Lysophosphatidic acid-sensitive intracellular Ca^{2+} store does not regulate Ca^{2+} entry at plasma membrane in Jurkat human T-cells

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In Jurkat T cells, the anti-CD3 antibody OKT3 and thapsigargin (TG) elevated the cytoplasmic free Ca^{2+} concentration ($[\text{Ca}^{2+}]_i$), after which it decreased to a sustained, elevated level. In contrast, lysophosphatidic acid (LPA) increased $[\text{Ca}^{2+}]_i$ only briefly and transiently, after which it declined to the resting level of $[\text{Ca}^{2+}]_i$ even in the presence of extracellular Ca^{2+} . OKT3 increased $\text{Ins}(1,4,5)\text{P}_3$ formation but neither LPA nor TG did. In the absence of extracellular Ca^{2+} , the addition of OKT3 did not affect an elevation of $[\text{Ca}^{2+}]_i$ induced by the subsequent addition of LPA and vice versa. In permeabilized Jurkat cells, the addition of $\text{Ins}(1,4,5)\text{P}_3$ released Ca^{2+} ; this was inhibited by heparin,

whereas LPA released Ca^{2+} even in the presence of heparin. cADP-ribose released Ca^{2+} ; this was additive with LPA-induced Ca^{2+} release and vice versa in permeabilized Jurkat cells. LPA did not stimulate Ca^{2+} entry and $^{45}\text{Ca}^{2+}$ uptake but OKT3 and TG did. LPA, OKT3 and TG did not affect the sustained elevation of $[\text{Ca}^{2+}]_i$ induced by ionomycin. The present results suggest that at least three kinds of intracellular Ca^{2+} stores, which are $\text{Ins}(1,4,5)\text{P}_3$ -, cADP-ribose- and LPA-sensitive, exist in Jurkat T cells, and that the LPA-sensitive intracellular Ca^{2+} store does not regulate Ca^{2+} entry at the plasma membrane.

INTRODUCTION

The activation of T lymphocytes represents a key step in physiological and pathological immune responses. These responses seem to be regulated by intracellular second messengers, the most important of which is known to be Ca^{2+} . An increase in the cytosolic free Ca^{2+} concentration ($[\text{Ca}^{2+}]_i$) contributes to an essential triggering signal for T-cell activation in the immune system [1,2]. This increase in $[\text{Ca}^{2+}]_i$ is due to the release of Ca^{2+} from intracellular stores and is sustained by the influx of extracellular Ca^{2+} [2,3]. At least four kinds of intracellular Ca^{2+} store exist in Jurkat T-cells; however, the physiological role of each store remains unclear [4]. In addition, the process involved in the activation of Ca^{2+} entry across the plasma membrane during the sustained increase in $[\text{Ca}^{2+}]_i$ is less known. Capacitative Ca^{2+} entry, by which depletion of Ca^{2+} in the $\text{Ins}(1,4,5)\text{P}_3$ -sensitive intracellular store activates Ca^{2+} entry, has been confirmed in Jurkat T-cells [5–7]. We have reported previously that the cAMP-sensitive intracellular Ca^{2+} store might also be important for capacitative Ca^{2+} entry [8]. Thus other intracellular Ca^{2+} stores, besides the $\text{Ins}(1,4,5)\text{P}_3$ -sensitive store, might regulate Ca^{2+} entry at the plasma membrane.

Lysophosphatidic acid (LPA) is believed to be a novel lipid mediator with a wide range of biological activities, including stimulation of cell proliferation, smooth-muscle contraction, platelet aggregation, neurite retraction and cell motility [9,10]. In addition, LPA might function as an agonist in that it exerts its own biological effects when added extracellularly to appropriate target cells [9]. In a variety of actions of LPA, it has been reported to cause Ca^{2+} mobilization in various cell types [11–14]. However, the effects of LPA on Ca^{2+} mobilization in lymphocytes is not known. We have reported that phosphatidic acid released Ca^{2+} from $\text{Ins}(1,4,5)\text{P}_3$ -insensitive intracellular stores but did not stimulate Ca^{2+} entry in Jurkat cells [15]. Because phosphatidic acid is known to be a precursor for LPA [9,16], LPA would be the final metabolite of phosphatidic acid for mobilizing Ca^{2+} in Jurkat cells. We report here that LPA releases Ca^{2+} from its own

sensitive intracellular store, and that the LPA-sensitive intracellular Ca^{2+} store does not regulate Ca^{2+} entry at the plasma membrane in Jurkat T-cells.

EXPERIMENTAL

Materials

$^{45}\text{CaCl}_2$ (5–50 mCi/mg of Ca) was from DuPont-New England Nuclear; fura 2 acetoxymethyl ester (fura 2/AM) and fura 2 pentapotassium salt were from Molecular Probes (Eugene, OR, U.S.A.); $\text{Ins}(1,4,5)\text{P}_3$ was from Dojindo Co. (Kumamoto, Japan). OKT3 was a gift from Dr. N. Satoh (Sapporo Medical University, Japan). All other reagents were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.).

Cell culture

Jurkat human leukaemia T-cells were maintained under O_2/CO_2 (19:1) in RPMI 1640 medium supplemented with 10% (v/v) fetal bovine serum, penicillin (50 i.u./ml), streptomycin (50 $\mu\text{g}/\text{ml}$) and L-glutamine (300 $\mu\text{g}/\text{ml}$) as described previously [17].

Measurement of $[\text{Ca}^{2+}]_i$

Measurement of $[\text{Ca}^{2+}]_i$ was performed as described previously [8]. Briefly, a Jurkat T-cell suspension (approx. 2×10^6 cells/ml) was loaded with 2 μM fura 2/AM for 30 min at room temperature. After the loading period, the T-cells were washed twice with Krebs–Ringer–Hepes (KRH) medium containing 0.2% BSA. The composition of KRH is as follows: 120 mM NaCl, 5.4 mM KCl, 1.0 mM CaCl_2 , 0.8 mM MgCl_2 , 11.1 mM glucose, 20 mM Hepes, pH 7.4. The fluorescence of the fura 2-loaded cell suspension was monitored in the cuvette at 35 °C with a Jasco

Abbreviations used: $[\text{Ca}^{2+}]_i$, cytosolic free Ca^{2+} concentration; KRH, Krebs–Ringer–Hepes; LPA, lysophosphatidic acid; TG, thapsigargin.

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CAF-110 (Japan Spectroscopic Co., Tokyo, Japan). The excitation wavelengths were 340 and 380 nm and emission was measured at 510 nm. $[Ca^{2+}]_i$ was estimated as described by Grynkiewicz et al. [18]. In the experiments in the absence of extracellular Ca^{2+} , 3 mM EGTA was added to the cell suspension 3 min before drug stimulation to chelate extracellular Ca^{2+} .

Measurement of $Ins(1,4,5)P_3$

Measurement of $Ins(1,4,5)P_3$ was performed as described previously [15]. Briefly, Jurkat cells (10^7 cells/ml) were washed twice with KRH, suspended in KRH containing 10 mM LiCl and preincubated for 10 min at 37 °C. The mixture after addition of drugs was further incubated for 30 s. The reaction was then terminated by equal volume of 15% (w/v) trichloroacetic acid. The preparations were kept on ice for 30 min and then centrifuged at 2000 *g* for 15 min at 4 °C. The supernatant was washed three times with 10 vol. of water-saturated diethyl ether to remove trichloroacetic acid from the solution. The resultant solutions were neutralized by titration to pH 7.5 with $NaHCO_3$. After centrifugation at 2000 *g* for 15 min at 4 °C, 100 μ l of supernatant was assayed with an $Ins(1,4,5)P_3$ assay kit (Amersham).

Preparation of permeabilized cells

Permeabilized Jurkat cells were prepared as described by Guse et al. (1992). Briefly, approx. 10^8 cells were incubated in a buffer composed of 110 mM KCl, 10 mM NaCl, 2 mM $MgCl_2$, 5 mM KH_2PO_4 , 2 mM EGTA, 1 mM dithiothreitol and 20 mM Hepes, pH 7.2, in the presence of 30 μ g/ml saponin for 5 min at 37 °C. After centrifugation at 180 *g* for 5 min at room temperature, the cell pellet was washed with an intracellular buffer composed of 110 mM KCl, 10 mM NaCl, 2 mM $MgCl_2$, 5 mM KH_2PO_4 and 20 mM Hepes, pH 7.2. The final pellet was resuspended in intracellular buffer containing 20 units/ml creatine kinase, 20 mM phosphocreatine and 10 μ g/ml oligomycin. The fluorescence of fura 2 free acid (1 μ M) was monitored as described above. When fluorescence had declined to a stable value after subsequent addition of 1 mM ATP, drugs were added.

Ca^{2+} entry into cells

Fura 2-loaded cells were washed twice with Ca^{2+} -free KRH containing 0.2 mM EGTA and then transferred to the cuvette. When an elevation of $[Ca^{2+}]_i$ induced by drugs had recovered to the resting level, 1.2 mM Ca^{2+} was added to the extracellular medium. Then $[Ca^{2+}]_i$ was measured until it reached a sustained, higher level of $[Ca^{2+}]_i$. Ca^{2+} entry was expressed as the difference between the $[Ca^{2+}]_i$ before and after the addition of Ca^{2+} .

$^{45}Ca^{2+}$ uptake into cells

Measurement of $^{45}Ca^{2+}$ uptake was performed as described previously [15]. Drugs and $^{45}Ca^{2+}$ (1 μ Ci) were added to 0.2 ml aliquots of cell suspension (2×10^6 cells/ml). After 5 min, $^{45}Ca^{2+}$ uptake was determined by addition of the aliquot to 1 ml of iced incubation solution containing 2 mM $LaCl_3$. The cells were separated by centrifugation at 1000 *g* for 1 min at 4 °C, washed twice with 1 ml of ice-cold incubation solution containing 1 mM EGTA and separated by further centrifugation using the same conditions. The cells were then resuspended in distilled water, and $^{45}Ca^{2+}$ in the cells was measured with standard liquid-scintillation counting techniques.

Ca^{2+} efflux

The effects of drugs on the sustained Ca^{2+} signal evoked by 10 μ M ionomycin were determined as described previously [19].

RESULTS

As shown in Figure 1, 10 μ g/ml OKT3, an anti-CD3 monoclonal antibody, and 100 nM thapsigargin (TG), a microsomal Ca^{2+} -ATPase inhibitor, elevated $[Ca^{2+}]_i$, after which it decreased to a sustained, elevated level. The sustained level of $[Ca^{2+}]_i$ induced by TG was higher than that by OKT3. In contrast, 10 μ M LPA increased $[Ca^{2+}]_i$ only transiently, after which it declined to the resting level of $[Ca^{2+}]_i$ even in the presence of extracellular Ca^{2+} .

Figure 1 Effects of 10 μ g/ml OKT3, 100 nM TG or 10 μ M LPA on $[Ca^{2+}]_i$ in Jurkat T-cells

$[Ca^{2+}]_i$ was measured in fura 2-loaded cells as described in the Materials and methods section. Each trace is representative of at least three experiments.

Table 1 Effects of 10 μ g/ml OKT3, 10 μ M LPA or 100 nM TG on $Ins(1,4,5)P_3$ formation in Jurkat cells

Jurkat cells (10^7 cells/ml) were washed twice with KRH, suspended in KRH containing 10 mM LiCl and preincubated for 10 min at 37 °C. The mixture, after addition of drugs, was further incubated for 30 s, and then the reaction was then terminated by the addition of 15% (w/v) trichloroacetic acid. The supernatant was washed three times with water-saturated diethyl ether to remove trichloroacetic acid from the solution. The resultant solutions were neutralized, and after centrifugation the supernatant was assayed with the $Ins(1,4,5)P_3$ assay system. The results shown are means $\pm$ S.E.M. for four determinations.

Treatment	$Ins(1,4,5)P_3$ (pmol/ 10^7 cells)
Control	2.13 ± 0.23
OKT3	8.44 ± 1.30
LPA	2.20 ± 0.57
TG	2.03 ± 0.66

Figure 2 Effect of 10 $\mu\text{g}/\text{ml}$ OKT3, 100 nM TG and 10 μM LPA on $[\text{Ca}^{2+}]_i$ in the absence of extracellular Ca^{2+}

$[\text{Ca}^{2+}]_i$ was measured in fura 2-loaded cells as described in the Materials and methods section. EGTA (3 mM) was added 3 min before addition of the first drug. Each trace is representative of at least three experiments.

This is consistent with the observation that phosphatidic acid mobilizes intracellular Ca^{2+} without subsequent Ca^{2+} influx at the plasma membrane in Jurkat cells [15,20]. LPA increases $\text{Ins}(1,4,5)\text{P}_3$ formation in cultured rat mesangial cells [21], avian nasal salt gland cells [22] and PC12 pheochromocytoma cells [23]. $\text{Ins}(1,4,5)\text{P}_3$ formation induced by LPA could cause Ca^{2+} mobilization in Jurkat cells. However, OKT3 increased

Figure 4 Ca^{2+} release from the cADP-ribose- and LPA-sensitive intracellular Ca^{2+} stores in permeabilized Jurkat cells

Jurkat cells were incubated as described in the Materials and methods section. Fura 2 free acid (1 μM), an ATP regenerating system and 30 $\mu\text{g}/\text{ml}$ saponin were added. When fluorescence had declined to a stable value, 10 μM cADP-ribose (cADPR) and 10 μM LPA were added. Each trace is representative of at least three experiments.

$\text{Ins}(1,4,5)\text{P}_3$ formation but neither LPA nor TG did, as shown in Table 1.

In the absence of extracellular Ca^{2+} , the elevation of $[\text{Ca}^{2+}]_i$ induced by OKT3 declined to a resting level before the addition of LPA (Figure 2). The $[\text{Ca}^{2+}]_i$ elevation by the subsequent addition of LPA in the presence of OKT3 was similar to that induced by LPA alone. Similarly, pretreatment with LPA did not affect the elevation of $[\text{Ca}^{2+}]_i$ induced by the subsequent addition of OKT3. This suggests that at least two kinds of intracellular

Figure 3 Ca^{2+} release from the $\text{Ins}(1,4,5)\text{P}_3$ - and LPA-sensitive intracellular Ca^{2+} stores in permeabilized Jurkat cells

Jurkat cells were incubated as described in the Materials and methods section. Fura 2 free acid (1 μM), an ATP regenerating system and 30 $\mu\text{g}/\text{ml}$ saponin were added. When fluorescence had declined to a stable value, 10 μM $\text{Ins}(1,4,5)\text{P}_3$, 10 μM LPA, 10 $\mu\text{g}/\text{ml}$ heparin (HP) and 100 nM TG were added as indicated by the arrows. Each trace is representative of at least three experiments.

Table 2 Effect of 10 $\mu\text{g/ml}$ OKT3, 10 μM LPA or 100 nM TG on Ca^{2+} entry and $^{45}\text{Ca}^{2+}$ uptake into Jurkat cells

Ca^{2+} entry and $^{45}\text{Ca}^{2+}$ uptake were measured as described in the Materials and methods section. Each result is the mean $\pm$ S.E.M. for four determinations.

Treatment	Ca^{2+} entry (nM)	$^{45}\text{Ca}^{2+}$ uptake (c.p.m./ 10^6 cells)
Control	49.8 $\pm$ 3.2	764.0 $\pm$ 35.0
OKT3	212.5 $\pm$ 19.8	1701.1 $\pm$ 92.6
LPA	52.0 $\pm$ 4.8	833.3 $\pm$ 62.8
TG	318.3 $\pm$ 30.6	3320.8 $\pm$ 337.6

Figure 5 Effects of LPA (10 μM), TG (100 nM) and OKT3 (10 $\mu\text{g/ml}$) on the sustained elevation of $[\text{Ca}^{2+}]_i$ induced by 10 μM ionomycin (ION)

$[\text{Ca}^{2+}]_i$ was measured in fura 2-loaded cells as described in the Materials and methods section. Each trace is representative of at least three experiments.

Ca^{2+} store, both of which are either $\text{Ins}(1,4,5)\text{P}_3$ - or LPA-sensitive, exist in Jurkat cells. We examined whether both stores have Ca^{2+} -ATPase by using TG. After pretreatment with TG in the absence of extracellular Ca^{2+} , neither OKT3 nor LPA elevated $[\text{Ca}^{2+}]_i$. We further studied whether two kinds of intracellular Ca^{2+} store exist in Jurkat cells by measuring the Ca^{2+} release from the permeabilized cells. As shown in Figure 3, the addition of $\text{Ins}(1,4,5)\text{P}_3$ or LPA released Ca^{2+} from non-mitochondrial stores. In addition, LPA released Ca^{2+} , which was additive with $\text{Ins}(1,4,5)\text{P}_3$ -evoked Ca^{2+} release and vice versa. In the presence of heparin, an antagonist of $\text{Ins}(1,4,5)\text{P}_3$ receptor, $\text{Ins}(1,4,5)\text{P}_3$ failed to release Ca^{2+} but LPA released Ca^{2+} to a level comparable to that released by LPA alone. The addition of TG gradually released Ca^{2+} and the subsequent addition of $\text{Ins}(1,4,5)\text{P}_3$ and LPA caused only a weak release of Ca^{2+} . Recently, Guse et al. [24] have reported that Jurkat cells contain additional Ca^{2+} stores that have ryanodine receptors and are sensitive to cADP-ribose. We further examined whether LPA mobilizes Ca^{2+} from cADP-ribose-sensitive Ca^{2+} stores in permeabilized Jurkat cells. As shown in Figure 4, the addition of cADP-ribose released Ca^{2+} from intracellular stores. LPA released Ca^{2+} which was additive with cADP-ribose-induced Ca^{2+} release and vice versa, suggesting

that LPA does not release Ca^{2+} from cADP-ribose-sensitive stores.

As shown in Table 2, OKT3 and TG increased Ca^{2+} entry and $^{45}\text{Ca}^{2+}$ uptake. The degrees of Ca^{2+} entry and $^{45}\text{Ca}^{2+}$ uptake evoked by TG were higher than those by OKT3. In contrast, LPA did not increase either Ca^{2+} entry or $^{45}\text{Ca}^{2+}$ uptake. The inability of LPA to increase Ca^{2+} entry might be explained by the activation of Ca^{2+} efflux at the plasma membrane. We checked this possibility by examining the effect of LPA on the sustained elevation of $[\text{Ca}^{2+}]_i$ induced by ionomycin [25]. LPA, OKT3 and TG did not affect the sustained elevation of $[\text{Ca}^{2+}]_i$ induced by ionomycin (Figure 5).

DISCUSSION

LPA is a novel lipid mediator with a wide range of biological activities, including stimulation of cell proliferation, smooth-muscle contraction, platelet aggregation, neurite retraction and cell motility [9,10]. The effects of LPA on immune cells are unclear. It is thought that LPA might belong to a new family of phospholipid mediators showing overlapping biological activities and acting on distinct receptors; conceivably, the ether-linked phospholipid platelet-activating factor and the mitogenic lipid sphingosine 1-phosphate might also belong to this putative family [9,10]. In addition, some evidence suggests the existence of a G-protein-coupled receptor for LPA on the cell surface [9]. However, the addition of platelet-activating factor (results not shown) and sphingosine 1-phosphate [15] did not increase $[\text{Ca}^{2+}]_i$ in Jurkat cells. Only the addition of LPA mobilized Ca^{2+} in intact and permeabilized Jurkat cells (Figures 1 and 3). Furthermore the present results showed that LPA increased $[\text{Ca}^{2+}]_i$ without an elevation of $\text{Ins}(1,4,5)\text{P}_3$ formation in Jurkat cells, although LPA increases $\text{Ins}(1,4,5)\text{P}_3$ production, which mobilizes Ca^{2+} in rat mesangial cells [21], avian nasal salt gland [22] and PC12 pheochromocytoma cells [23]. The $\text{Ins}(1,4,5)\text{P}_3$ -generating action of LPA in these cells is thought to result from the activation of phospholipase C through G-protein [10,11]. Inability of LPA to produce $\text{Ins}(1,4,5)\text{P}_3$ in Jurkat cells could be explained by the following reasoning. First, stimulation of the T-cell receptor through tyrosine kinase, but not through G-protein, activates phospholipase C- γ 1, which yields $\text{Ins}(1,4,5)\text{P}_3$ and diacylglycerol from the hydrolysis of phosphatidylinositol bisphosphate [26]. Secondly, the receptor for LPA might be on the intracellular Ca^{2+} store rather than on the cell surface in Jurkat cells.

Although Ca^{2+} entry causing a sustained increase in $[\text{Ca}^{2+}]_i$ has an important role in cell function in lymphocytes, the mechanism of Ca^{2+} entry is unknown. Putney [27] proposed capacitative Ca^{2+} entry as a mechanism by which depletion of Ca^{2+} in the intracellular store activates Ca^{2+} entry. Using TG, we demonstrated that the intracellular Ca^{2+} store regulates Ca^{2+} entry at the plasma membrane [28]. This capacitative Ca^{2+} entry, related to the $\text{Ins}(1,4,5)\text{P}_3$ -sensitive store, exists in T-cells [5–7]. In addition, Randriamampita and Tsien [29] reported that the emptying of intracellular Ca^{2+} stores releases a Ca^{2+} influx factor to activate Ca^{2+} entry at the plasma membrane in Jurkat cells. The present results clearly suggest that depletion of Ca^{2+} in the $\text{Ins}(1,4,5)\text{P}_3$ - but not LPA-sensitive intracellular store activated Ca^{2+} entry. With regard to the physiological function of the Ca^{2+} signalling pathway for OKT3, which causes a sustained increase in $[\text{Ca}^{2+}]_i$, the function of the T-cell receptor could be via the action of $\text{Ins}(1,4,5)\text{P}_3$ on the $\text{Ins}(1,4,5)\text{P}_3$ -sensitive Ca^{2+} intracellular store followed by the sustained activation of Ca^{2+} entry.

We have reported that phosphatidic acid released Ca^{2+} from the $\text{Ins}(1,4,5)\text{P}_3$ -insensitive intracellular store but did not stimu-

late Ca^{2+} entry in Jurkat cells [15]. Because phosphatidic acid is known to be a precursor for LPA [9,16], formed mainly through phospholipase A_2 -mediated deacylation of newly generated phosphatidic acid [9,10,16], LPA is likely to be the final metabolite of phosphatidic acid for mobilizing Ca^{2+} in Jurkat cells. Jalink et al. [12] have reported that LPA (up to $50 \mu\text{g/ml}$) was ineffective in increasing $[\text{Ca}^{2+}]_i$ in Jurkat cells. The reason for the difference between their results and ours is unclear. In the absence of extracellular Ca^{2+} , the subsequent addition of phytohaemagglutinin, an $\text{Ins}(1,4,5)\text{P}_3$ -generating agent, did not increase $[\text{Ca}^{2+}]_i$ after the pretreatment of Jurkat cells with OKT3 (results not shown). This suggests that the $\text{Ins}(1,4,5)\text{P}_3$ -sensitive intracellular store is depleted of Ca^{2+} after the addition of OKT3 in the absence of extracellular Ca^{2+} . Under these conditions, LPA was able to release Ca^{2+} from the intracellular store (Figure 2), suggesting that at least two kinds of intracellular Ca^{2+} store exist in Jurkat T-cells, both of which are either $\text{Ins}(1,4,5)\text{P}_3$ - or LPA-sensitive. This is supported by the observation that LPA alone releases Ca^{2+} from permeabilized cells even in the presence of heparin (Figure 3). Furthermore both stores seem to have a Ca^{2+} -ATPase that is inhibited by TG. cADP-ribose has been considered as an endogenous regulator of Ca^{2+} -induced Ca^{2+} release through a ryanodine receptor rather than through an $\text{Ins}(1,4,5)\text{P}_3$ receptor [31–33]. Guse et al. [24] showed that Jurkat cells have endogenous cADP-ribose that releases Ca^{2+} from $\text{Ins}(1,4,5)\text{P}_3$ -insensitive stores. However, LPA did not release Ca^{2+} from cADP-ribose-sensitive stores (Figure 4), suggesting that LPA releases Ca^{2+} from its own intracellular store. We also reported that the cAMP-sensitive intracellular Ca^{2+} store exists in Jurkat cells [8]. Guse et al. [4] suggested that at least four kinds of intracellular Ca^{2+} store exist in Jurkat cells. In addition, an increase in $[\text{Ca}^{2+}]_i$ contributes to an essential triggering signal for T-cell activation [1,2]. Thus Ca^{2+} release from the LPA-sensitive intracellular store might be important for triggering signals for T-cell activation.

In summary, we report that at least three kinds of intracellular store, which are $\text{Ins}(1,4,5)\text{P}_3$ -, cADP-ribose- and LPA-sensitive, exist in Jurkat human T-cells. Although the physiological roles of these stores remain unclear, the present results show for the first time that the depletion of Ca^{2+} in the LPA-sensitive intracellular store does not cause capacitative Ca^{2+} entry in Jurkat T-cells.

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