplastic rat liver epithelial 261B-cells (Hill *et al.*, 1988), or by releasing Ca^{2+} independently of $\text{Ins}(1,4,5)\text{P}_3$, as demonstrated in bovine adrenal microsomes (Ely *et al.*, 1990). In *Xenopus laevis* oocytes Ca^{2+} release in response to microinjected $\text{Ins}(1,3,4,5)\text{P}_4$ has been observed (Parker & Miledi, 1987*a,b*). However, these effects may have been due alternatively to metabolically derived $\text{Ins}(1,3,4,6)\text{P}_4$ (Guse *et al.*, 1991), which was able to induce Ca^{2+} release in *Xenopus laevis* oocytes with high potency (Ivorra *et al.*, 1991).

To re-investigate the role of $\text{Ins}(1,4,5)\text{P}_3$ and $\text{Ins}(1,3,4,5)\text{P}_4$ in the regulation of $[\text{Ca}^{2+}]_i$ in human T-lymphocytes and monocytes, we have combined Ca^{2+} -release studies in permeabilized cells, crude microsomes and enriched plasma membranes with studies on the purity and metabolism of $\text{Ins}(1,3,4,5)\text{P}_4$ in the microsomal preparation. From previous experiments carried out in Jurkat T-lymphocytes it became clear that $\text{Ins}(1,4,5)\text{P}_3$ cannot account for the total increase in $[\text{Ca}^{2+}]_i$ in response to T-cell receptor/CD3 stimulation, since $\text{Ins}(1,4,5)\text{P}_3$ is only transiently elevated (Guse & Emmrich, 1992), whereas $[\text{Ca}^{2+}]_i$ remains high for at least 1 h. Here we report that $\text{Ins}(1,3,4,5)\text{P}_4$, which remains elevated in Jurkat T-lymphocytes much longer than $\text{Ins}(1,4,5)\text{P}_3$ (Guse & Emmrich, 1991), independently of $\text{Ins}(1,4,5)\text{P}_3$ releases Ca^{2+} from crude microsomes and from enriched vesicular plasma membranes, but not from intracellular stores in permeabilized cells. Thus we present direct and indirect evidence that $\text{Ins}(1,3,4,5)\text{P}_4$ independently of $\text{Ins}(1,4,5)\text{P}_3$ induces a Ca^{2+} flux via the membrane compartment that is functionally destroyed during the permeabilization of the cells, namely the plasma membrane.

EXPERIMENTAL

Materials

$\text{Ins}(1,3,4,5)\text{P}_4$, $\text{Ins}(2,4,5)\text{P}_3$ and creatine kinase were purchased

Abbreviations used: $[\text{Ca}^{2+}]_i$, free cytosolic Ca^{2+} concentration; InsP_3 and InsP_4 , inositol tris- and tetrakis-phosphates with isomeric positioning of phosphate groups as indicated and assumed to be D enantiomers unless otherwise stated.

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Fig. 1. Effects of $\text{Ins}(1,4,5)\text{P}_3$ and $\text{Ins}(1,3,4,5)\text{P}_4$ on Ca^{2+} release from intracellular stores in permeabilized cells

Saponin-permeabilized U937 (upper tracing), Jurkat (middle tracing) and HPB.ALL cells (lower tracing) were challenged with $\text{Ins}(1,4,5)\text{P}_3$ ($4 \mu\text{M}$, a) or $\text{Ins}(1,3,4,5)\text{P}_4$ ($4 \mu\text{M}$, b) in an intracellular buffer, in the presence of ATP, an energy-conserving system, oligomycin and Fura-2 free acid for measurement of the free Ca^{2+} concentration by fluorimetric monitoring. IM indicates the addition of ionomycin ($2 \mu\text{M}$).

Fig. 2. Dose-response curve of the effect of $\text{Ins}(1,4,5)\text{P}_3$ on Ca^{2+} release from intracellular stores in permeabilized cells

For experimental conditions see the legend to Fig. 1. Various concentrations of $\text{Ins}(1,4,5)\text{P}_3$ were added to the permeabilized cells as indicated. Data are presented as means $\pm$ s.d. ($n = 5$) for U937 ($\bullet$), Jurkat ($\blacktriangledown$) and HPB.ALL (∇) cells. Data are based on the maximal Ca^{2+} release in each individual experiment as demonstrated in Fig. 1.

from Boehringer, Mannheim, Germany. Dithiothreitol, saponin, oligomycin, phosphocreatine and ATP were bought from Sigma Chemicals, Deisenhofen, Germany. Fura-2 free acid, ionomycin, $\text{Ins}(1,3,4)\text{P}_3$ and $\text{Ins}(1,4,5)\text{P}_3$ were from Calbiochem, Frankfurt, Germany.

Preparation of permeabilized cells

The human cell lines were cultured as described by Guse &

Emmrich (1991). For preparation of permeabilized cells, 2×10^8 cells were incubated in 6 ml of buffer A (20 mM-Hepes, 110 mM-KCl, 10 mM-NaCl, 2 mM- MgCl_2 , 5 mM- KH_2PO_4 , 2 mM-EGTA, 1 mM-dithiothreitol, pH 7.2) in the presence of saponin ($30 \mu\text{g}/\text{ml}$) for 5 min at 37°C . After a first centrifugation (450 g , 6 min), the cell pellet was rinsed three times with an intracellular buffer (110 mM-KCl, 10 mM-NaCl, 2 mM- MgCl_2 , 5 mM- KH_2PO_4 , 20 mM-Hepes, pH 7.2), then resuspended in 6 ml of the same buffer and again centrifuged. The final pellet was resuspended in 6 ml of the intracellular buffer until used. Ca^{2+} release from intracellular stores was measured in a LS-3B fluorimeter (Perkin-Elmer, Überlingen, Germany) with wavelength settings of $340 \pm 5 \text{ nm}$ (excitation) and $500 \pm 5 \text{ nm}$ (emission) in a thermostatically controlled quartz cuvette (37°C) with automated stirring. A 1.9 ml portion of the permeabilized cell suspension [$(6-7 \times 10^7)$ cells] was transferred to the cuvette. An energy-conserving system consisting of creatine kinase (20 units/ml) and phosphocreatine (20 mM) was added. Oligomycin ($10 \mu\text{g}/\text{ml}$) was also included to inhibit the mitochondrial ATPase. Before addition of Fura-2 free acid ($1.5 \mu\text{M}$), the cuvette was prewarmed to 37°C for 5 min. Subsequent addition of ATP to a final concentration of 1 mM resulted in a decrease in the fluorescence, indicating Ca^{2+} uptake by the intracellular stores. After baseline stabilization, inositol phosphates were added in small volumes (2–10 μl). At the end of each experiment, the free Ca^{2+} concentration was calibrated by addition of Ca^{2+} (1 mM)/ionomycin ($2 \mu\text{M}$) and subsequently by addition of EGTA (4 mM)/Tris (40 mM).

Preparation of crude microsomes

The human cell lines were cultured as described by Guse & Emmrich (1991), and microsomes were prepared from these lines as described by Ely *et al.* (1990). In brief, 6×10^8 cells were pelleted (450 g , 2 min, 18°C), resuspended in 7.2 ml of buffer A (20 mM-Hepes, 110 mM-KCl, 10 mM-NaCl, 2 mM- MgCl_2 , 5 mM-

Fig. 3. Effects of Ins(1,4,5) P_3 and Ins(1,3,4,5) P_4 on Ca^{2+} release from isolated crude microsomes

Crude microsomes from U937 (upper tracings), Jurkat (middle tracings) or HPB.ALL cells (lower tracings) were challenged with Ins(1,4,5) P_3 (4 μ M; a1, a2) or Ins(1,3,4,5) P_4 (4 μ M; b1, b2) in an intracellular buffer in the presence of ATP, an energy-conserving system, oligomycin and Fura-2 free acid. IM indicates the addition of ionomycin (2 μ M). Panels (a2) and (b2) show the dose-response relationships. Data are presented as means $\pm$ S.D. ($n = 5$) for U937 cells ($\bullet$), Jurkat ($\blacktriangledown$) and HPB.ALL (∇).

KH_2PO_4 , 2 mM-EGTA, 1 mM-dithiothreitol, pH 7.2) and homogenized with a Potter-Elvehjem homogenizer. After centrifugation (500 g , 15 min, 4 $^{\circ}C$) the supernatant was again centrifuged (35000 g , 2 min, 4 $^{\circ}C$). The pellet was washed once and resuspended in 7.2 ml of buffer B (buffer A without EGTA) and again centrifuged (35000 g , 20 min, 4 $^{\circ}C$). The final pellet was resuspended in 4 ml of buffer C (110 mM-KCl, 10 mM-NaCl, 2 mM-MgCl $_2$, 5 mM-KH $_2$ PO $_4$, 20 mM-Hepes, pH 7.2) and kept on ice until used. Ca^{2+} release from isolated microsomes (1.95 ml of suspension) was measured and calibrated as described above.

Preparation of enriched plasma membranes

Enriched plasma membranes were prepared as described by

Jett *et al.* (1977). In brief, microsomes from 4×10^8 cells were prepared as described above, except that mitochondria were removed by one additional centrifugation step (4200 g , 10 min, 4 $^{\circ}C$). The crude microsomal fraction (9.5 ml) was then layered on a 35%-sucrose solution (1.5 ml) containing 40 mM-KCl, 20 mM-Hepes, pH 7.0, 50 ng of phenylmethanesulphonyl fluoride/ml and 50 ng of antipain/ml and centrifuged at 95000 g for 90 min at 4 $^{\circ}C$ (Centrikon T-2070, swing-out rotor TST41.14; Kontron Instruments, München, Germany). The enriched plasma membranes in the interphase of the gradient were then dialysed overnight against 140 mM-KCl/20 mM-Hepes (pH 7.0) containing 50 ng of phenylmethanesulphonyl fluoride/ml and 50 ng of antipain/ml to remove the sucrose. The Ca^{2+}

measurements were carried out as described for the crude microsomes. 5'-Nucleotidase activity was measured in parallel (Ogilvie *et al.*, 1989).

H.p.l.c. analysis of Ins(1,3,4,5) P_4 metabolism

The metabolism of Ins(1,3,4,5) P_4 by the crude microsome preparation was measured under conditions identical with those described for the Ca^{2+} -release experiments. Microsomes (1.95 ml suspension in the intracellular buffer described above) prepared from 3×10^8 cells were incubated with Ins(1,3,4,5) P_4 for 0 s and 30 s, and the incubation period was terminated by addition of ice-cold $HClO_4$ (10%, w/v). The samples were processed and analysed by using anion-exchange h.p.l.c. and a post-column complexometric dye as described previously (Mayr, 1988; Guse & Emmrich, 1991).

RESULTS

The function of Ins(1,3,4,5) P_4 and Ins(1,4,5) P_3 was studied in three model systems. First, saponin-permeabilized cells were prepared in an intracellular buffer with a low Ca^{2+} concentration (approx. 150 nM) and the effect of increasing concentrations of either Ins(1,4,5) P_3 or Ins(1,3,4,5) P_4 on Ca^{2+} release from intracellular stores was monitored with the fluorescent dye Fura-2 free acid (Figs. 1 and 2). Ins(1,4,5) P_3 induced a Ca^{2+} release in all three cell types (Figs. 1a and 2), with differences in the amount of Ca^{2+} released (Fig. 1a). In contrast, when Ins(1,3,4,5) P_4 was added to the preparations no Ca^{2+} release could be detected. HPB.ALL cells showed only a slight increase, which never exceeded a difference of 30 nM ($n = 10$). Its biological significance remains to be established (Fig. 1b).

As a second model, crude microsomes were prepared from the three cell lines. These microsomes contained endoplasmic-reticulum membranes (Lambert, 1987) as well as plasma membranes; this was revealed by 5'-nucleotidase activity (Ogilvie *et al.*, 1989). Ca^{2+} -reloaded microsomes from each of the three cell types released Ca^{2+} in a dose-dependent response to Ins(1,4,5) P_3 (Figs. 3a1 and 3a2) with EC_{50} values quite similar to those obtained in permeabilized cells (Table 1). Most interestingly, Ins(1,3,4,5) P_4 also induced a dose-dependent release of Ca^{2+} from the microsomal preparations (Figs. 3b1 and 3b2). In the microsomal preparation the EC_{50} value for Ins(1,4,5) P_3 was about 10-fold smaller than for Ins(1,3,4,5) P_4 , indicating either (i) two independent mechanisms of Ca^{2+} release or (ii), less likely, an effect of Ins(1,3,4,5) P_4 via the Ins(1,4,5) P_3 receptor. This second possibility could be excluded by showing that the effects of Ins(1,4,5) P_3 and Ins(1,3,4,5) P_4 occurred independently of each other. This was done by challenging crude microsomes (i) with maximal concentrations of the non-hydrolysable isomer Ins(2,4,5) P_3 alone, or (ii) with Ins(1,3,4,5) P_4 alone, or (iii) with a combination of Ins(2,4,5) P_3 and Ins(1,3,4,5) P_4 . The statistical analysis revealed that $19 \pm 7\%$ ($n = 3$) of the Ins(2,4,5) P_3 /Ins(1,3,4,5) P_4 -sensitive Ca^{2+} pool was released by Ins(1,3,4,5) P_4 , whereas the remaining portion, $80 \pm 11\%$ ($n = 3$), was released by Ins(2,4,5) P_3 (Fig. 4). According to Student's *t* test for unpaired data, a significant difference ($P \leq 0.05$) was observed between effects of the combined addition of Ins(2,4,5) P_3 /Ins(1,3,4,5) P_4 and the addition of Ins(2,4,5) P_3 alone. It should be noted that additivity was observed only if the basal Ca^{2+} concentration in the cuvette was below 200 nM. At higher Ca^{2+} concentration, which were observed in a number of preparations, the effects of combined addition of Ins(2,4,5) P_3 /Ins(1,3,4,5) P_4 were significantly smaller than those observed after addition of Ins(2,4,5) P_3 (results not shown).

However, preliminary experiments using enriched vesicular plasma membranes from U937 cells confirmed the results obtained in the crude microsomal preparations. The enriched plasma-membrane preparations revealed a 4–6-fold higher 5'-nucleotidase activity than in crude microsomes. With the same experimental set-up Ins(1,3,4,5) P_4 dose-dependently released

Table 1. EC_{50} values for Ins(1,4,5) P_3 - and Ins(1,3,4,5) P_4 -induced Ca^{2+} release

Dose-response curves for Ins(1,4,5) P_3 and Ins(1,3,4,5) P_4 were obtained in permeabilized cells or crude microsomes as detailed in Figs. 1–3.

Addition		EC_{50} value (μM)		
		Jurkat	HPB.ALL	U937
Ins(1,4,5) P_3	Permeabilized cells	0.84	0.11	0.41
	Microsomes	0.16	0.16	0.16
Ins(1,3,4,5) P_4	Permeabilized cells	—	—	—
	Microsomes	1.22	2.29	1.35

Fig. 4. Ins(1,3,4,5) P_4 - and Ins(2,4,5) P_3 -mediated effects on Ca^{2+} release are independent of each other

Ca^{2+} efflux from crude microsomes of Jurkat T cells was stimulated by addition of a maximal concentration of Ins(1,3,4,5) P_4 (8 μM), or by addition of a maximal concentration of Ins(2,4,5) P_3 (4 μM) or by addition of Ins(1,3,4,5) P_4 and Ins(2,4,5) P_3 at the same time (8 μM /4 μM). Traces from one typical experiment out of three are displayed.

Fig. 5. Ins(1,3,4,5) P_4 -induced Ca^{2+} release from enriched vesicular plasma membranes of U937 cells

Enriched vesicular plasma membranes from U937 cells were challenged with Ins(1,3,4,5) P_4 in an intracellular buffer in the presence of ATP, an energy-conserving system, oligomycin and Fura-2 free acid. Addition of Ins(1,4,5) P_3 (IP_3) after a maximal concentration of Ins(1,3,4,5) P_4 (IP_4) did not result in further Ca^{2+} release. IM indicates the addition of ionomycin (2 μM).

Fig. 6. Purity control of Ins(1,3,4,5) P_4 used for Ca^{2+} -release experiments

(a) Ins(1,3,4,5) P_4 (4 μM) was added to a prepared cuvette containing all the solutions as described for measuring microsomal Ca^{2+} release, except that intracellular buffer instead of microsomes was included. (b) Ins(1,3,4,5) P_4 (1 nmol injected) was analysed by anion-exchange h.p.l.c. and a complexometric post-column dye system for non-radioactive detection as described by Guse & Emmrich (1991). The elution positions of Ins(1,4,5) P_3 and Ins(1,3,4) P_3 are indicated. (c) Ins(1,3,4,5) P_4 (4 μM) was incubated with crude microsomes in an intracellular buffer under conditions similar to those in the Ca^{2+} -release experiments for 30 s. The sample was processed and analysed by anion-exchange h.p.l.c. and a complexometric post-column dye system as described (Guse & Emmrich, 1991). The elution positions of Ins(1,4,5) P_3 and Ins(1,3,4) P_3 are indicated.

Ca^{2+} from enriched vesicular plasma membranes at a similar concentration range as for crude microsomes (Fig. 5). In contrast, Ins(1,4,5) P_3 was only effective in the plasma membranes at much higher concentrations (results not shown).

Several alternative interpretations of the findings could be excluded by the following controls: Ins(1,3,4,5) P_4 was checked for its purity (i) with regard to contaminating Ca^{2+} in the Ins(1,3,4,5) P_4 preparation by measuring the Ca^{2+} content fluorimetrically, (ii) with regard to contaminating Ins(1,4,5) P_3 by analysis on anion-exchange h.p.l.c. and a post-column dye system as described previously (Mayr, 1988; Guse & Emmrich, 1992), and (iii) by investigating whether an Ins(1,3,4,5) P_4 3-phosphatase (Hoer *et al.*, 1988) was present in the microsomal preparations. Our Ins(1,3,4,5) P_4 preparation contained neither Ca^{2+} (sensitivity threshold 5–10 nM; Fig. 6a) or Ins(1,4,5) P_3 at a sensitivity threshold of the anion-exchange h.p.l.c. system of 5 pmol (signal-to-noise ratio 2.5:1; Fig. 6b). The Ins(1,3,4,5) P_4 preparation was

not metabolized to detectable amounts of Ins(1,4,5) P_3 during incubation with the microsomes (Fig. 6c).

DISCUSSION

In conclusion, our results demonstrate that Ins(1,3,4,5) P_4 , independently of (1,4,5) P_3 , is able to release Ca^{2+} from crude microsomes. In these preparations the plasma-membrane fragments together with endoplasmic-reticulum membrane may form either 'mixed' vesicles or alternatively separate vesicles. In either case an intact Ca^{2+} gradient across the microsomal membrane was re-established. However, if the Ca^{2+} gradient across the plasma membrane was abolished, e.g. in permeabilized cells, no Ca^{2+} release by Ins(1,3,4,5) P_4 could be observed. The extracellular and intracellular Ca^{2+} concentrations were equilibrated in these cells and Ca^{2+} gradients were only maintained across membranes of the endoplasmic reticulum, which seem to be restricted in their susceptibility to Ins(1,4,5) P_3 . Ins(1,3,4,5) P_4 , on the other hand, requires an intact Ca^{2+} gradient across the plasma membrane, and induces a plasmalemmal Ca^{2+} flux. The Ca^{2+} flux appears to be unidirectional from the extracellular space to the cytosol (or into membrane vesicles closely associated with the plasma membrane), i.e. the microsomal lumen corresponds to the extracellular space in this model of inside-out vesicles. Such a system allows demonstration of the action of a cytosolic second messenger such as Ins(1,3,4,5) P_4 , which has to approach its target from the cytosol in the physiological situation. Vesicles with an opposite orientation could not have responded in the assay (which does not necessarily imply that they are not present in the microsomal preparation). This more indirect evidence was then confirmed by showing directly that enriched vesicular plasma membranes respond to Ins(1,3,4,5) P_4 , at least in U937 cells. Thus our data suggest an Ins(1,3,4,5) P_4 -regulated Ca^{2+} channel in the plasma membrane of the cell lines investigated. Biochemical support for our hypothesis comes from a recently published study demonstrating the presence of a novel Ins(1,4,5) P_3 -receptor in the plasma membrane of Jurkat T-lymphocytes (Khan *et al.*, 1992). This novel Ins(1,4,5) P_3 receptor appears to be closely related to the cerebellar Ins(1,4,5) P_3 receptor, since it cross-reacts with anti-Ins(1,4,5) P_3 -receptor antisera in Western-blot experiments (Khan *et al.*, 1992). However, the novel Ins(1,4,5) P_3 receptor, which is present in Jurkat plasma membranes, as demonstrated by binding studies and the presence of sialic acid in the carbohydrate side chains (Khan *et al.*, 1992), has different binding characteristics from those of the cerebellar receptor. Whereas at the cerebellar receptor as well as at the microsomal receptor from Jurkat cells Ins(1,4,5) P_3 displaced [3H]Ins(1,4,5) P_3 approx. 500-fold better than Ins(1,3,4,5) P_4 , this factor decreases to 2–3 in the Jurkat-cell plasma membrane (Khan *et al.*, 1992). Therefore, our finding of an Ins(1,3,4,5) P_4 -sensitive Ca^{2+} -release mechanism in the plasma membrane of T-lymphocytes and monocytes is in agreement with the hypothesis that the novel Ins(1,4,5) P_3 receptor mediates Ins(1,3,4,5) P_4 -induced Ca^{2+} entry (Fig. 7).

A different model, namely an Ins(1,3,4,5) P_4 -sensitive channel connecting plasma-membrane-associated vesicles and calciosomes, was recently proposed by Irvine (1989, 1990). The model is based on experiments where Ins(1,3,4,5) P_4 enhances the effect of Ins(1,4,5) P_3 in permeabilized cell preparations without itself having much effect. However, whether this model also applies to T-lymphocytes and monocytes remains to be investigated.

In the past, only very few independent effects of Ins(1,3,4,5) P_4 have been described in the literature. In *Xenopus laevis* oocytes Ins(1,3,4,5) P_4 mobilized intracellular Ca^{2+} under conditions where no Ins(1,4,5) P_3 was formed (Guse *et al.*, 1991). This is

Fig. 7. Hypothetical model to explain the effects of $\text{Ins}(1,4,5)\text{P}_3$ and $(1,3,4,5)\text{P}_4$ on Ca^{2+} release and Ca^{2+} entry in Jurkat T-lymphocytes and U937 monocytes

Abbreviation: ER, endoplasmic reticulum. $\text{Ins}(1,4,5)\text{P}_3$ receptors I and II are the endoplasmic-reticulum and plasmalemmal isoforms of the receptor.

obviously in contrast with our results in the T-lymphocyte and monocytoïd cell lines. However, an explanation may be a different location of the $\text{Ins}(1,3,4,5)\text{P}_4$ receptor in the very large *X. laevis* oocytes.

An independent effect of $\text{Ins}(1,3,4,5)\text{P}_4$ was also reported on bovine adrenal microsomes (Ely *et al.*, 1990). These microsomes were prepared similarly to those used in our study. The kinetics of the $\text{Ins}(1,3,4,5)\text{P}_4$ - and $\text{Ins}(1,4,5)\text{P}_3$ -induced Ca^{2+} release seem to be very similar in the two systems. Whereas the $\text{Ins}(1,4,5)\text{P}_3$ -induced increase was more or less short-lived, the $\text{Ins}(1,3,4,5)\text{P}_4$ -induced rise in the Ca^{2+} concentration stayed elevated (Ely *et al.*, 1990; see the Results section). This phenomenon indicates different regulatory mechanisms being present in $\text{Ins}(1,3,4,5)\text{P}_4$ -sensitive and in $\text{Ins}(1,4,5)\text{P}_3$ -sensitive vesicles. One possible explanation would be a less active Ca^{2+} -ATPase in the $\text{Ins}(1,3,4,5)\text{P}_4$ -sensitive vesicles. Another similarity between the results of Ely *et al.* (1990) and our own was the observation that high concentrations of $\text{Ins}(1,3,4,5)\text{P}_4$ released less Ca^{2+} from the microsomes than did identical concentrations of $\text{Ins}(1,4,5)\text{P}_3$. Very recently a direct effect of $\text{Ins}(1,3,4,5)\text{P}_4$, but not of $\text{Ins}(1,4,5)\text{P}_3$ on a Ca^{2+} -permeable channel in plasma membranes from endothelial cells was demonstrated (Lückhoff & Clapham, 1992). This important observation is consistent with our finding

that $\text{Ins}(1,3,4,5)\text{P}_4$ independently acts on a Ca^{2+} -release mechanism in crude T-lymphocyte and monocyte microsomes and monocyte plasma membranes. However, $\text{Ins}(1,4,5)\text{P}_3$ / $\text{Ins}(2,4,5)\text{P}_3$ also seem to play a role in regulating Ca^{2+} entry in some cell types. In mouse lacrimal acinar cells $\text{Ins}(2,4,5)\text{P}_3$ induced Ca^{2+} release from internal stores as well as Ca^{2+} entry (Bird *et al.*, 1991). These different results in different cell systems indicate a cell-type-specific expression of $\text{Ins}(1,4,5)\text{P}_3$ - or $\text{Ins}(1,3,4,5)\text{P}_4$ -gated Ca^{2+} channels in the plasma membrane.

Another system where $\text{Ins}(1,3,4,5)\text{P}_4$ induced an independent effect of $\text{Ins}(1,4,5)\text{P}_3$ is the neuroblastoma cell line SH-SY5Y (Gawler *et al.*, 1990, 1991). In contrast with our results, in that system $\text{Ins}(1,3,4,5)\text{P}_4$ induced a Ca^{2+} release from intracellular, non-mitochondrial, stores in permeabilized cells, with an EC_{50} of $1\text{ }\mu\text{M}$. Gawler *et al.* (1990, 1991) also used saponin for permeabilization of the cells, but, in contrast with our study, used a different read-out system to measure Ca^{2+} efflux from intracellular stores, namely release of $^{45}\text{Ca}^{2+}$. The different results in their study and in ours may be explained by (i) the more sensitive method of measuring the Ca^{2+} efflux used by Gawler *et al.* (1990, 1991), or (ii) a different location of an $\text{Ins}(1,3,4,5)\text{P}_4$ receptor in SH-SY5Y cells as compared with the cell lines investigated in our study.

In conclusion, we have described for the cell lines investigated (Jurkat, HPB.ALL, U937) that $\text{Ins}(1,3,4,5)\text{P}_4$ activates a Ca^{2+} efflux from crude microsomal preparations and enriched vesicular plasma membranes, which is independent of the $\text{Ins}(1,4,5)\text{P}_3$ effect. Since $\text{Ins}(1,3,4,5)\text{P}_4$ does not function in permeabilized preparations of the same cells, we suggest the requirement for an intact Ca^{2+} gradient across the plasma membrane and an $\text{Ins}(1,3,4,5)\text{P}_4$ -susceptible Ca^{2+} channel in the plasma membrane.

A suggestive metaphor was proposed by Irvine (1989), describing the co-operative function of $\text{Ins}(1,4,5)\text{P}_3$ and $\text{Ins}(1,3,4,5)\text{P}_4$ as a 'duet'. Extending on this idea, each vocalist may be meaningful to a different audience, thereby providing a way to regulate differentially influx versus internal mobilization of Ca^{2+} .

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