Acta Biochimica Polonica

75th Anniversary of the M. Nencki Institute of Experimental Biology

Vol. 40 No. 3/1993

QUARTERLY

Minireview

Thapsigargin: potent inhibitor of Ca^{2+} transport ATP-ases of endoplasmic and sarcoplasmic reticulum*

Paweł Sabała, Małgorzata Czarny, Jan Paweł Woronczak and Jolanta Barańska

Department of Cellular Biochemistry, M. Nencki Institute of Experimental Biology, L. Pasteura 3, 02–093 Warsaw, Poland

Received 17 May, 1993

GENERAL CHARACTERISTICS

Thapsigargin and thapsigargicin are two major active components found in root extracts of the umbelliferous (*Apiaceae*) plant *Thapsia garganica* L. This root extract has been known from ancient times for its skin irritation effect and was used for treatment of muscles and joint inflammations. Rasmussen *et al.* [1] isolated these two compounds and showed that thapsigargin and thapsigargicin release histamine in rat mast cells. Thapsigargin, the most abundant of the two compounds, is a sesquiterpene lactone whose structure has been studied in detail by NMR, circular dichroism spectroscopy and crystallographic methods [2 - 5] (Fig. 1). Thapsigargin is known to promote tumorigenesis in mouse skin [6] independently of protein kinase C activation or protein phosphatases inhibition [7, 8]. Most recently, Wong *et al.* [9] have shown that thapsigargin-induced tumorigenesis can be caused by rapid inhibition of mRNA translation, protein processing and gene expression. Thapsigargin is a neurotoxin in perinatal rodent brain causing irreversible brain injury [10], and totally blocks the induction of long-term potentiation in rat hippocampal slices [11]. It has been reported that thapsigargin induces acute responses in a large variety of cell types.

Thapsigargin-induced cellular activation appears in all cases to be initiated by a single common event: a rapid and pronounced increase in the concentration of cytosolic free Ca^{2+} that occurs *via* a direct discharge of intracellular $InsP_3$-sensitive Ca^{2+} stores without hydrolysis of inositolphospholipids [8, 12 - 14]. Since this discovery, the interest in thapsigargin studies has increased, especially when it was established that thapsigargin is a high affinity inhibitor of all the endoplasmic reticulum Ca^{2+} transport ATPases tested [15 - 18]. Thus, for its abilities of identification and selective interference with intracellular Ca^{2+} pools, thapsigargin became a very convenient experimental tool.

ACTION OF THAPSIGARGIN ON Ca^{2+} TRANSPORT ATPases

In 1987 Thastrup *et al.* [12, 13] found that thapsigargin produced elevation of intracellular calcium concentration by emptying $InsP_3$-sensitive stores without generation of phosphoinositides. Three years later Thastrup and coworkers [19] revealed that this effect was due to the inhibition of calcium uptake into the

*This work was supported by the State Committee for Scientific Research by a Statutable grant to the Nencki Institute and grant No. 406109101 to the authors

[1]Abbreviations: SERCA, sarcoplasmic and endoplasmic reticulum calcium ATPases; $InsP_3$, inositol 1,4,5-trisphosphate

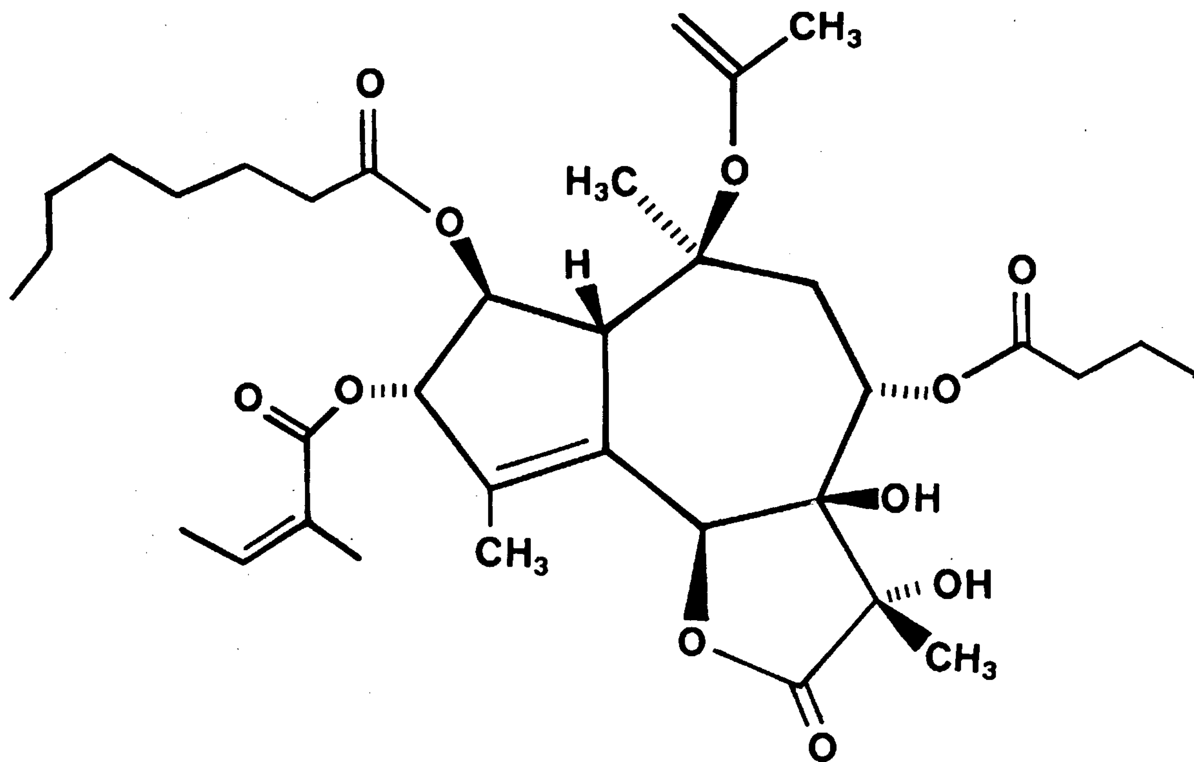


Fig. 1. Structure of thapsigargin.

endoplasmic reticulum by blocking its ATP-dependent calcium pump. In 1991 Lytton *et al.* [17] showed that thapsigargin acted on all sarcoplasmic and endoplasmic reticulum Ca^{2+} transport ATPases. The mechanism of this inhibition was explained by Sagara & Inesi [15] in the same year.

SERCA ATPases

Ca^{2+} is loaded into the endoplasmic reticulum by means of intracellular adenosine triphosphate (ATP)-dependent Ca^{2+} pumps of the highly homologous SERCA[1] (sarcoplasmic or endoplasmic reticulum Ca^{2+}-ATPase) family. Recently three mammalian SERCA genes (SERCA-1, SERCA-2 and SERCA-3) have been identified. It has been found that transcripts of SERCA-1 and SERCA-2 exist in two alternatively spliced forms [20], which produce at least five different protein products. The calcium dependent ATPase whose activity is essential for the Ca^{2+} pump's function is a 110 kDa polypeptide chain placed asymmetrically across the endoplasmic reticulum membrane with a large extramembranous region on the cytosolic side [21, 22]. The enzyme binds two Ca^{2+} ions and ATP from cytosol and releases the bound Ca^{2+} into the lumen of the endoplasmic reticulum by utilization of ATP. Calcium binding to the high affinity sites of the ATPase promotes ATP binding and hydrolysis, the enzyme is phosphorylated and a series of conformational changes causes Ca^{2+} translocation to the luminal side of the endoplasmic reticulum [23, 24]. The mechanism of action of the Ca^{2+}-ATPase may be described according to a model with two major states: E_1 and E_2, shown in Fig. 2.

As shown in the schematic representation of the endoplasmic reticulum Ca^{2+}-ATPase (Fig. 3) the phosphorylation domain (Asp 351) resides in the extramembranous region of the enzyme and the Ca^{2+} binding domain resides within the transmembrane region and consists of four helices [22]. It is likely that four helices form a channel for Ca^{2+} which is controlled by the phosphorylation site [24].

Mechanism of action

The inhibitory effect of thapsigargin on Ca^{2+}-ATPase can be observed at very low, subnanomolar concentrations [15] and at various isoforms of SERCA pumps [16,17]. The plasma membrane ATPases and Na^+/K^+ ATPases are not inhibited [17]. The dead-end thapsigargin*ATPase complex is formed when the

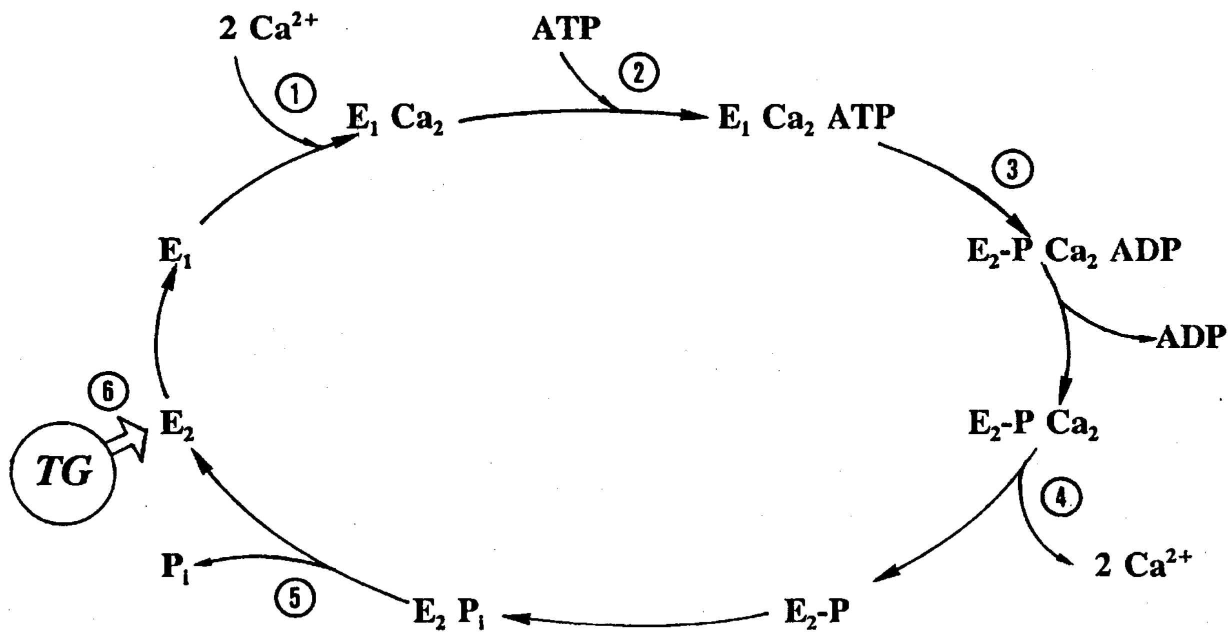


Fig. 2. Calcium pumping cycle of sarcoplasmic and endoplasmic reticulum ATPase.
The binding of the two Ca^{2+} from the cytosol (1) to the high affinity sites of Ca^{2+}-ATPase (E_1 state) is strictly required for starting its pumping action. When these sites are occupied ATP binds to the enzyme (2) which is then phosphorylated by a transfer of ATP terminal phosphate (3). The series of conformational changes in the enzyme leads to a translocation of bound Ca^{2+} and a 3-order magnitude reduction in affinity for these ions. Finally, calcium dissociates to the luminal spaces of the endoplasmic reticulum (4) and the phosphorylated intermediate is hydrolysed (5). All presented reactions are reversible. Thus, the defined sequence of chemical and vectorial reactions with utilization of free energy from ATP produces ion transition and reduction of the affinity for Ca^{2+}. The postulated inhibition of thapsigargin (TG) starts when the enzyme is in the E_2, Ca^{2+} free form (6). E_1 and E_2 correspond to different conformational forms of Ca^{2+}-ATPase. In fact there may be many more conformational changes in the cycle for calcium pumping by the enzyme [23, 80].

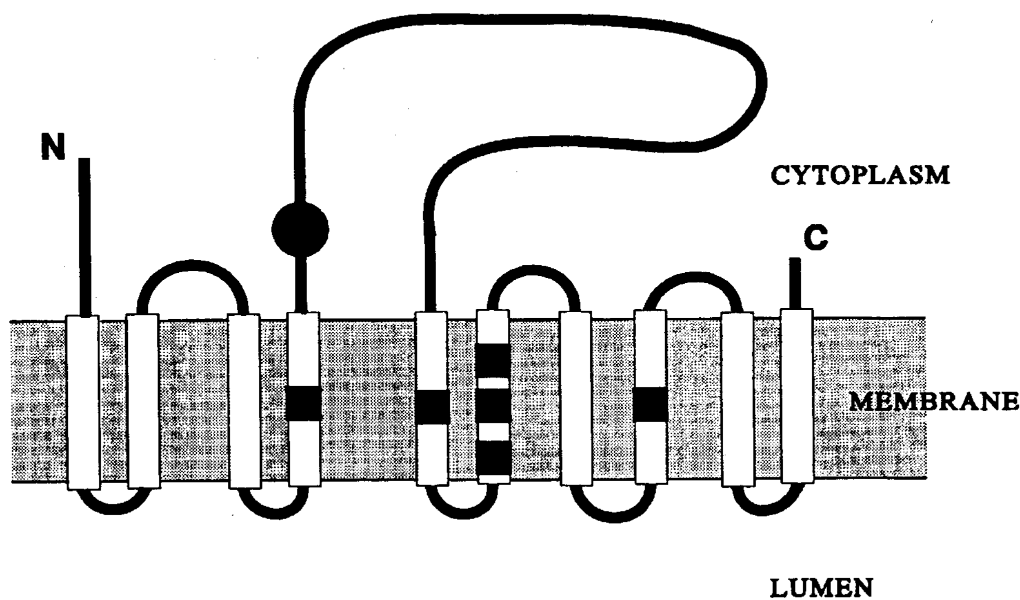


Fig. 3. Schematic representation of SERCA pump.
SERCA pump consists of ten transmembranous domains. Four of them (black squares) are involved in Ca^{2+} binding. The largest cytosolic fragment is responsible for ATP binding; the black circle represents the phosphorylation site (Asp 351).

enzyme is in a state without bound Ca^{2+}; it occurs when bound Ca^{2+} dissociates as a consequence of ATP utilization during the action of the pump. Thapsigargin produces a global influence on the enzyme resulting in a strong inhibition of Ca^{2+} binding and ATPase activity (including ATP binding and partial reactions in the absence of Ca^{2+}) [18, 21, 25]. Thapsigargin is less effective in blocking Ca^{2+} binding to the E_1 form of the enzyme, and higher concentrations of free Ca^{2+} have a protective effect on Ca^{2+} binding to the ATPase [18]. Under these conditions, the phosphorylated enzyme is formed even in the presence of an excess of thapsigargin until the enzyme reaches the E_2, state. In fact, Ca^{2+}-ATPase is susceptible to thapsigargin in its E_2, Ca^{2+}-free form [18, 21]. Thapsigargin acts with the enzyme stoichiometrically of 1 mol of inhibitor per mol of ATPase. Its interactions with E_2 produce a global inhibition of the Ca^{2+} transport ATPase activities which is manifested by total blocking of high affinity Ca^{2+} binding, loss of Ca^{2+} transport, ATP binding and phosphorylated enzyme formation [18, 21]. The reversed action of the ATPase cycle is also inhibited; in the presence of thapsigargin there is a very low (in favorable conditions) phosphorylated enzyme formation from P_i [21], and ADP-dependent Ca^{2+} efflux from loaded sarcoplasmic reticulum vesicles (with ATP synthesis) is prevented [15, 26].

Thus, thapsigargin is a specific and irreversible blocker of Ca^{2+}-ATPases in both muscle (skeletal [15, 18, 21], smooth [27, 28], cardiac [29 - 31]) and nonmuscle cells [17, 19, 32, 33]. The mechanism of the action of this drug on the Ca^{2+} pump protein of sarcoplasmic reticulum vesicles purified from rabbit skeletal muscle was, as mentioned above, the subject of studies of Sagara & Inesi [15]. Similar results of Ca^{2+}-ATPase blocking by thapsigargin in cardiac miocytes were achieved by Wrzosek *et al.* [29]. The effect of thapsigargin on sarcoplasmic reticulum Ca^{2+} content and contractions in single cardiac myocytes of guinea-pig heart was also reported by Lewartowski & Wolska [30]. They showed that thapsigargin completely blocked Ca^{2+} uptake by the sarcoplasmic reticulum of these cells. Despite of the sarcoplasmic reticulum depletion of Ca^{2+}, the amplitude of electrically stimulated contraction was decreased only by 30%, what suggests that in this type of cells not only sarcoplasmic reticulum, but also sarcolemmal Ca^{2+} may activate the contractile system. In vascular smooth muscle [28], thapsigargin inhibits sarcoplasmic reticulum Ca^{2+}-ATPase and stimulates calcium entry. Thapsigargin has also been shown to inhibit the endoplasmic reticulum Ca^{2+}-ATPase, to mobilize intracellular Ca^{2+}, and to activate Ca^{2+} entry in nonmuscle cells [14, 19]. It is generally accepted that calcium concentration in the endoplasmic reticulum might depend on two opposed events: active transport of Ca^{2+} by the ATPase and its passive leak from the endoplasmic reticulum [19]. Thapsigargin by blocking Ca^{2+} uptake may change the equilibrium towards leak from the endoplasmic and sarcoplasmic reticulum in nonmuscle and muscle cells, respectively. The nature of the Ca^{2+} leak remains unknown, but it appears to be widespread in eukaryotic cells. Depletion of internal stores is somehow coupled to the entry of extracellular calcium. All of these events lead to a distinct and sustained increase in intracellular Ca^{2+} levels in nonmuscle and smooth muscle cells, producing a variety of cellular responses.

THAPSIGARGIN AS A NEW MOLECULAR PROBE

Thapsigargin proved to be a valuable tool for the evaluation of many physiological, calcium-related cellular events, such as agonist and hormone activation, immunological response, muscle contraction-relaxation cycle, fertilization, secretion, phospholipid synthesis, intercellular communication and many others. Table 1 presents the areas of research where thapsigargin was engaged to elucidate mechanisms of various cellular events.

Thanks to the discovery and explanation of thapsigargin action on Ca^{2+}-ATPases it became clear that the sarcoplasmic and endoplasmic reticulum are the most important Ca^{2+} sequestering organelle in the cell. It was also possible to distinguish different calcium pools within this heterogeneous structure. Extracellular signals can trigger elevation of intracellular calcium concentration by inducing its influx from the extracellular space and/or by releasing Ca^{2+} from intracellular stores. It has been found that thapsigargin can mimic this action,

Table 1

Application of thapsigargin as a tool for elucidation of mechanisms of various cellular processes

Areas of research	References*
Calcium homeostasis and dynamics	
* identification and characterization of intracellular Ca^{2+} pools	[34 - 44]
* cytoplasmic Ca^{2+} signals after agonist or hormone stimulation	[45 - 52]
* calcium oscillations	[53 - 56]
Intercellular communication	[57]
Secretion	[58 - 62]
Calcium pumps	[21, 29, 63, 64]
Muscle contraction-relaxation cycle	[29 - 31, 65 - 67]
Phosphatidylserine synthesis	[68, 69]
Tumorigenesis	[9, 70 - 73]

* Literature data published in 1992 and 1993

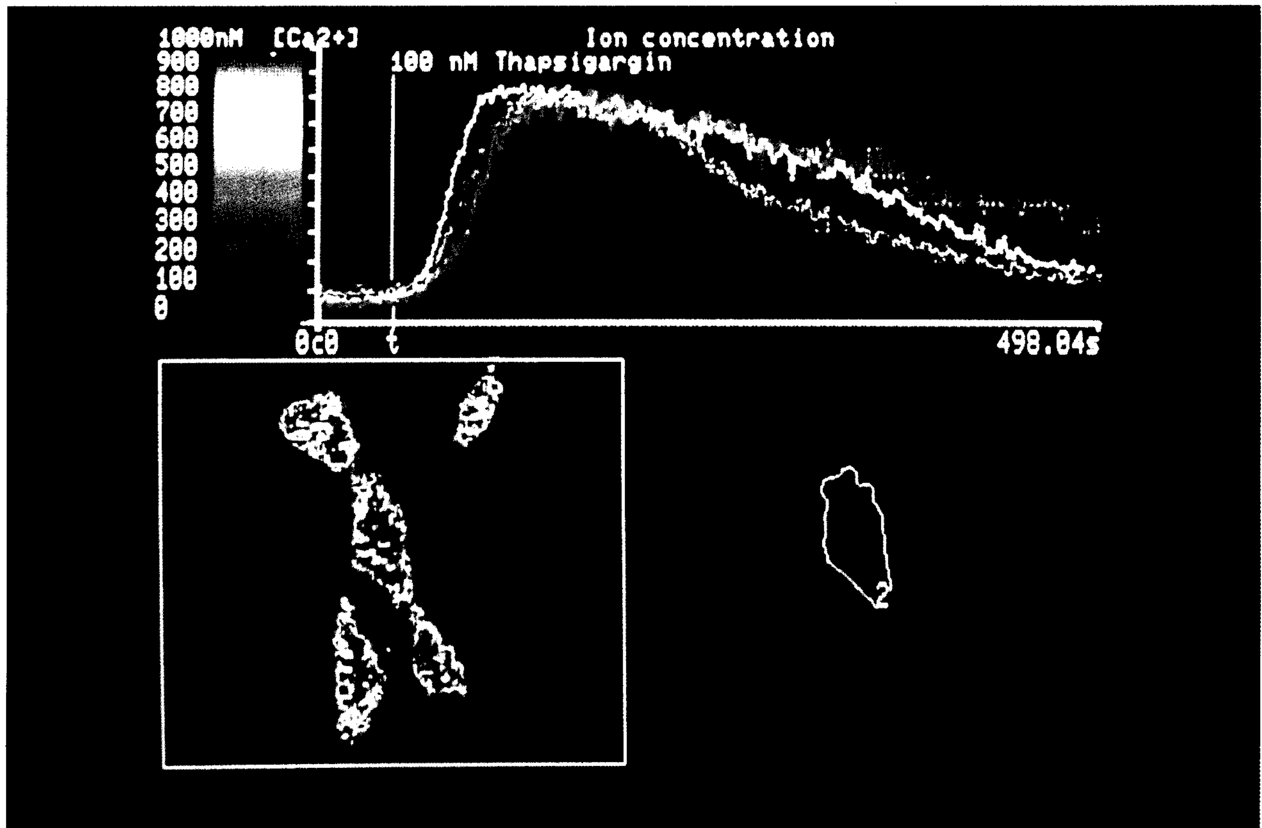
depleting intracellular $InsP_3$-sensitive and thapsigargin-sensitive calcium stores [27, 32].

In a variety of nonmuscle cells a relationship between release of Ca^{2+} from intracellular stores and increased entry of Ca^{2+} across the plasma membrane was observed [14, 33, 74]; the mechanism which triggers this calcium influx remains unknown but two possibilities have been suggested. Some authors believed that the increase of plasma membrane permeability was connected with the elevated Ca^{2+} concentration in the cytosol in response to emptying the endoplasmic stores [75, 76]. Others have proposed that the entry pathway of Ca^{2+} across the plasma membrane is responsive to the Ca^{2+} content of the stores [14, 77]. According to this hypothesis, concentration of the cytosolic Ca^{2+} is irrelevant to this process. In 1986, Putney [77] developed a "capacitative" model of Ca^{2+} entry and postulated that plasmalemmal permeability is controlled by the degree of filling of an intracellular Ca^{2+} storage compartment. Support for this hypothesis has come from experiments in which thapsigargin was used. Inhibition of Ca^{2+}-ATPase by thapsigargin produces the leak of calcium from the endoplasmic reticulum. It has been found that the depletion of these intracellular Ca^{2+} pools, independent of receptor activation, increases Ca^{2+} permeability of the plasma membrane and is responsible for the influx of Ca^{2+} into the cell.

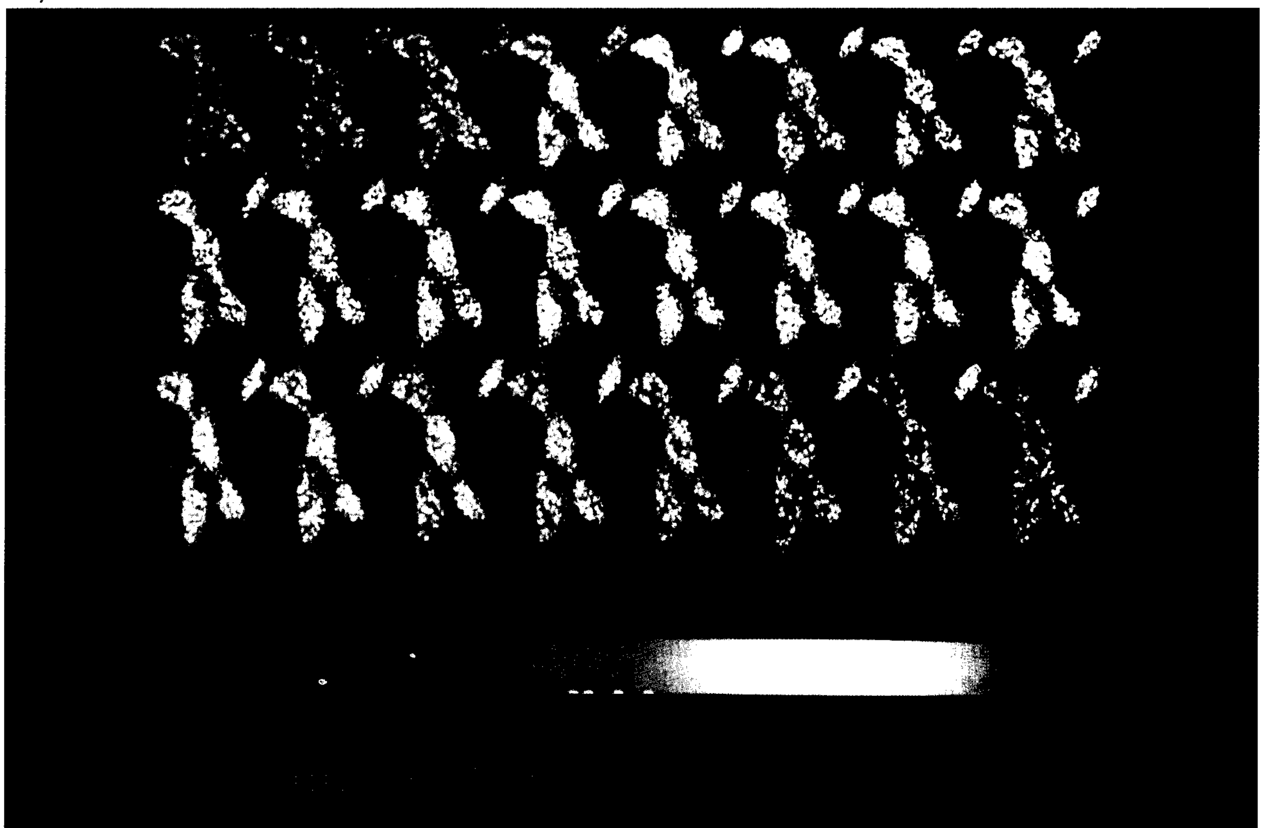
The introduction by Grynkiewicz *et al.* [78] of fluorescent dyes, whose excitation and emission properties are altered when they bind Ca^{2+}, has greatly enhanced the ability to monitor intracellular Ca^{2+} dynamics. The use of fluorescent probes and digital image analysis systems allows for monitoring of intracellular calcium levels in a single, living cell.

Figure 4 illustrates the effect of thapsigargin on intracellular calcium levels in intact glioma C6 cells [68]. In our study the measurements of changes in Ca^{2+} level in cytosol after the thapsigargin treatment were performed simultaneously with the determination of the effect of this drug on phosphatidylserine synthesis. We found [68] that thapsigargin, in addition to increasing Ca^{2+} concentration in the cytoplasm, strongly diminished phosphatidylserine synthesis. Since the synthesis of this phospholipid depends on Ca^{2+} and takes place in the endoplasmic reticulum [79], the inhibitory effect of thapsigargin on this process allowed us to conclude that phosphatidylserine synthesis occurs on the luminal side of this structure and has to be regulated by Ca^{2+}-ATPases. Similar results on phosphatidylserine synthesis in Jurkat T lymphocytes were recently presented by Pellasy *et al.* [69].

The broad spectrum of research in which thapsigargin is used as a molecular probe to elucidate various intracellular processes (Table 1) is connected with the universal role of Ca^{2+} as a second messenger. The fact that thapsigargin is a potent and specific inhibitor of Ca^{2+} transport ATPases in both the sarcoplasmic and endoplasmic reticulum revealed the similarity



(1)



(2)

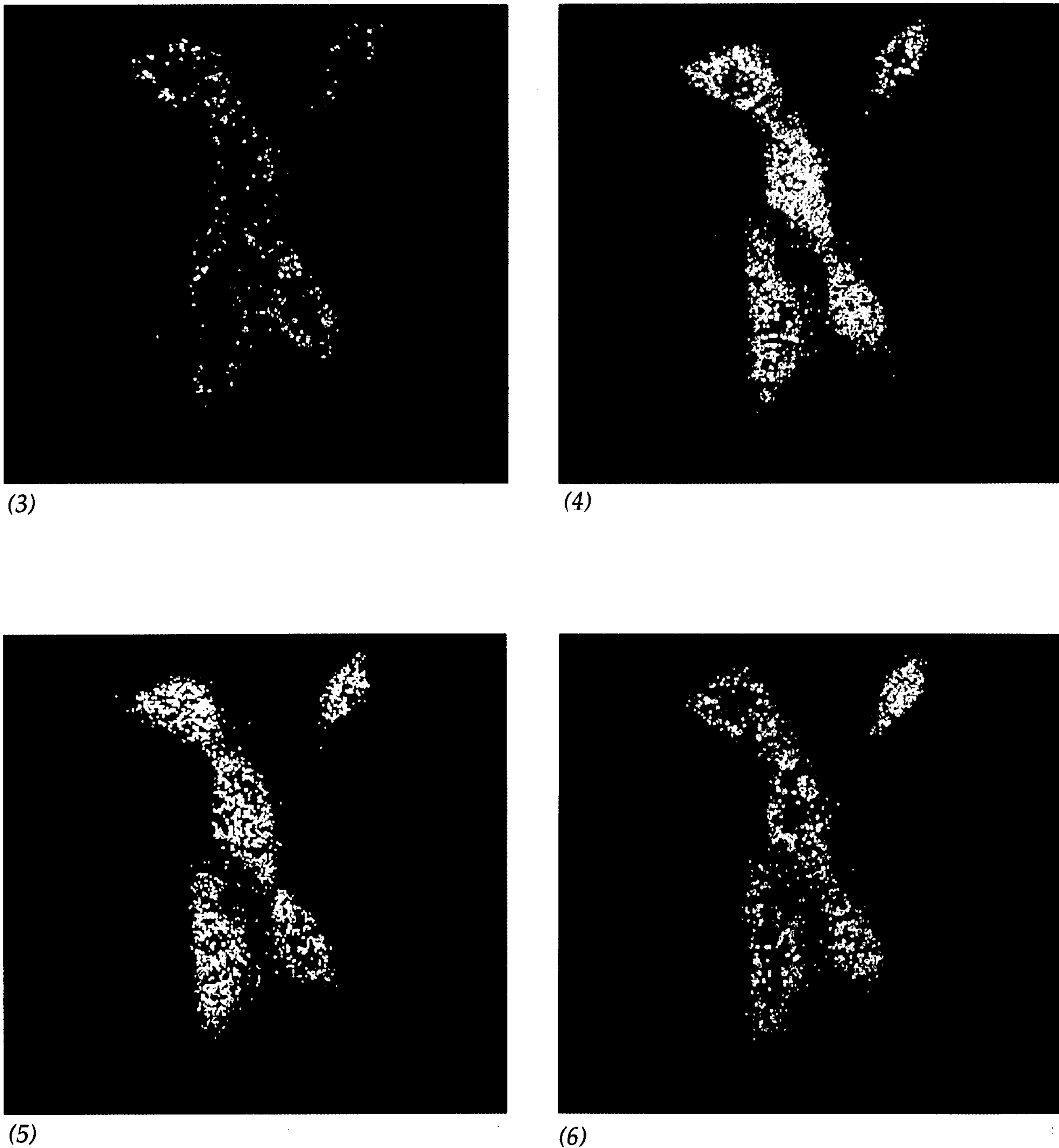


Fig. 4. Pseudo-color images illustrating the effect of thapsigargin on intracellular calcium levels in intact glioma C6 cells.

Images show the increase in intracellular calcium concentration after 100 nM thapsigargin treatment. The pseudo-color scale covering the 0 - 1000 nM range is shown. Cells were cultured on glass coverslips and loaded with the intracellular Ca^{2+} indicator Fura-2 during 30 min incubation with 1 μM of its acetoxymethyl ester. (1) Shows the action of thapsigargin on a single glioma C6 cell during 500 s. As it is seen, treatment of Fura-2 labelled cells by thapsigargin caused an increase in cytosolic Ca^{2+} and after a lag time period of 20 s a sustained $[Ca^{2+}]_i$ rise. This changing of the cytosolic calcium levels are represented by curves (1) and by the changing of pseudocolors cf. (2). (3) – (6) Represent Fura-2 loaded, thapsigargin treated cells after subsequent periods of time. All of these pictures originate from an experiment carried out on a Joyce-Loebl Ltd. computerizing image-processing system Magiscan for calcium analysis using Tardis V6.2.

of these organelles which serve as major intracellular Ca^{2+} stores in eukaryotic cells. In conclusion, the elucidation of the mechanism of thapsigargin action allows us to gain better insight into the Ca^{2+}-related molecular events in the cell.

REFERENCES

1. Rasmussen, U., Christensen, S.B. & Sandberg, F. (1978) Thapsigargin and thapsigargicin, two new histamine liberators from *Thapsia garganica* L. *Acta Pharm. Suec.* **15**, 133 - 140.
2. Christensen, S.B., Rasmussen, U. & Christophersen, C. (1980) Thapsigargin, constitution of a sesquiterpene lactone histamine liberator from *Thapsia garganica*. *Tetrahedron Lett.* **21**, 3829 - 3832.
3. Christensen, S.B., Larsen, I.K. & Rasmussen, U. (1982) Thapsigargin and thapsigargicin, two histamine liberating sesquiterpene lactones from *Thapsia garganica*. X-Ray analysis of the 7,11-epoxide of thapsigargin. *J. Org. Chem.* **47**, 649 - 652.
4. Christensen, S.B. & Norup, E. (1985) Absolute configuration of the histamine liberating sesquiterpene lactones thapsigargin and trilobolide. *Tetrahedron Lett.* **26**, 107 - 110.
5. Christensen, S.B. (1988) Interpretation of the NMR and circular dichroism data of sesquiterpene lactone thapsigargin. *Acta Chem. Scand. B* **42**, 623 - 628.
6. Hakii, H., Fujiki, H., Suganuma, M., Nakayasu, M., Tahira, T., Sugimura, T., Scheuer, P.J. & Christensen, S.B. (1986) Thapsigargin, a histamine secretagogue, is a non-12-*O*- tetradecanoylphorbol-13-acetate (TPA) type tumor promoter in two-stage mouse skin carcinogenesis. *J. Cancer Res. Clin. Oncol.* **111**, 177 - 181.
7. Fujiki, H., Suganuma, M., Nakayasu, M., Hakii, H., Horiuchi, T., Takayama, S. & Sugimura, T. (1986) Palytoxin is a non-12-*O*-tetradecanoylphorbol-13-acetate type tumor promoter in two-stage mouse skin carcinogenesis. *Carcinogenesis* **7**, 707 - 710.
8. Jackson, T.R., Patterson, S.I., Thastrup, O. & Hanley, M.R. (1988) A novel tumor promoter, thapsigargin, transiently increases cytoplasmic free Ca^{2+} without generation of inositol phosphates in NG115-401L neuronal cells. *Biochem. J.* **253**, 81 - 86.
9. Wong, W.L., Brostrom, M.A., Kuznetsov, G., Gmitter-Yellen, D. & Brostrom, C.O. (1993) Inhibition of protein synthesis and early protein processing by thapsigargin in cultured cells. *Biochem. J.* **289**, 71 - 79.
10. Silverstein, F.S. & Nelson, C. (1992) The microsomal calcium-ATPase inhibitor thapsigargin is a neurotoxin in perinatal rodent brain. *Neurosci. Lett.* **145**, 157 - 160.
11. Harvey, J. & Collingridge, G.L. (1992) Thapsigargin blocks the induction of long-term potentiation in rat hippocampal slices. *Neurosci. Lett.* **139**, 197 - 200.
12. Thastrup, O., Foder, B. & Scharff, O. (1987) The calcium mobilizing tumor promoting agent, thapsigargin elevates the platelet cytoplasmic free calcium concentration to a higher steady state level. A possible mechanism of action for the tumor promotion. *Biochem. Biophys. Res. Commun.* **142**, 654 - 660.
13. Thastrup, O., Linnebjerg, H., Bjerrum, P.J., Knudsen, J.B. & Christensen, S.B. (1987) The inflammatory and tumor-promoting sesquiterpene lactone, thapsigargin, activates platelets by selective mobilization of calcium as shown by protein phosphorylations. *Biochim. Biophys. Acta* **927**, 65 - 73.
14. Takemura, H., Hughes, A.R., Thastrup, O. & Putney, J.W.J. (1989) Activation of calcium entry by the tumor promoter thapsigargin in parotid acinar cells. Evidence that an intracellular calcium pool and not an inositol phosphate regulates calcium fluxes at the plasma membrane. *J. Biol. Chem.* **264**, 12266 - 12271.
15. Sagara, Y. & Inesi, G. (1991) Inhibition of the sarcoplasmic reticulum Ca^{2+} transport ATPase by thapsigargin at subnanomolar concentrations. *J. Biol. Chem.* **266**, 13503 - 13506.
16. Campbell, A.M., Kessler, P.D., Sagara, Y., Inesi, G. & Fambrough, D.M. (1991) Nucleotide sequences of avian cardiac and brain SR/ER Ca^{2+}ATPases and functional comparison with fast twitch Ca^{2+}ATPase. Calcium affinities and inhibitor effects. *J. Biol. Chem.* **266**, 16050 - 16055.
17. Lytton, J., Westlin, M. & Hanley, M.R. (1991) Thapsigargin inhibits the sarcoplasmic or endoplasmic reticulum Ca-ATPase family of calcium pumps. *J. Biol. Chem.* **266**, 17067 - 17071.
18. Kijima, Y., Ogunbunmi, E. & Fleischer, S. (1991) Drug action of thapsigargin on the Ca^{2+} pump protein of sarcoplasmic reticulum. *J. Biol. Chem.* **266**, 22912 - 22918.
19. Thastrup, O., Cullen, P.J., Drobak, B.K., Hanley, M.R. & Dawson, A.P. (1990) Thapsigargin, a tumor promoter, discharges intracellular Ca^{2+} stores by specific inhibition of the endoplasmic reticulum Ca^{2+}ATPase. *Proc. Natl. Acad. Sci. U.S.A.* **87**, 2466 - 2470.

20. Grover, A.K. & Khan, I. (1992) Calcium pump isoforms: diversity, selectivity and plasticity. *Cell Calcium* **13**, 9 - 17.

21. Sagara, Y., Fernandez-Belda, F., de Meis, L. & Inesi, G. (1992) Characterization of the inhibition of intracellular Ca^{2+} transport ATPases by thapsigargin. *J. Biol. Chem.* **267**, 12606 - 12613.

22. Clarke, D.M., Loo, T.W., Inesi, G. & MacLean, D.H. (1989) Location of high affinity Ca^{2+} binding sites within the predicted transmembrane domain of the sarcoplasmic reticulum Ca^{2+}ATPase. *Nature (London)* **339**, 476 - 478.

23. Pickart, C.M. & Jencks, W.P. (1984) Energetics of Calcium-transporting ATPase. *J. Biol. Chem.* **259**, 1629 - 1643.

24. Chen, Z., Coan, C., Fielding, L. & Cassafer, G. (1991) Interaction of CrATP with the phosphorylation site of the sarcoplasmic reticulum ATPase. *J. Biol. Chem.* **266**, 12386 - 12394.

25. Inesi, G. & Sagara, Y. (1992) Thapsigargin, a high affinity and global inhibitor of intracellular Ca^{2+} transport ATPases. *Arch. Biochem. Biophys.* **298**, 313 - 317.

26. de Meis, L. & Inesi, G. (1992) Functional evidence of a transmembrane channel within the Ca^{2+} transport ATPase of sarcoplasmic reticulum. *FEBS Lett.* **299**, 33 - 35.

27. Bian, J.H., Ghosh, T.K., Wang, J.C. & Gill, D.L. (1991) Identification of intracellular calcium pools. Selective modification by thapsigargin. *J. Biol. Chem.* **266**, 8801 - 8806.

28. Xuan, Y.T., Wang, O.L., Whorton, A.R. (1992) Thapsigargin stimulates Ca^{2+} entry in vascular smooth muscle cells: nicardipine-sensitive and -insensitive pathways. *Am. J. Physiol.* **262**, C1258 - C1265.

29. Wrzosek, A., Schneider, H., Grueninger, S. & Chiesi, M. (1992) Effect of thapsigargin on cardiac muscle cells. *Cell Calcium* **13**, 281 - 292.

30. Lewartowski, B. & Wolska, B.M. (1993) The effect of thapsigargin on sarcoplasmic reticulum Ca^{2+} content and contractions in single myocytes of guinea-pig heart. *J. Mol. Cell. Cardiol.* **25**, 23 - 29.

31. Kirby, M.S., Sagara, Y., Gaa, S., Inesi, G., Lederer, W.J. & Rogers, T.B. (1992) Thapsigargin inhibits contraction and Ca^{2+} transient in cardiac cells by specific inhibition of the sarcoplasmic reticulum Ca^{2+} pump. *J. Biol. Chem.* **267**, 12545 - 12551.

32. Ely, J.A., Ambroz, C., Baukal, A.J., Christensen, S.B., Balla, T. & Catt, K.J. (1991) Relationship between agonist- and thapsigargin-sensitive calcium pools in adrenal glomerulosa cells. Thapsigargin-induced Ca^{2+} mobilization and entry. *J. Biol. Chem.* **266**, 18635 - 18641.

33. Takemura, H., Thastrup, O. & Putney, J.W.J. (1990) Calcium efflux across the plasma membrane of rat parotid acinar cells is unaffected by receptor activation or by the microsomal calcium ATPase inhibitor, thapsigargin. *Cell Calcium* **11**, 11 - 17.

34. Rotnes, J.S. & Iversen, J.G. (1992) Thapsigargin reveals evidence for fMLP-insensitive calcium pools in human leukocytes. *Cell Calcium* **13**, 487 - 500.

35. Robinson, I.M., Cheek, T.R. & Burgoyne, R.D. (1992) Ca^{2+} influx induced by the Ca(2+)- ATPase inhibitors 2,5-di-(t-butyl)-1,4-benzohydroquinone and thapsigargin in bovine adrenal chromaffin cells. *Biochem. J.* **288**, 457 - 463.

36. Shuttleworth, T.J. & Thompson, J.L. (1992) Modulation of inositol(1,4,5)trisphosphate-sensitive calcium store content during continuous receptor activation and its effects on calcium entry. *Cell Calcium* **13**, 541 - 551.

37. Schilling, W.P., Cabello, O.A. & Rajan, L. (1992) Depletion of the inositol 1,4,5-trisphosphate--sensitive intracellular Ca^{2+} store in vascular endothelial cells activates the agonist-sensitive Ca(2+)-influx pathway. *Biochem. J.* **284**, 521 - 530.

38. Rosales, C. & Brown, E.J. (1992) Signal transduction by neutrophil immunoglobulin G Fc receptors. Dissociation of intracytoplasmic calcium concentration rise from inositol 1,4,5-trisphosphate. *J. Biol. Chem.* **267**, 5265 - 5271.

39. Neylon, C.B., Nickashin, A., Little, P.J., Tkachuk, V.A. & Bobik, A. (1992) Thrombin-induced Ca^{2+} mobilization in vascular smooth muscle utilizes a slowly ribosylating pertussis toxin-sensitive G protein. Evidence for the involvement of a G protein in inositol trisphosphate-dependent Ca^{2+} release. *J. Biol. Chem.* **267**, 7295 - 7302.

40. Missiaen, L., De Smedt, H., Droogmans, G. & Casteels, R. (1992) Ca^{2+} release induced by inositol 1,4,5-trisphosphate is a steady-state phenomenon controlled by luminal Ca^{2+} in permeabilized cells. *Nature (London)* **357**, 599 - 602.

41. Llopis, J., Kass, G.E., Gahm, A. & Orrenius, S. (1992) Evidence for two pathways of receptor--mediated Ca^{2+} entry in hepatocytes. *Biochem. J.* **284**, 243 - 247.

42. Hoth, M. & Penner, R. (1992) Depletion of intracellular calcium stores activates a calcium current in mast cells. *Nature (London)* **355**, 353 - 356.

43. Glennon, M.C., Bird, G.S., Takemura, H., Thastrup, O., Leslie, B.A. & Putney, J.W.J. (1992) In situ imaging of agonist-sensitive calcium pools in AR4-2J pancreatoma cells. Evidence for an agonist- and inositol 1,4,5-trisphosphate--sensitive calcium pool in or closely associated with the nuclear envelope. *J. Biol. Chem.* **267**, 25568 - 25575.

44. Bird, G.J., Obie, J.F. & Putney, J.W.J. (1992) Functional homogeneity of the non-mitochondrial Ca^{2+} pool in intact mouse lacrimal acinar cells. *J. Biol. Chem.* **267**, 18382 - 18386.

45. Roche, S., Bali, J.P. & Magous, R. (1993) Receptor-operated Ca^{2+} channels in gastric parietal cells: gastrin and carbachol induce Ca^{2+} influx in depleting intracellular Ca^{2+} stores. *Biochem. J.* **289**, 117 - 124.

46. Toescu, E.C., Lawrie, A.M., Petersen, O.H. & Gallacher, D.V. (1992) Spatial and temporal distribution of agonist-evoked cytoplasmic Ca^{2+} signals in exocrine acinar cells analyzed by digital image microscopy. *EMBO J.* **11**, 1623 - 1629.

47. Sargeant, P., Clarkson, W.D., Sage, S.O. & Heemskerk, J.W. (1992) Calcium influx evoked by Ca^{2+} store depletion in human platelets is more susceptible to cytochrome P-450 inhibitors than receptor- mediated calcium entry. *Cell Calcium* **13**, 553 - 564.

48. Sayer, R.J., Schwindt, P.C. & Crill, W.E. (1992) Metabotropic glutamate receptor-mediated suppression of L-type calcium current in acutely isolated neocortical neurons. *J. Neurophysiol.* **68**, 833 - 842.

49. Irving, A.J., Collingridge, G.L. & Schofield, J.G. (1992) L-Glutamate and acetylcholine mobilize Ca^{2+} from the same intracellular pool in cerebellar granule cells using transduction mechanisms with different Ca^{2+} sensitivities. *Cell Calcium* **13**, 293 - 301.

50. Glennon, M.C., Bird, G.S., Kwan, C.Y. & Putney, J.W.J. (1992) Actions of vasopressin and the Ca(2+)-ATPase inhibitor, thapsigargin, on Ca^{2+} signaling in hepatocytes. *J. Biol. Chem.* **267**, 8230 - 8233.

51. Fatatis, A., Bassi, A., Monsurro, M.R., Sorrentino, G., Mita, G.D., Di Renzo, G.F. & Annunziato, L. (1992) LAN-1: a human neuroblastoma cell line with M1 and M3 muscarinic receptor subtypes coupled to intracellular Ca^{2+} elevation and lacking Ca^{2+} channels activated by membrane depolarization. *J. Neurochem.* **59**, 1 - 9.

52. Clementi, E., Scheer, H., Zacchetti, D., Fasolato, C., Pozzan, T. & Meldolesi, J. (1992) Receptor--activated Ca^{2+} influx. Two independently regulated mechanisms of influx stimulation coexist in neurosecretory PC12 cells. *J. Biol. Chem.* **267**, 2164 - 2172.

53. Reiser, G., Cesar, M. & Binmoller, F.J. (1992) Bradykinin and muscarine induce Ca(2+)-dependent oscillations of membrane potential in rat glioma cells indicating a rhythmic Ca^{2+} release from internal stores: thapsigargin and 2,5-di(tert-butyl)-1,4-benzohydroquinone deplete InsP3-sensitive Ca^{2+} stores in glioma and in neuroblastoma-glioma hybrid cells. *Exp. Cell Res.* **202**, 440 - 449.

54. Laskey, R.E., Adams, D.J., Cannell, M. & van Breemen, C. (1992) Calcium entry-dependent oscillations of cytoplasmic calcium concentration in cultured endothelial cell monolayers. *Proc. Natl. Acad. Sci. U.S.A.* **89**, 1690 - 1694.

55. Kline, D. & Kline, J.T. (1992) Thapsigargin activates a calcium influx pathway in the unfertilized mouse egg and suppresses repetitive calcium transients in the fertilized egg. *J. Biol. Chem.* **267**, 17624 - 17630.

56. Donnadieu, E., Bismuth, G. & Trautmann, A. (1992) Calcium fluxes in T lymphocytes. *J. Biol. Chem.* **267**, 25864 - 25872.

57. Boitano, S., Driksen, E.R. & Sanderson, M.J. (1992) Intercellular propagation of calcium waves mediated by inositol trisphosphate. *Science* **258**, 292 - 295.

58. MacGlashan, D.J. & Botana, L.M. (1993) Biphasic Ca^{2+} responses in human basophils. Evidence that the initial transient elevation associated with the mobilization of intracellular calcium is an insufficient signal for degranulation. *J. Immunol.* **150**, 980 - 991.

59. Taylor, M.J. & Clark, C.L. (1992) Evidence that basal secretion of relaxin by individual cultured large luteal cells is influenced by mobilization of intracellular calcium: analysis by a reverse hemolytic plaque assay. *Cell Calcium* **13**, 571 - 580.

60. Rodriguez Del Castillo, A., Vitale, M.L. & Trifaro, J.M. (1992) Ca^{2+} and pH determine the interaction of chromaffin cell scinderin with phosphatidylserine and phosphatidylinositol 4,5,-biphosphate and its cellular distribution during nicotinic-receptor stimulation and protein kinase C activation. *J. Cell. Biol.* **119**, 797 - 810.

61. Metz, D.C., Patto, R.J., Mrozinski, J.E.J., Jensen, R.T., Turner, R.J. & Gardner, J.D. (1992) Thapsigargin defines the roles of cellular calcium in secretagogue-stimulated enzyme secretion from pancreatic acini. *J. Biol. Chem.* **267**, 20620 - 20629.

62. Islam, M.S., Rorsman, P. & Berggren, P.O. (1992) Ca(2+)-induced Ca^{2+} release in insulin-secreting cells. *FEBS Lett.* **296**, 287 - 291.

63. Sagara, Y., Wade, J.B. & Inesi, G. (1992) A conformational mechanism for formation of a dead-end complex by the sarcoplasmic reticulum ATPase with thapsigargin. *J. Biol. Chem.* **267**, 1286 - 1292.

64. Lanini, L., Bachs, O. & Carafoli, E. (1992) The calcium pump of the liver nuclear membrane is identical to that of endoplasmic reticulum. *J. Biol. Chem.* **267**, 11548 - 11552.

65. Vigne, P., Breittmayer, J.P. & Frelin, C. (1992) Thapsigargin, a new inotropic agent, antagonizes action of endothelin-1 in rat atrial cells. *Am. J. Physiol.* **263**, H1689 - H1694.

66. Mikkelsen, E.O., Poulsen, S.H. & Christensen, S.B. (1992) Comparison of the effects of thapsigargin and BAY K 8644 on spontaneous mechanical activity in rat portal vein and contractile responses of rat cardiac muscle. *Pharmacol. Toxicol.* **70**, 152 - 156.

67. Wolska, B.M. & Lewartowski, B. (1993) The role of sarcoplasmic reticulum and Na-Ca exchange in the Ca^{2+} extrusion from the resting myocytes of guinea-pig heart: comparison with rat. *J. Mol. Cell Cardiol.* **25**, 75 - 91.

68. Czarny, M., Sabała, P., Ucieklak, A., Kaczmarek, L. & Barańska, J. (1992) Inhibition of phosphatidylserine synthesis by glutamate, acetylcholine, thapsigargin and ionophore A23187 in glioma C6 cells. *Biochem. Biophys. Res. Commun.* **186**, 1582 - 1587.

69. Pelassy, C., Breittmayer, J.P. & Aussel, C. (1992) Agonist-induced inhibition of phosphatidylserine synthesis is secondary to the emptying of intracellular Ca^{2+} stores in Jurkat T-cells. *Biochem. J.* **288**, 785 - 789.

70. Preston, S.F. & Berlin, R.D. (1992) An intracellular calcium store regulates protein synthesis in HeLa cells, but it is not the hormone-sensitive store. *Cell Calcium* **13**, 303 - 312.

71. Kiley, S.C., Parker, P.J., Fabbro, D. & Jaken, S. (1992) Selective redistribution of protein kinase C isozymes by thapsigargin and staurosporine. *Carcinogenesis* **13**, 1997 - 2001.

72. Hennings, H., Lowry, D.T., Robinson, V.A., Morgan, D.L., Fujiki, H. & Yuspa, S.H. (1992) Activity of diverse tumor promoters in a keratinocyte co-culture model of initiated epidermis. *Carcinogenesis* **13**, 2145 - 2151.

73. Bargon, J., Trapnell, B.C., Chu, C.S., Rosenthal, E.R., Yoshimura, K., Guggino, W.B., Dalemans, W., Pavirani, A., Lecocq, J.P. & Crystal, R.G. (1992) Down-regulation of cystic fibrosis transmembrane conductance regulator gene expression by agents that modulate intracellular divalent cations. *Mol. Cell Biol.* **12**, 1872 - 1878.

74. Ng, J., Fredholm, B.B., Jondal, M. & Andersson, T. (1988) Regulation of receptor-mediated calcium influx across the plasma membrane in a human leukemic T-cell line: evidence of its dependence on an initial calcium mobilization from intracellular stores. *Biochim. Biophys. Acta* **971**, 207 - 214.

75. von Tscharner, V., Prod'hom, B., Baggiolini, M. & Reuter, H. (1986) Ion channels in human neutrophils activated by a rise in free cytosolic calcium concentration. *Nature (London)* **324**, 369 - 372.

76. Ng, J., Gustavsson, J., Jondal, M. & Andersson, T. (1990) Regulation of calcium influx across the plasma membrane of human T-leukemic cell line, JURKAT: Dependence on a rise in cytosolic free calcium can be dissociated from formation of inositol phosphates. *Biochim. Biophys. Acta* **1053**, 97 - 105.

77. Putney, J.W., Jr. (1986) A model for receptor--regulated calcium entry. *Cell Calcium* **7**, 1 - 12.

78. Grynkiewicz, G., Poenie, M. & Tsien, R.Y. (1985) A new generation of Ca^{2+} indicators with greatly improved fluorescence properties. *J. Biol. Chem.* **260**, 3440 - 3450.

79. Barańska, J. (1982) Biosynthesis and transport of phosphatidylserine in the cell. *Adv. Lipid Res.* **19**, 163 - 183.

80. Jencks, W.P. (1989) How does a calcium pump pump calcium? *J. Biol. Chem.* **264**, 18855 - 18858.