J. Membrane Biol. 141, 1–6 (1994)

The Journal of
Membrane
Biology

Topical Review

Specific Inhibitors of Intracellular Ca^{2+} Transport ATPases

G. Inesi, Y. Sagara
Department of Biological Chemistry, University of Maryland School of Medicine, Baltimore, Maryland 21201

Received: 2 February 1994/Revised: 11 March 1994

Introduction

In addition to intracellular Ca^{2+} entry through the plasmamembrane, intracellular Ca^{2+} stores play an important role in regulation of the cytosolic Ca^{2+} concentration by releasing stored Ca^{2+} and rendering possible its function of second messenger (Carafoli, 1987). In turn, the intracellular stores are filled by active transport of cytosolic Ca^{2+} through the intervention of membrane bound ATPases. The first of these enzymes to be discovered was the Ca^{2+} transport ATPase of sarcoplasmic reticulum (SR) (Hasselbach & Makinose, 1961; Ebashi & Lippman, 1962). Subsequently, cloning of cDNAs encoding homologous ATPases (MacLennan et al., 1985; Brandl et al., 1986; Gunteski-Hamblin, Green & Shull, 1988; Burk et al., 1989; Lytton et al., 1989; Campbell et al., 1991) demonstrated the presence of several isoforms not only in SR, but also in the endoplasmic reticulum (ER) of a wide variety of cells. Owing to their association with *S*arco- and *E*ndoplasmic *R*eticulum, and their Ca^{2+} dependence, these enzymes are referred to as SERCA ATPases. The SERCA isoforms display quite similar functional behaviors, although some differences have been noted in their functional regulation, turnover number and Ca^{2+} concentration dependence (Lytton et al., 1992).

It has been recently reported that various chemical compounds inhibit specifically the SERCA ATPases. The most specific and potent of these inhibitors is thapsigargin (TG), a sesquiterpene lactone (Fig. 1*A*) extracted from the root of the umbelliferous plant *Thapsia garganica* (Rasmussen et al., 1978; Christensen, Larsen & Rasmussen, 1982; Christensen, 1988). It was found that TG produces elevation of cytosolic Ca^{2+} (Thastrup et al., 1987*a,b*) and depletes intracellular Ca^{2+} stores (Jackson et al., 1988). It was then established that TG is a specific inhibitor of all tested SERCA ATPases (Campbell et al., 1991; Kijima, Ogunbunmi & Fleischer, 1991; Lytton et al., 1991; Sagara & Inesi, 1991), and not of other cation ATPases including the plasma membrane Na^+,K^+ and Ca^{2+} ATPases (Lytton et al., 1991). Its chemical analogue thapsigargicin (*TGC;* Fig. 1*B*), also extracted from the same plant, appears to produce similar effects.

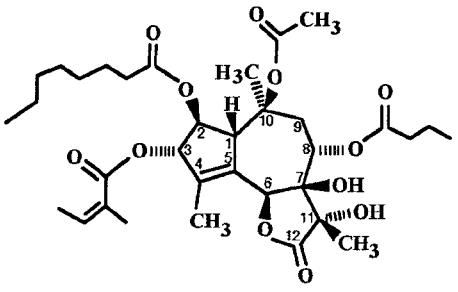
Another SERCA inhibitor is cyclopiazonic acid (*CPA;* Fig. 1*C*) which is one of the toxic metabolites produced by the molds *Pencillium cyclopium* and *Aspergillus flavus* (Holzapfel, 1968). Goeger, Riley and Dorner (1988) found that CPA is a potent inhibitor of Ca^{2+} sequestration and ATPase activity in SR of rat skeletal muscle, and suggested that this inhibition accounts for CPA toxicity. Yet another compound of interest is 2,5,-di(t-butyl) hydroquinone (*DBHQ;* Fig. 1*D*). Hydroquinones are important industrial chemicals which are used as antioxidants, sunscreen components, and intermediates of some dyes (Murphy et al., 1992). DBHQ was found to inhibit ATP-dependent Ca^{2+} by liver microsomes, while it was ineffective on Ca^{2+} transport by mitochondria or plasma membrane (Moore et al., 1987).

The inhibitors of intracellular Ca^{2+} ATPases constitute a quite interesting and useful group of chemical compounds inasmuch as they provide important means of probing the catalytic and transport mechanism of these transport enzymes, their expression and regulation in cells, and the role of cytosolic Ca^{2+} stores in cell biology.

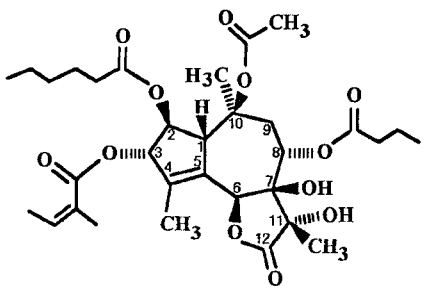
Key words: Ca^{2+} transport — Ca^{2+} ATPases — Thapsigargin — Ca^{2+} transport inhibitors

Correspondence to: G. Inesi

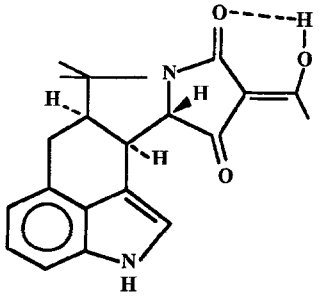
A. Thapsigargin (TG)



B. Thapsigargicin (TGC)



C. Cyclopiazonic Acid (CPA)



D. 2,5-Di(tert-butyl)hydroquinone (DBHQ)

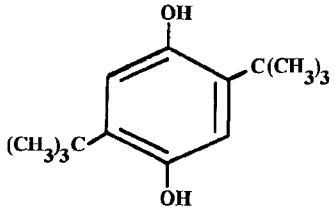


Fig. 1. Structures of Ca^{2+} ATPase inhibitors. (*A*) Thapsigargin (*TG*); (*B*) thapsigargicin (*TGC*); (*C*) cyclopiazonic acid (*CPA*); (*D*) 2,5-di-(t-butyl)-hydroquinone (*DBHQ*).

Formation of a Dead-End Enzyme-Inhibitor Complex

The inhibitory effect of TG is obtained by stoichiometric titration of the ATPase at concentrations as low as nanomolar or lower, and the resulting inhibition affects both Ca^{2+} transport and ATPase activity (Sagara & Inesi, 1991). It is of interest that if TG is added to enzyme preincubated with a Ca^{2+} chelator (e.g., EGTA), and then Ca^{2+} and ATP are added to start the reaction, the enzyme appears fully inhibited and no activity is observed. On the other hand, if TG is added to enzyme preincubated with Ca^{2+}, and then ATP is added to start the reaction, a single cycle of ATP utilization is observed before the inhibitory effect of TG takes place (Sagara et al., 1992*a*). This behavior is due to a requirement for Ca^{2+} dissociation, either by equilibration with EGTA or by utilization of ATP, in order to obtain the TG sensitive state of the enzyme. This can be viewed diagrammatically in Fig. 2 where it is shown that the enzyme (*E*) is activated by high affinity binding of cytosolic Ca^{2+} in exchange for H^+. ATP is then utilized by formation of a phosphorylated enzyme intermediate, which is followed by vectorial dissociation of the bound Ca^{2+} in exchange for H^+. Finally, hydrolytic cleavage of the intermediate takes place. TG interacts only with the Ca^{2+} free enzyme state, thereby forming a dead-end inhibitory complex. If the enzyme resides in the $E \cdot Ca_2$ state when TG is added, a full cycle of ATP utilization must occur before the $H_2 \cdot E$ state is obtained, to render the enzyme sensitive to TG.

Another interesting feature of the TG inhibition is its global character, involving the entire ATPase molecule. In fact, it is possible to study separately the Ca^{2+} binding reaction and the phosphorylation reaction of the ATPase cycle: the former reaction is studied by equilibrating the enzyme with Ca^{2+} in the absence of ATP; the latter reaction is studied by equilibrating the enzyme with Pi in the absence of Ca^{2+} and ATP. Under these conditions, either reaction occurs independently of the other and in the absence of enzyme turnover. It is then possible to demonstrate that both Ca^{2+} binding and the phosphorylation reaction are inhibited by TG (Sagara et al., 1992*a*) with a competitive behavior with respect to

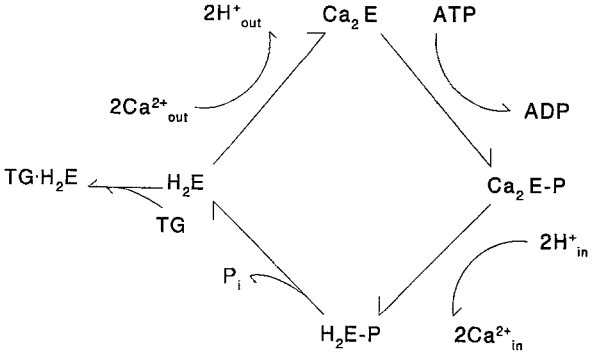


Fig. 2. Diagram of the Ca^{2+} ATPase cycle and TG interaction. The enzyme (E) is activated by high affinity binding of cytosolic Ca^{2+} in exchange for H^+. ATP utilization is followed by formation of a phosphorylated enzyme intermediate, and then by vectorial translocation of bound Ca^{2+}. TG reacts specifically with the Ca^{2+} free enzyme ($H_2 \cdot E$) and forms a dead-end complex.

Ca^{2+} and Pi. Since these two reactions occur in enzyme domains which are distant from each other (Inesi, Sumbilla & Kirtley, 1990), it is apparent that the effect of a single bound TG extends over the entire ATPase molecule. The global effect of TG on the ATPase protein is also demonstrated by its induction of ordered enzyme arrays (Sagara, Wade & Inesi, 1992*b* and Fig. 3).

From the standpoint of the TG chemical structure, the inhibitory activity of TG is strongly configuration dependent, with a major contribution from the ester groups at C_3, C_8 and C_{10}, and an apparently minor contribution from the lactose ring substituents (Christensen et al., 1993). It is most interesting that simple epimerization of the ester bound at C_8 produces a three order of magnitude reduction of inhibitory potency. With regard to the location of TG binding within the ATPase molecule, it was found that chimeric enzymes in which the large cytosolic domain of the Ca^{2+} ATPase (containing the catalytic domain) was replaced by the corresponding domain of the Na^+,K^+ ATPase, retain TG sensitivity (Sumbilla et al., 1993). Since the Na^+,K^+ ATPase is not sensitive to TG, it appears that TG sensitivity must be conferred to the chimeric proteins by either one of the segments derived from the Ca^{2+} ATPase, i.e., the transmembrane region and a small cytosolic loop near the amino terminal. Definitive identification of the TG binding domain must wait for the availability of chemically reactive TG analogues to be used in direct labeling experiments.

As for the mechanism of the other inhibitors, it can be argued that TGC must act quite similarly to TG, owing to its analogous chemical structure. On the other hand, CPA and DHBQ appear to have a lower affinity than TG, as they are effective in the micromolar range compared to the nanomolar range of TG. It was reported that CPA is a stoichiometric inhibitor, whose interaction with the SR ATPase is favored by low ATP concentrations (Seidler et al., 1989). DBHQ was shown to inhibit SERCA ATPases (Kass et al., 1989) by a mechanism quite similar to that of TG (Wictome et al., 1992).

Intracellular Ca^{2+} ATPase Inhibitors Interfere with Cell Proliferation and Several Specific Cell Functions

Numerous cell functions triggered by signals originating from the plasma membrane, through stimulation of G protein or tyrosine kinase linked receptors, are activated through signaling pathways involving intracellular ryanodine or inositol triphosphate receptors and leading to Ca^{2+} release from intracellular stores (Berridge, 1993). The consequent elevation of cytosolic Ca^{2+} occurs with specific spatiotemporal patterns which are involved in control and/or regulation of functions such as contractility, sensory perception, neuromodulation, fertility, development, growth and transformation. In turn, the availability of intracellular Ca^{2+} is dependent on the function of Ca^{2+} transport ATPases, which are bound to sarco- and endoplasmic reticulum membranes and operate active transport of cytosolic Ca^{2+} into intracellular compartments.

It was observed in early experiments that TG and TGC produce histamine release in various cell types (Rasmussen, Christensen & Sandburg, 1978; Ali et al., 1985), activate platelets (Thastrup et al., 1987*a,b*), and affect proliferation of lymphocytes (Scharff et al., 1988). It was reported that these effects were mediated by transient elevation of cytosolic Ca^{2+} (Jackson et al., 1988; Scharff et al., 1988) which was not due to activation of specific Ca^{2+} release mechanisms, but rather to inhibition of transport ATPases which are responsible for steady-state filling of intracellular stores (Thastrup et al., 1990). A clear case of specific functional interference produced by the emptying of intracellular Ca^{2+} stores by TG was observed with regards to contractile activation of cardiac myocytes (Kirby et al., 1992). Furthermore, TG has been reported to affect EGF receptor phosphorylation (Takishima et al., 1988), and c-*fos* and c-*jun* proto-oncogene expression (Schönthal et al., 1991). More generally, exposure of cells to TG and DBHQ results in depletion of intracellular Ca^{2+} stores and inhibition of cell proliferation (Ghosh et al., 1991; Short et al., 1993; *see* Fig. 4). Reports on the use of TG for experimental perturbation of the cytosolic Ca^{2+} homeostasis are quite numerous, and beyond the scope of this brief review. It is of interest that emptying of intracellular Ca^{2+} stores by TG or DBHQ appears to stimulate entry of extracellular Ca^{2+} into the cytoplasm through plasmalemmal channels (Takemura et al.,

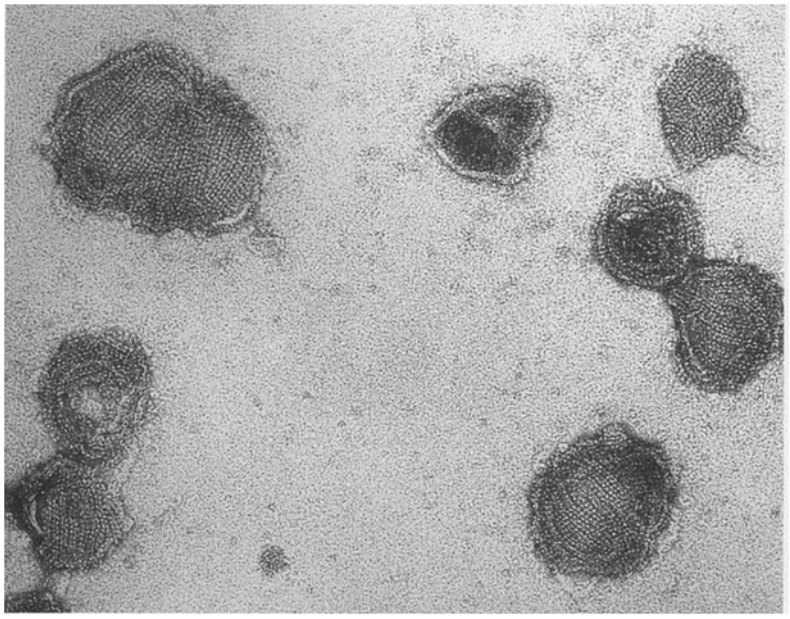


Fig. 3. The global effect of TG is reflected by facilitation of ATPase bidimensional ordering. SR vesicles were suspended in 20 mM MOPS buffer, pH 7.0, 80 mM KCl, 5 mM $Mg Cl_2$, 0.20 mM EGTA, 0.25 mM $Ca Cl_2$, 0.1 μM TG, and 5 mM decavanadate. A control in the absence of TG did not form ordered arrays. The samples were stained with uranyl acetate and examined by electron microscopy (Sagara et al., 1992*b*). 155,000 × magnification.

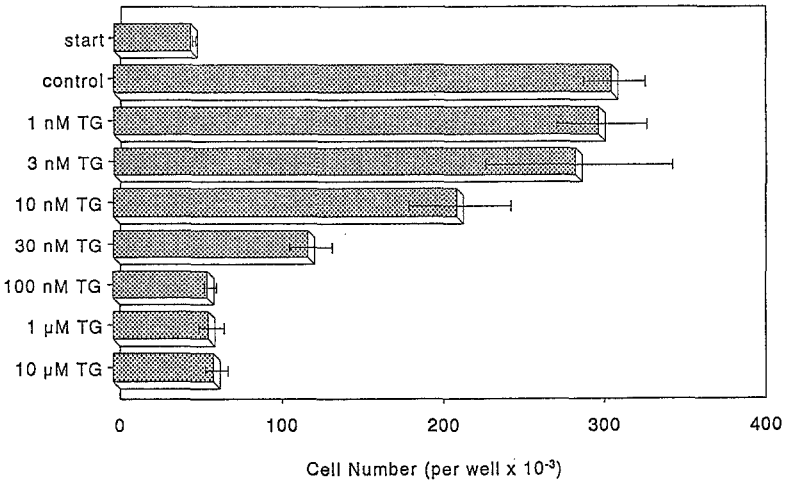


Fig. 4. Inhibition of cultured DDT, MF-2 cells proliferation by thapsigargin. The number of cells per well is shown as a function of the TG concentration in the culture medium (Ghosh et al., 1991).

1989; Bird et al., 1991; Clementi et al., 1992; Hoth & Penner, 1992; Robinson et al., 1992). Such a mechanism, referred to as "capacitative calcium entry" (Putney, 1993), has been related to the intervention of a cytosolic messenger (Randriamampita & Tsien, 1993; Parekh, Terlau & Stühmer, 1993).

Caution on the interpretation of TG effects on whole cells is dictated by the possibility that the intracellular Ca^{2+} stores are heterogeneous and not equally affected by TG (Rossier & Putney, 1991; Gill et al., 1992). Furthermore, development of cell lines acquiring resistance to TG either by overexpression of Ca^{2+} transport ATPase, or by intervention of scavenging proteins such as the multidrug resistance p-glycoprotein has been reported (Gutheil et al., 1994). Given a demonstrated effect on the whole cell, it is then necessary to exclude that another system in addition to the Ca^{2+} transport ATPases is involved, especially when relatively high concentrations of inhibitors are used. For instance, in addition to a major effect on the dense tubular Ca^{2+} transport ATPase, TG has also minor effects on the plasmamembrane Ca^{2+} transport ATPase and plasmamembrane Ca^{2+} leak of platelets (Tao & Haynes, 1992).

Conclusive Remarks

It has been recently discovered that the intracellular Ca^{2+} transport (SERCA) ATPases can be inhibited by a number of compounds among which the plant extract TG appears to be the most potent and specific. TG interacts stoichiometrically with the state acquired by the enzyme in the absence of Ca^{2+}, and forms a dead-end

complex. The consequent conformational effect is reflected by facilitation of the enzyme's ability to form bidimensional crystals. The ATPase inhibitors interfere with the function of SR and ER Ca^{2+} stores, and with cytosolic Ca^{2+} signaling pathways. Consequently, a variety of cell functions linked to Ca^{2+} signaling are also interfered with. The inhibitors of intracellular Ca^{2+} ATPases promise to be very useful agents in molecular studies of ATPase mechanisms, as well as in studies of cell functions which are regulated by cytosolic Ca^{2+}.

Support from the National Institutes of Health and the American Heart Association is gratefully acknowledged.

References

Ali, H., Christensen, S.B., Foreman, J.C., Pearce, F.L., Piotrowski, W., Thastrup, O. 1985. The ability of thapsigargin and thapsigargicin to activate cells involved in the inflammatory response. *Br. J. Pharmacol.* **85:**705–712

Berridge, M.J. 1993. Inositol trisphospate and calcium signalling. *Nature* **361:**315–325

Bird, G.S., Rossier, M.F., Hughes, A.R., Shears, S.B., Armstrong, D.L., Putney, J.W. 1991. Activation of Ca^{2+} entry into acinar cells by a non-phosphorylatable inositol trisphosphate. *Nature* **352:**162–165

Brandl, C.J., Green, N.M., Korczak, B., MacLennan, D.H. 1986. Two Ca^{2+}-ATPase genes: homologies and mechanistic implications of deduced amino acid sequences. *Cell* **44:**597–607

Burk, S.E., Lytton, J., MacLennan, D.H., Shull, G.E. 1989. cDNA cloning, functional expression, and mRNA tissue distribution of a third organellar Ca^{2+} pump. *J. Biol. Chem.* **264:**18561–18568

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 comparisons with fast twitch Ca^{2+}-ATPase. Calcium affinities and inhibitor effects. *J. Biol. Chem.* **266:**16050–16055

Carafoli, E. 1987. Intracellular calcium homeostasis. *Annu. Rev. Biochem.* **56:**395–433

Christensen, S.B. 1988. Interpretation of the NMR and circular dichroic data of the sesquiterpene lactone thapsigargin. *Acta Chemica Scandinavica* **B42:**623–628

Christensen, S.B., Andersen, A., Poulsen, J.-C.J., Treiman, M. 1993. Derivatives of thapsigargin as probes of its binding site on endoplasmic reticulum Ca^{2+} ATPase: Stereoselectivity and important functional groups. *FEBS Lett.* **335:**345–348

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

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(4):**2164–2172

Ebashi, S., Lippman, F. 1962. Adenosine triphosphate-linked concentration of calcium ions in a particulate fraction of rabbit muscle. *J. Cell Biol.* **14:**389–400

Ghosh, T.K., Bian, J., Short, A.D., Rybak, S.L., Gill, D.L. 1991. Persistent intracellular calcium pool depletion by thapsigargin and its influence on cell growth. *J. Biol. Chem.* **266:**24690–24697

Gill, D.L., Ghosh, T.K., Bian, J., Short, A.D., Waldron, R.T., Rybak, S.L. 1992. Function and organization of the inositol-1,4,5-triphosphate-sensitive pools. *Adv. Second Mess. and Phosphoprot. Res.* **26:**265–308

Goeger, D.E., Riley, R.T., Dorner, J.W. 1988. Cyclopiazonic acid inhibition of the Ca^{2+}-transport ATPase in rat skeletal muscle sarcoplasmic reticulum vesicles. *Biochem. Pharmacol.* **37(5):**978–981

Gunteski-Hamblin, A.-M., Greeb, J., Shull, G.E. 1988. A novel Ca^{2+} pump expressed in brain, kidney, and stomach is encoded by an alternative transcript of the slow-twitch muscle sarcoplasmic reticulum Ca-ATPase gene. Identification of cDNAs encoding Ca^{2+} and other cation-transporting ATPases using an oligonucleotide probe derived from the ATP-binding site. *J. Biol. Chem.* **263:**15032–15040

Gutheil, J.C., Hart, S.R., Belani, C.P., Melera, P.W., Hussain, A. 1994. Alterations in Ca^{2+} transport ATPase and p-glycoprotein expression can mediate resistance to thapsigargin. *J. Biol. Chem.* (*in press*)

Hasselbach, W., Makinose, M. 1961. Die Calciumpumpe der "Erschlaffungsgrana" des Muskels and ihre Abhangigkeit von der ATP-spaltung. *Biochem. Z.* **333:**518–528

Holzapfel, C.W. 1968. The isolation and structure of cyclopiazonic acid, a toxic metabolite of penicillium cyclopium westling. *Tetrahedron* **24(5):**2101–2119

Hoth, M., Penner, R. 1992. Depletion of intracellular calcium stores activates a calcium current in mast cells. *Nature* **355:**353–356

Inesi, G., Sumbilla, C., Kirtley, M.E. 1990. Relationships of molecular structure and function in the Ca^{2+} transport ATPase. *Physiol. Rev.* **70:**749–760

Jackson, T.R., Patterson, S.I., Thastrup, O., Hanley, M.R. 1988. A novel tumour promoter, thapsigargin, transiently increases cytoplasmic free Ca^{2+} without generation of inositol phosphates in NG115-401L neuronal cells. *Biochem. J.* **253:**81–86

Kass, G.E.N., Duddy, S.K., Moore, G.A., Orrenius, S. 1989. 2,5-Di(tert-butyl)-1,4-benzohydroquinone rapidly elevates cytosolic Ca^{2+} concentration by mobilizing the inositol 1,4,5-triphosphate-sensitive Ca^{2+} pool. *J. Biol. Chem.* **264:**15192–15198

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

Kirby, M.S., Sagara, Y., Gaa, S.T., 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(18):**12545–12551

Lytton, J., Westlin, M., Burk, S.E., Shull, G.E., MacLennan, D.H. 1992. Functional comparisons between isoforms of the sarcoplasmic or endoplasmic reticulum family of calcium pumps. *J. Biol. Chem.* **267:**14483–14489

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

Lytton, J., Zarain-Herzberg, A., Periasamy, M., MacLennan, D.H. 1989. Molecular cloning of the mammalian smooth muscle sarco(endo)plasmic reticulum Ca^{2+}-ATPases. *J. Biol. Chem.* **264:**7059–7065

MacLennan, D.H., Brandl, C.J., Korczak, B., Green, N.M. 1985. Amino-acid sequence of a Ca^{2+} + Mg^{2+} dependent ATPase from rabbit muscle sarcoplasmic reticulum, deduced from its complementary DNA sequence. *Nature* **316:**696–700

Moore, G.A., McConkey, D.J., Kass, G.E., O'Brien, P.J., Orrenius, S. 1987. 2,5-di(tert-butyl)-1,4-benzohydroquinone—a novel inhibitor of liver microsomal Ca^{2+} sequestration. *FEBS Lett.* **224(2):**331–336

Murphy, S.J., Schroeder, R.E., Blacker, A.M., Krasavage, W.J.,

English, J.C. 1992. A study of developmental toxicity of hydroquinone in the rabbit. *Fund. App. Toxicol.* **19(2):**214–221

Parekh, A.B., Terlau, H., Stühmer, W. 1993. Depletion of InsP3 stores activates a Ca^{2+} and K^+ current by means of a phosphatase and a diffusible messenger. *Nature* **364:**814–818

Putney, J.W. 1993. Excitement about calcium signaling in inexcitable cells. *Science* **262:**676–678

Randriamampita, C., Tsien, R.Y. 1993. Emptying of intracellular Ca^{2+} stores releases a novel small messenger that stimulates Ca^{2+} influx. *Science* **364:**809–814

Rasmussen, U., Christensen, S.B., Sandberg, F. 1978. Thapsigargin and thapsigargicin, two new histamine liberators from *Thapsiagarganica. Acta Pharmaceut. Suec.* **15:**133–140

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

Rossier, M.F., Putney, J.W. 1991. The identity of the calcium-storing, inositol 1,4,5-triphosphate-sensitive organelle in non-muscle cells: calciosome, endoplasmic reticulum . . . or both? *TINS* **14(7):** 310–314

Sagara, Y., Fernandez-Belda, F., De Meis, L., Inesi, G. 1992*a*. Characterization of the inhibition of intracellular Ca^{2+} transport ATPases by thapsigargin. *J. Biol. Chem.* **267:**12606–12613

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

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

Scharff, O., Foder, B., Thastrup, O., Hofman, B., Miler, J., Ryder, L.P., Jacobsen, K.D., Langhoff, E., Dickweiss, E., Christensen, S.B. 1988. Effect of thapsigargin on cytoplasmic Ca^{2+} and proliferation of human lymphocytes in relation to AIDS. *Biochim. Biophys. Acta* **972:**257–264

Schönthal, A., Sugarman, J., Brown, J.H., Hanley, M.R., Feramisco, J.R. 1991. Regulation of c-*fos* and c-*jun* protooncogene expression by the Ca^{2+}-ATPase inhibitor thapsigargin. *Proc. Natl. Acad. Sci. USA* **88:**7096–7100

Seidler, N.W., Jona, I., Vegh, M., Martonosi, A. 1989. Cyclopiazonic acid is a specific inhibitor of the Ca^{2+}-ATPase of sarcoplasmic reticulum. *J. Biol. Chem.* **264:**17816–17823

Short, A.D., Bian, J., Ghosh, T.K., Waldron, R.T., Rybak, S.L., Gill, D.L. 1993. Intracellular Ca^{2+} pool content is linked to control of cell growth. *Proc. Natl. Acad. Sci. USA* **90:**4986–4990

Sumbilla, C., Lu, L., Inesi, G., Ishii, T., Takeyasu, K., Fang, Y., Fambrough, D.M. 1993. Ca^{2+} dependent and thapsigargin-inhibited phosphorylation of Na^+,K^+-ATPases catalytic domain following chimeric recombination with the Ca^{2+}-ATPase. *J. Biol. Chem.* **268:**21185–21192

Takemura, H., Hughes, A.R., Thastrup, O., Putney, J.W., Jr. 1989. Activation of calcium enty by the tumor promoter thapsigargin in parotid acinar cells. *J. Biol. Chem.* **264:**12266–12271

Takishima, K., Friedman, B., Fujiki, H., Rosner, M.R. 1988. Thapsigargin, a novel promoter, phosphorylates the epidermal growth factor receptor at threonine 669. *Biochem. Biophys. Res. Commun.* **157(2):**740–746

Tao, J., Haynes D.H. 1992. Actions of thapsigargin on the Ca^{2+} handling systems of the human platelet. *J. Biol. Chem.* **267:**24972–24982

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. *Proc. Natl. Acad. Sci. USA* **87:**2466–2470

Thastrup, O., Foder, B., Scharff, O. 1987*a*. The calcium mobilizing and tumour promoting agent, thapsigargin, elevates the platelet cytoplasmic free calcium concentration to a higher steady state level. A possible mechanism of action for the tumour promotion. *Biochem. Biophys. Res. Commun.* **142:**654–660

Thastrup, O., Linnebjerg, P.J., Bjerrum, P.J., Knudson, C.M., Christensen, S.B. 1987*b*. The inflammatory and tumor-promoting sesquiterpene lactone thapsigargin activates platelets by selective mobilization of calcium as shown by protein phosphorylation. *Biochim. Biophys. Acta.* **927:**65–73

Wictome, M., Michelangeli, F., Lee, A.G., East, J.M. 1992. The inhibitors thapsigargin and 2,5-di(tert-butyl)-1,4-benzohydroquinone favour the E2 form of the Ca^{2+}, Mg^{2+}-ATPases. *FEBS Lett.* **304:** 109–113