Agonist-induced inhibition of phosphatidylserine synthesis is secondary to the emptying of intracellular Ca^{2+} stores in Jurkat T-cells

Claudette PELASSY, Jean-Philippe BREITTMAYER and Claude AUSSEL*

Interactions Cellulaires en Immunologie, INSERM U343, Faculté de Médecine, 06107 Nice Cédex 02, France

The biosynthesis of phosphatidylserine (PtdSer) by the serine base-exchange enzyme system, in Jurkat T-lymphocytes, was inhibited in intact cells maintained in low- Ca^{2+} -containing buffer ($< 10 \mu\text{M}-\text{Ca}^{2+}$) by using Ca^{2+} ionophores (A23187 or ionomycin). The rise in cytosolic Ca^{2+} concentration under these experimental conditions was only due to the release of Ca^{2+} from intracellular compartments, suggesting that the inhibition of PtdSer synthesis was correlated with the emptying of intracellular Ca^{2+} pools. This was further studied in saponin-permeabilized cells, in which PtdSer synthesis was found to be inhibited by EGTA, Ca^{2+} ionophores (A23187 or ionomycin) and Ca^{2+} -ATPase inhibitors [thapsigargin or 2,5-di-(*t*-butyl)-benzohydroquinone]. Since Ca^{2+} -ATPase inhibitors impaired refilling of the Ca^{2+} stores with Ca^{2+} , and since in CD3-activated Jurkat T-cells the Ca^{2+} stores remained empty after 1 h of treatment with anti-CD3 monoclonal antibodies, we suggest that PtdSer synthesis is mainly regulated by the level of Ca^{2+} in the intracellular compartments and that the Ca^{2+} -dependent serine base-exchange system responsible for PtdSer synthesis is probably located within or close to a Ca^{2+} -storage organelle.

INTRODUCTION

Jurkat T-cells respond to phytohaemagglutinin or to monoclonal antibodies (mAbs) reacting with surface receptors (the CD3–TCR complex or CD2 molecules) in combination with the phorbol ester phorbol 12-myristate 13-acetate by producing interleukin-2. The transmembrane signalling pathway activated by CD3 or CD2 mAbs involves a phospholipase C that cleaves phosphatidylinositol bisphosphate into diacylglycerol and inositol 1,4,5-trisphosphate (InsP_3). The second step of T-cell activation is an augmentation of the concentration of cytosolic Ca^{2+} , which is due both to a release of Ca^{2+} from intracellular stores, probably mediated by InsP_3 , and to an external Ca^{2+} influx (Imboden & Stobo, 1985; Weiss *et al.*, 1986; Pantaleo *et al.*, 1987).

We have previously shown that these early events of T-cell activation are accompanied by a marked decrease in phosphatidylserine (PtdSer) synthesis (Pelassy *et al.*, 1989). A pharmacological approach had demonstrated that blocking the Ca^{2+} influx by using nardihydroguaiaretic acid, tosylphenylalanyl-chloromethane or phorbol 12-myristate 13-acetate did not modify the decrease in PtdSer synthesis induced by phytohaemagglutinin or CD3 mAb (Aussel *et al.*, 1991). In contrast, blocking the release of Ca^{2+} from intracellular stores with *N*-ethylmaleimide restored PtdSer synthesis to a level quite comparable with that observed in control cells (Aussel *et al.*, 1991). These facts suggested that the release of Ca^{2+} from intracellular stores plays a role in the regulation of PtdSer synthesis.

The biosynthesis of PtdSer by mammalian cells appears to occur solely by an exchange of L-serine for either the ethanolamine moiety of phosphatidylethanolamine or the choline moiety of phosphatidylcholine (Kanfer, 1972; Miura & Kanfer, 1976). The use of rat brain and liver microsomes (Bjerve, 1973*a,b*; Gaiti *et al.*, 1974) as well as Jurkat cells (Aussel *et al.*, 1991), has demonstrated that PtdSer synthesis is a Ca^{2+} -dependent process. The base-exchange enzyme system responsible for PtdSer syn-

thesis needs Ca^{2+} for its activity. The fact that PtdSer synthesis was inhibited when cytosolic Ca^{2+} increased prompted us to study in more detail the relationships between Ca^{2+} and PtdSer synthesis in activated Jurkat cells. Recent advances in the regulation of Ca^{2+} -ATPase by thapsigargin (TG), a plant-derived sesquiterpene lactone (Rasmussen *et al.*, 1978), and 2,5-di-(*t*-butyl)-1,4-benzohydroquinone (DTBuHQ) (Llopis *et al.*, 1991), in a number of cells (Takemura *et al.*, 1989; Mason *et al.*, 1991), including T-lymphocytes (Gouy *et al.*, 1990; Sarkadi *et al.*, 1991), had led to the conclusion that both TG and DTBuHQ are quite able to release Ca^{2+} from intracellular compartments, independently of physiological stimuli. These drugs are believed to act predominantly by the inhibition of the endoplasmic-reticulum Ca^{2+} pump, and thus allow the release of Ca^{2+} without InsP_3 generation. It was thus of interest to study the effects of TG and DTBuHQ on PtdSer synthesis in T-cells.

MATERIALS AND METHODS

Cells

Jurkat D: the human T cell line Jurkat was kindly supplied by Dr. A. M. Schmitt-Verhulst (Centre d'Immunologie, Marseille-Luminy, France). Cells were cloned by limiting dilution. Clone D was selected on the base of the interleukin-2 production when activated with phytohaemagglutinin and phorbol 12-myristate 13-acetate. Cells were cultured in RPMI 1640 (Seromed, Lille, France) supplemented with 5% (v/v) fetal-calf serum, 50 units of penicillin/ml, 50 μg of streptomycin/ml, 2 mM-L-glutamine, 1 mM-pyruvate and 0.1 $\mu\text{M}-\beta$ -mercaptoethanol.

Chemicals

Ca^{2+} -ionophores (A23187 and ionomycin), TG and InsP_3 were purchased from Sigma, St. Louis, MI, U.S.A. DTBuHQ was from Aldrich Chimie, Strasbourg, France. [^3H]Serine (0.37–1.1 TBq/mmol) was purchased from either Amersham International (U.K.) or Dositek (Orsay, France).

Abbreviations used: PtdSer, phosphatidylserine; mAbs, monoclonal antibodies; TG, thapsigargin; DTBuHQ, 2,5-di-(*t*-butyl)-1,4-benzohydroquinone; InsP_3 , inositol 1,4,5-trisphosphate.

* To whom all correspondence should be addressed.

PtdSer synthesis in intact cells

Jurkat cells (2×10^6) were maintained in 0.5 ml of a buffer (pH 7.4) containing 137 mM-NaCl, 2.7 mM-KCl, 2.5 mM-glucose, 20 mM-Hepes, 0.1% BSA, 1 mM-MgCl₂ and CaCl₂ as indicated in the Figure legends, at 37 °C in the presence of 4 μ Ci of [³H]serine/ml and effectors (for concentrations, see the Figure legends). After an incubation period of 0–2 h the cells were rapidly sedimented in an Eppendorf centrifuge, the supernatants were discarded and the cell phospholipids were extracted with chloroform/methanol (Bligh & Dyer, 1959). The lipid extracts were analysed by t.l.c. in the solvent system chloroform/methanol/acetic acid/water (25:15:4:1, by vol.). Authentic phospholipid standards (Sigma) were run in parallel and were detected with iodine vapours. Radioactivity in lipid spots was determined with an automatic linear radiochromatography analyser (Berthold).

PtdSer synthesis in permeabilized cells

For permeabilization, the cells were washed and then maintained in the presence of saponin (50 μ g/ml) for 5 min at 37 °C in a medium containing 140 mM-KCl, 10 mM-NaCl, 2.5 mM-MgCl₂, 0.1 μ M-CaCl₂ and 20 mM-Hepes (pH 7.4). The efficiency of permeabilization was verified with Trypan Blue. The cells were then harvested and maintained in the same buffer (without saponin) in the presence of 4 μ Ci of [³H]serine (Amersham)/ml and different effectors (for concentrations see the Figure legends) for different periods of time. In order to maintain the ATP pool, the buffer was supplemented with 2 mM-ATP, 5 mM-phosphocreatine and 1 unit of creatine kinase as described by Voelker (1990). At the end of the incubation period, phospholipids were extracted and analysed as described above.

Measurements of changes in Ca²⁺

Cytosolic Ca²⁺ was assayed with Indo-1 (Calbiochem) (Grynkiewicz *et al.*, 1985). Cells (5×10^6 /ml) were loaded with 5 μ M-Indo-1 at 37 °C in the dark for 1 h, then washed and resuspended in medium containing the different effectors. The analyses were performed on a fluorescence-activated cell sorter (FACStar Plus; Becton Dickinson). The fluorescence intensity at 480 nm, corresponding to the free Indo-1 concentration, as well as the fluorescence at 400 nm, corresponding to the complex Ca²⁺-Indo-1, were measured. The ratio fluorescence at 400 nm/fluorescence at 480 nm allows the evaluation of changes in cytosolic free Ca²⁺ concentration, independently of the cell size and the intracellular Indo-1 concentration.

RESULTS

Ca²⁺ ionophores decreased PtdSer synthesis in cells maintained at low extracellular Ca²⁺ concentration

Activation of Jurkat T-cells with lectins, mAbs directed against CD3 or Ca²⁺ ionophores is accompanied by an increase in cytoplasmic free Ca²⁺ concentration, due both to release of Ca²⁺ from intracellular stores and to Ca²⁺ influx. The Ca²⁺ influx could be easily suppressed, either by adding EGTA to the culture medium or by using buffers containing less than 10 μ M-Ca²⁺. These low free Ca²⁺ concentrations were established in EGTA-containing medium in accordance with Bers (1982). In these experimental conditions, T-cell activators, including the Ca²⁺ ionophores (A23187 or ionomycin), remain able to mobilize Ca²⁺ from intracellular stores (Fig. 1). In order to eliminate the eventual participation of Ca²⁺ influx when measuring PtdSer synthesis, we have studied the incorporation of [³H]serine into PtdSer in buffers containing decreasing amounts of Ca²⁺. The results obtained demonstrated that PtdSer synthesis decreased slowly when extracellular Ca²⁺ concentration was lowered, then

remained stable at Ca²⁺ concentrations below 10 μ M (results not shown). Addition of Ca²⁺ ionophores (either A23187 or ionomycin at 0.1 μ M) under these experimental conditions (1 or 0.1 μ M-Ca²⁺) markedly decreased PtdSer synthesis (Table 1), confirming previous results that demonstrated firstly that the Ca²⁺ influx generated by both T-cell activators and Ca²⁺ ionophores was not linked to the inhibition of PtdSer (Aussel *et al.*, 1991) and secondly that the release of Ca²⁺ from intracellular stores was accompanied by a decreased incorporation of [³H]serine into PtdSer.

PtdSer synthesis in permeabilized cells

In the following experiments, and in order to confirm the participation of release of Ca²⁺ from intracellular compartments in inhibition of PtdSer synthesis, we used saponin-permeabilized cells maintained in a low-Ca²⁺-containing medium. Under these experimental conditions, the changes observed in PtdSer metabolism would be independent both of changes in cytosolic free Ca²⁺ concentration and of an eventual effect on the uptake of [³H]serine by the cells.

As shown in Fig. 2, addition of EGTA (0.5 mM) totally abrogated PtdSer synthesis, a fact in agreement with the need for Ca²⁺ to obtain a full activation of the base-exchange enzyme system responsible for the synthesis of PtdSer. Upon addition of Ca²⁺ (1 mM) in the incubation medium, PtdSer synthesis was recovered. These results were quite compatible with the fact that EGTA, by complexing Ca²⁺, inhibited the base-exchange enzyme system, and that by-passing the buffering capacity of EGTA by addition of Ca²⁺ activated the enzyme. This experiment could be interpreted as follows: in the presence of EGTA, Ca²⁺

Fig. 1. Changes in cytosolic Ca²⁺ induced by 0.1 μ M-ionomycin in Jurkat T-cells

Cells were loaded with 5 μ M-Indo-1, washed, and then maintained in buffers containing decreasing amounts of Ca²⁺ (○, 3 mM; ●, 1 mM; □, 1 μ M; ■, 0.1 μ M) and treated at zero time with Ca²⁺ ionophore. The fluorescence ratio 400 nm/480 nm was monitored by cytofluorimetry.

Table 1. Effect of Ca²⁺ ionophores on PtdSer synthesis in Jurkat T-cell

PtdSer synthesis in Jurkat T-cells maintained in buffer containing either 1 or 0.1 μ M-Ca²⁺. The effects of A23187 (0.1 μ M) and ionomycin (0.1 μ M) are shown. Results (means $\pm$ S.D., $n = 6$) are expressed as c.p.m. of [³H]serine incorporated into PtdSer during an incubation period of 1 h and measured after phospholipid analysis by t.l.c.

[Ca ²⁺]	Control	A23187	Ionomycin
1 μ M	334 $\pm$ 24	183 $\pm$ 12	163 $\pm$ 15
0.1 μ M	364 $\pm$ 27	146 $\pm$ 10	155 $\pm$ 16

Fig. 2. PtdSer synthesis in permeabilized Jurkat T-cells

The effect of EGTA (0.5 mM) is shown as a function of time: ●, control cells; ○, EGTA-treated cells. At 60 min, 1 mM- Ca^{2+} was added to the incubation medium and PtdSer synthesis was monitored for a further 1 h (□). Results are expressed as c.p.m. (means $\pm$ S.D.). Other details were as in Table 1.

Fig. 3. Effect of A23187 and ionomycin on permeabilized cells

Jurkat T-cells were permeabilized with saponin as described in the Materials and methods section, then treated with either 0.1 μM -A23187 (○) or 0.1 μM -ionomycin (□) and incubated for various times. The incorporation of [^3H]serine into PtdSer in control (●) and ionophore-treated cells is shown as c.p.m. (means $\pm$ S.D., $n = 3$).

concentration (in the nM range) is low enough to deplete Ca^{2+} stores by impairing their refilling by Ca^{2+} -ATPase, whereas bypassing the buffering capacity of EGTA allows refilling of Ca^{2+} stores. In cells permeabilized in low- Ca^{2+} -containing buffer (below 1 μM - Ca^{2+}), Ca^{2+} ionophores act by emptying intracellular stores (see Fig. 1). As shown in Fig. 3, both A23187 and ionomycin at 0.1 μM strongly decreased PtdSer synthesis, suggesting that intracellular store depletion is responsible for the inhibition of PtdSer synthesis.

Effect of TG and DTBuHQ on PtdSer synthesis

It has been previously demonstrated (Gouy *et al.*, 1990; Sarkadi *et al.*, 1991) that TG and DTBuHQ by blocking the Ca^{2+} -translocase of the sarcoplasmic reticulum are able to release Ca^{2+} from intracellular stores in Jurkat T-cells. In order to confirm that depletion of intracellular Ca^{2+} stores might be responsible for the inhibition of PtdSer synthesis, we have studied the effects of these two Ca^{2+} -translocase inhibitors. It was found that both TG and DTBuHQ at concentrations of 1 μM and 20 μM respectively strongly inhibited PtdSer synthesis in permeabilized Jurkat T-cells (Fig. 4).

Effect of InsP_3 on PtdSer synthesis in permeabilized cells

Since it is generally acknowledged that the second messenger

Fig. 4. Effect of Ca^{2+} , Mg^{2+} -ATPase inhibitors on PtdSer synthesis

Jurkat T-cells were permeabilized, and then incubated as a function of time in the absence (○, control) or presence of either 1 μM -TG (■) or 20 μM -DTbHQ (●). Other details are given in the Materials and methods section.

InsP_3 generated from the hydrolysis of phosphatidylinositides participates in the release of Ca^{2+} from intracellular stores, we have studied the effect of InsP_3 on PtdSer synthesis in permeabilized Jurkat cells. As shown in Fig. 5, InsP_3 diminished PtdSer synthesis, but was found to be much less efficient than Ca^{2+} ionophores or Ca^{2+} -translocase inhibitors.

Status of Ca^{2+} stores after CD3-induced T-cell activation

It is well known that CD3 mAbs, as well as other T-cell activators, induce Ca^{2+} release from intracellular stores and that this release culminates in less than 2 min. Measurement of PtdSer synthesis, for technical reasons such as moderate sensitivity and the need for [^3H]serine to enter the cells before its utilization as substrate for PtdSer synthesis, could be monitored only after a 15 min period. In addition, we have previously shown that inhibition of PtdSer synthesis was maintained for hours. It was thus decided to study the state of Ca^{2+} stores after CD3 activation of Jurkat cells. This was done with the following experiments. Cells were treated with CD3 mAb in the presence of 1 mM- Ca^{2+} in the medium. Then, at different periods of time ranging from 0 to 1 h, EGTA (2 mM) was added to the cell suspension and 0.1 μM -ionomycin was immediately added in order to measure the amount of Ca^{2+} still releasable from the intracellular stores. As shown in Fig. 6, at 15, 30 or 60 min after CD3 activation, ionomycin released a very small amount of Ca^{2+} , indicating that

Fig. 5. Effect of InsP_3 (10 μM) on PtdSer synthesis in saponin-permeabilized Jurkat cells

Experimental details were as in Fig. 4. ○, Control cells; ●, InsP_3 -treated cells. Results are expressed as c.p.m. (means $\pm$ S.D., $n = 6$) from two experiments done in triplicate.

Fig. 6. Measurement of Ca^{2+} releasable from intracellular stores in controls ($t = 0$, ∇) and at 15, 30 or 60 min after CD3 treatment

Cells were treated with $1 \mu\text{g}$ of CD3 mAb/ml in medium containing 1 mM - Ca^{2+} , and then after 15 min ($\square$), 30 min ($\blacksquare$) or 60 min ($\circ$) external Ca^{2+} was chelated with 2 mM -EGTA, and the cells were immediately treated with ionomycin ($0.1 \mu\text{M}$) in order to measure Ca^{2+} remaining in intracellular stores.

Fig. 7. Changes in cytosolic Ca^{2+} induced by $0.1 \mu\text{M}$ -TG in Jurkat T-cells maintained either in 1 mM - Ca^{2+} ($+\text{Ca}^{2+}$) or in the presence of 2 mM -EGTA added at zero time ($-\text{Ca}^{2+}$)

The fluorescence ratio $400 \text{ nm}/480 \text{ nm}$ of Indo-1-loaded cells was monitored as described in the Materials and methods section.

CD3 mAbs not only released Ca^{2+} from intracellular compartments but also prevented refilling of the stores with Ca^{2+} . This probably explains why PtdSer synthesis remains inhibited for a long period of time after T-cell activation.

Effect of TG on intact Jurkat cells

It has been previously shown that TG, the blocker of Ca^{2+} -ATPase, induces in T-cells both a release of Ca^{2+} from intracellular stores and a Ca^{2+} influx (Gouy *et al.*, 1990). In the presence of TG, Ca^{2+} stores are believed to be empty, first because Ca^{2+} -ATPase is blocked by the drug and secondly, since it has been demonstrated that after TG treatment of T-cells, CD3 mAb as well as CD2 mAbs were unable to induce Ca^{2+} release from intracellular stores (Gouy *et al.*, 1990). This indicates that TG and CD3 mAbs mobilize Ca^{2+} from a common intracellular pool. In our Jurkat T-cell clone, TG induced similar effects (Fig. 7), i.e. a release of Ca^{2+} from intracellular compartments and a Ca^{2+} influx. Measurements of PtdSer synthesis in TG-treated Jurkat cells indicated that [^3H]serine incorporation into PtdSer was strongly inhibited (75% inhibition at $0.1 \mu\text{M}$ -TG).

DISCUSSION

T-cell activation obtained by triggering the T-cell receptor complex with CD3 mAbs involves the stimulation of phospholipase C activity. Possibly phospholipase Cyl

phosphorylated on a tyrosine residue (Mustelin *et al.*, 1990; Weiss *et al.*, 1991; Granja *et al.*, 1991) is responsible for the generation of the two second messengers, diacylglycerol and InsP_3 . InsP_3 has been shown to open Ca^{2+} channels on the endoplasmic reticulum in a number of cells, and thus has been suggested to be responsible for the release of Ca^{2+} . Besides InsP_3 , other mechanisms probably occur; for example, we have shown that phosphatidic acid was also able to release Ca^{2+} from intracellular stores in Jurkat T-cells (Breittmayer *et al.*, 1991). We have previously shown that T-cell activation is accompanied by a marked decrease in PtdSer synthesis in the absence of significant changes in the uptake of [^3H]serine (Pelassy *et al.*, 1989). In Jurkat cells, as in other mammalian cells, PtdSer synthesis occurs by an exchange of serine with another phospholipid polar head group such as choline or ethanolamine in pre-existing phospholipids (Kanfer, 1989). This biosynthetic route is a Ca^{2+} -dependent process (Bjerve, 1973b; Gaiti *et al.*, 1974). It was thus of interest to study how PtdSer is regulated during T-cell activation, since lymphocyte activators were known to induce a rise in cytosolic Ca^{2+} concentration. A previous report by our group (Aussel *et al.*, 1991) had shown that impairing Ca^{2+} influx in Jurkat cells does not reverse the inhibition of PtdSer synthesis, whereas blocking the release of Ca^{2+} from intracellular stores maintained PtdSer synthesis to a level comparable with that of non-activated cells. In the present paper, we have used a different approach. First, we used very low extracellular Ca^{2+} concentration, in order to avoid a Ca^{2+} influx even when the cells were treated with Ca^{2+} ionophores; secondly, we have done experiments in saponin-permeabilized cells in order to avoid both Ca^{2+} influx and eventual changes in the uptake of [^3H]serine. The results obtained clearly showed that (1) A23187 and ionomycin were able to induce inhibition of PtdSer synthesis, (2) that depleting Ca^{2+} stores with EGTA impaired PtdSer synthesis, whereas refilling the stores with Ca^{2+} restored the synthesis of this phospholipid, and (3) depleting Ca^{2+} stores with TG or DTBuHQ, two inhibitors of the endoplasmic-reticulum Ca^{2+} translocase, also inhibited PtdSer synthesis. The use of InsP_3 , which is generally acknowledged to be responsible for the opening of Ca^{2+} channels on the endoplasmic-reticulum membranes, also caused a noticeable inhibition of PtdSer synthesis, although much less than that caused by A23187, ionomycin, TG or DTBuHQ. This is probably due to the fact that InsP_3 -induced Ca^{2+} release is partial, whereas the other drugs induced a total release of sequestered Ca^{2+} . A similar phenomenon has been previously described by Hirata *et al.* (1984), using saponin-permeabilized macrophages treated with similar amounts of either InsP_3 or A23187.

We have previously shown that, after CD3 treatment of Jurkat cells, PtdSer synthesis remained inhibited for at least 1 h. If this long-lasting inhibition was due to the release of Ca^{2+} from intracellular stores, as hypothesized in the present work, it was of interest to study the status of Ca^{2+} stores after CD3 treatment. This was done in the experiment described in Fig. 6. In this experiment, CD3-treated cells were maintained in a medium containing 1 mM - Ca^{2+} for 15, 30 or 60 min, i.e. a time sufficient to allow refilling of the stores provided that this process occurs after CD3 treatment. As shown in Fig. 6, ionomycin was unable to induce a Ca^{2+} release after CD3 treatment, whereas under the same experimental conditions (but in the absence of CD3, i.e. at time 0) this ionophore is quite able to induce a large release of Ca^{2+} from intracellular stores. A possible artefact induced by the presence of EGTA added 20 s before ionomycin appears unlikely, since EGTA was also added at a zero time and served as a control for this experiment. In addition, it is well known that EGTA, when used to empty intracellular Ca^{2+} stores in intact cells, must be used in the cold to impair refilling of the stores by

Ca^{2+} -ATPase, as this enzyme is inhibited at low temperature. Accordingly, we suggest that PtdSer synthesis remains inhibited, because CD3 not only causes a release of Ca^{2+} from intracellular compartments but also prevents refilling of the stores with Ca^{2+} . In addition, TG when used in intact Jurkat cells induces changes in cytoplasmic Ca^{2+} very similar to those observed in the presence of CD3, i.e. a release of Ca^{2+} from intracellular stores and a Ca^{2+} influx. TG is also able to decrease PtdSer synthesis markedly in intact Jurkat cells maintained in medium containing 1 mM- Ca^{2+} . Since TG blocks Ca^{2+} -ATPase and thus is believed to impair refilling of Ca^{2+} stores, it appears that the inhibition of PtdSer synthesis is mainly the result of empty Ca^{2+} stores. This is in good agreement with the previous work by Gouy *et al.* (1990), demonstrating that, after CD3 activation of T-cells, TG is unable to mobilize Ca^{2+} from intracellular compartments and that, after a first treatment with TG, CD3 does not mobilize Ca^{2+} , indicating that the same intracellular pool Ca^{2+} is affected by both CD3 and TG. As demonstrated by Sarkadi *et al.* (1991), ionomycin also mobilizes Ca^{2+} from the same intracellular pool. Inhibition of PtdSer synthesis as the result of emptying intracellular Ca^{2+} -storage organelles might be a special property of T-cells, since in other cells, and particularly in excitable cells, Ca^{2+} stores appear to be refilled with Ca^{2+} after activation, because the cells can be re-activated a short time after a first exposure to an effector (Vigne *et al.*, 1988). This is not true with T-cells, which after CD3 activation become refractory to re-activation with either CD3 or CD2 mAbs (Pantaleo *et al.*, 1987).

In previous papers (Aussel *et al.*, 1990; Pelassy *et al.*, 1992), we have shown that cationic drugs such as K^{+} -channel blockers were able to induce a strong PtdSer synthesis in both Jurkat and THP1 cells. These drugs do not change Ca^{2+} movements in Jurkat cells (C. Pelassy, J.-P. Breittmayer & C. Aussel, unpublished work), and thus are believed to act on PtdSer synthesis by a distinct and at present uncharacterized mechanism.

Studies on the subcellular distribution of the base-exchange enzyme system in rat liver had led to the conclusion that the enzyme(s) is/are localized in the microsomal fraction (Bjerve, 1973a). Similar results were obtained with rat brain (Buchanan & Kanfer, 1980). Further studies have concluded that the choline base-exchange activity is localized on the outer surface of rat brain microsomal membranes (Arienti *et al.*, 1985), whereas the enzymes responsible for serine and ethanolamine base exchange were preferentially located on the luminal side of the microsomes. More recent results (Baranska, 1989) suggested that the serine base-exchange reaction occurs at the luminal leaflet of the endoplasmic reticulum and would be mainly regulated by the activity of Ca^{2+} , Mg^{2+} -ATPase, which maintains high Ca^{2+} levels (in the mM range, compared with 0.1–0.2 μM in the cytosol) in the endoplasmic reticulum of liver cells and CHO-K1 cells. Our results, based on the use of Ca^{2+} ionophores and inhibitors of Ca^{2+} sequestration, strongly suggest that in Jurkat T-lymphocytes

PtdSer synthesis is regulated by the level of Ca^{2+} in the intracellular Ca^{2+} stores, and that the serine base-exchange enzyme system is located within or close to the intracellular Ca^{2+} stores.

REFERENCES

- Arienti, G., Corazzi, L., Freysz, L., Binaglia, L., Roberti, R. & Porcellati, G. (1985) *J. Neurochem.* **44**, 38–41
- Aussel, C., Pelassy, C., Mary, D., Choquet, D. & Rossi, B. (1990) *Immunopharmacology* **20**, 97–103
- Aussel, C., Pelassy, C., Mary, D., Breittmayer, J. P., Cousin, J. L. & Rossi, B. (1991) *J. Lipid Mediators* **3**, 267–282
- Baranska, J. (1989) *FEBS Lett.* **256**, 33–37
- Bers, D. M. (1982) *Am. J. Physiol.* **242**, C404–C408
- Bjerve, K. S. (1973a) *Biochim. Biophys. Acta* **296**, 549–562
- Bjerve, K. S. (1973b) *Biochim. Biophys. Acta* **306**, 396–402
- Bligh, E. G. & Dyer, W. J. (1959) *Can. J. Biochem.* **37**, 911–916
- Breittmayer, J. P., Aussel, C., Farahifar, D., Cousin, J. L. & Fehlmann, M. (1991) *Immunology* **73**, 134–139
- Buchanan, A. G. & Kanfer, J. N. (1980) *J. Neurochem.* **34**, 720–725
- Gaiti, A., De Medio, E., Brunetti, M., Amaducci, L. & Porcellati, G. (1974) *J. Neurochem.* **23**, 1153–1159
- Gouy, H., Cefai, D., Christensen, S., Debré, P. & Bismuth, G. (1990) *Eur. J. Immunol.* **20**, 2269–2275
- Granja, C., Lin, L. L., Yunis, E. J., Relias, V. & Dasgupta, J. D. (1991) *J. Biol. Chem.* **266**, 16277–16280
- Gryniewicz, G., Poenie, M. & Tsien, R. (1985) *J. Biol. Chem.* **260**, 3440–3446
- Hirata, M., Suematsu, E., Hashimoto, T., Hamachi, T. & Koga, T. (1984) *Biochem. J.* **223**, 229–236
- Imboden, J. & Stobo, J. (1985) *J. Exp. Med.* **161**, 446–456
- Kanfer, J. (1972) *J. Lipid Res.* **13**, 468–476
- Kanfer, J. (1989) in *Phosphatidylcholine Metabolism* (Vance, D. E., ed.), pp. 65–86, CRC Press, Boca Raton, FL
- Llopis, J., Chow, S. B., Kass, G. E. N., Gham, A. & Orrenius, S. (1991) *Biochem. J.* **277**, 553–556
- Mason, M. J., Garcia-Rodriguez, C. & Grinstein, S. (1991) *J. Biol. Chem.* **266**, 20856–20862
- Miura, T. & Kanfer, J. (1976) *Arch. Biochem. Biophys.* **175**, 654–660
- Mustelin, T., Coggeshall, K. M., Isakov, N. & Altman, A. (1990) *Science* **247**, 1584–1587
- Pantaleo, G., Olive, D., Poggi, A., Kosumbo, W. J., Moretta, L. & Moretta, A. (1987) *Eur. J. Immunol.* **17**, 55–60
- Pelassy, C., Aussel, C. & Fehlmann, M. (1989) *Cell. Signalling* **1**, 99–105
- Pelassy, C., Cattani, N. & Aussel, C. (1992) *Biochem. J.* **282**, 443–446
- Rasmussen, U., Christensen, S. B. & Sanberg, F. (1978) *Acta Pharm. Suec.* **15**, 133–140
- Sarkadi, B., Tordai, A., Homolya, L., Scharff, O. & Gardos, G. (1991) *J. Membr. Biol.* **123**, 9–21
- Takemura, H., Hugues, A. R., Thastrup, O. & Putney, J., Jr. (1989) *J. Biol. Chem.* **264**, 12266–12271
- Vigne, P., Breittmayer, J. P., Lazdunski, M. & Frelin, C. (1988) *Eur. J. Biochem.* **176**, 47–52
- Voelker, D. R. (1990) *J. Biol. Chem.* **265**, 14340–14346
- Weiss, A., Imboden, J., Hardy, K., Manger, B., Terhorst, C. & Stobo, J. (1986) *Annu. Rev. Immunol.* **4**, 593–603
- Weiss, A., Koretzky, G., Schatzman, R. C. & Kadlecsek, T. (1991) *Proc. Natl. Acad. Sci. U.S.A.* **88**, 5484–5488

Received 6 April 1992/18 June 1992; accepted 25 June 1992