Jasmonates: Regulation of Ca\(^{2+}\)-ATPase and role in calcium homeostasis

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ABSTRACT: The active transport of Ca\(^{2+}\) across the membrane of sarcoplasmic reticulum has received much attention for several decades from biochemical and biophysical points of view. The protein composition of this membrane system has been extensively studied and four major proteins have been identified. The molecular mechanisms by which the chemical energy of ATP hydrolysis is channelled into the vectorial movement of Ca\(^{2+}\) via the Ca\(^{2+}\)-transporting ATPases have also been the subject of intensive research for several years. As a result, a great deal of information on the biochemical properties of the enzymes involved have accumulated. However, the precise mechanism by which these enzymes translocate Ca\(^{2+}\) is just beginning to emerge as a result of the recent report on the crystal structure of skeletal muscle sarcoplasmic reticulum Ca\(^{2+}\)-ATPase. The intracellular Ca\(^{2+}\) concentration controls (directly or indirectly) many important cellular processes such as muscle contraction and relaxation, nervous excitation, exocrine and endocrine secretions, as well as complicated processes such as cell proliferation and fertilization. The discovery of the molecular function of jasmonates in regulating calcium homeostasis has given new insights into this exciting field. The emergence of jasmonates as key players in integrating intracellular calcium homeostasis, and their roles in our understanding of the calcium pumping event, is the main focus of this review.

Key Words: Calcium pump; Ca\(^{2+}\) homeostasis; Ca\(^{2+}\)-ATPases; Jasmone; Methyl jasmonate; Phospholamban.

Introduction

The regulation of intracellular calcium

The calcium ion (Ca\(^{2+}\)) is perhaps the most versatile intracellular messenger in living cells. The regulation of intracellular Ca\(^{2+}\) concentration continues to attract widespread interest as a result of the important role of Ca\(^{2+}\) in mediating a wide variety of cellular processes (Tada \textit{et al}., 1978; Carafoli, 1987; Bewaji, 1995; Lee and East, 2001). In the liver, cytosolic Ca\(^{2+}\) concentration is maintained at approximately 2 µM (Carafoli, 1987). This is achieved by the concerted activities of a plasma membrane Ca\(^{2+}\) pump which extrudes Ca\(^{2+}\) from the cytoplasmic compartment, and the active Ca\(^{2+}\) sequestration by mitochondria and endoplasmic reticulum (Carafoli, 1991; Bababunmi \textit{et al}., 1994).

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In muscle cells, the major reservoir of Ca$^{2+}$ is the sarcoplasmic reticulum (SR). The release of Ca$^{2+}$ from the SR causes contraction while the subsequent active transport of Ca$^{2+}$ into the SR, through the activity of membrane-bound Ca$^{2+}$-ATPase, causes relaxation (Tada et al., 1978). The report on the crystal structure of the Ca$^{2+}$-ATPase of skeletal muscle sarcoplasmic reticulum in its Ca$^{2+}$-bound form (Toyoshima et al. 2000) has given us an insight on how to interpret the vast amount of kinetic data in structural terms.

The Ca$^{2+}$-pumping ATPase of the sarcoplasmic reticulum with a molecular weight of about 100,000 daltons accounts for up to 90 per cent of the total protein. Other proteins include calsequestrin, a high-affinity Ca$^{2+}$-binding protein, and a proteolipid known as phospholamban (Tada et al., 1978).

The abundance of the Ca$^{2+}$-ATPase in this membrane system makes it an excellent protein for the study of structure-function relationship. It has been shown that when the intact 100,000 dalton polypeptide is cleaved by trypsin in situ, two fragments are produced with molecular weights of 55,000 and 45,000 daltons without loss of Ca$^{2+}$ transport function (Klip et al., 1980). The 55,000-dalton fragment is subsequently cleaved into 30,000- and 20,000-dalton fragments. However, this latter cleavage is accompanied by loss of Ca$^{2+}$ transport activity but no loss of ATPase activity.

A consequence of the messenger role of Ca$^{2+}$ is the necessity for its precise regulation. The control of the intracellular level of Ca$^{2+}$ is also an essential step in metabolic regulation. It is generally accepted that in mammalian cells the steady-state concentration of Ca$^{2+}$ is about 10$^{-8}$ to 10$^{-7}$ M. This is three or four orders of magnitude lower than the free Ca$^{2+}$ concentration in the extracellular environment, which has been estimated to be about 1.5 mM (Rasmussen and Goodman, 1977; Carafoli and Crompton, 1978).

Intracellular Ca$^{2+}$ concentration can be regulated by transporting the ion across the plasma membrane or by redistribution within subcellular organelles. The cytosol also contains a large number of non-membraneous ligands which are able to bind Ca$^{2+}$. Some of these ligands are simple compounds such as adenine nucleotides, inorganic phosphate and citrate. Others are proteins which are able to bind Ca$^{2+}$ with high affinity and specificity (Carafoli, 1991). However, these ligands may not be effective in the modulation of the cytosolic free Ca$^{2+}$ concentrations in concert with physiological demands. The maintenance of intracellular Ca$^{2+}$ concentrations at a level much lower than in the extracellular medium must, therefore, depend on its extrusion through the plasma membrane or sequestration within intracellular organelles.

The regulatory role of Ca$^{2+}$ has been extended to a broad range of cellular reactions and processes. The work of several investigators, reviewed by Carafoli (1991), have shown that Ca$^{2+}$ participates in such biological processes as cell motility, muscle contraction, axonal flow, cytoplasmic streaming, chromosome movement, neurotransmitter release, endocytosis and exocytosis.

**Calcium pumping ATPases**

Ca$^{2+}$-transporting ATPases (or Ca$^{2+}$ pumps) located on various membrane types serve to translocate Ca$^{2+}$ ions across these membranes against very steep concentration gradients (Lee and East, 2001; Bababunmi et al., 1994). Ca$^{2+}$ fluxes between the cytoplasm, intracellular Ca$^{2+}$ storage organelles and the plasma membrane play a major role in cellular homeostasis and signal transduction. The stimulation of a wide variety of receptors causes Ca$^{2+}$ to enter the cytoplasm from intracellular storage compartments as well as from external medium through Ca$^{2+}$ channels. In a reverse process, Ca$^{2+}$ can also be resequestered into intracellular storage pools and eliminated from the cytoplasm through the plasma membrane as a result of the activity of ATP-dependent Ca$^{2+}$-transporting ATPases (Ca$^{2+}$ pumps).

Two types of Ca$^{2+}$-pumps have been described in animal tissues: the plasma membrane type Ca$^{2+}$-ATPases (PMCA), and the sarco(endo)plasmic reticulum type Ca$^{2+}$-ATPases (SERCA) (Carafoli 1991; Bewaji and Bababunmi, 1987; Bewaji, 1993; Bababunmi et al. 1994; Bewaji and Dawson, 1995). Several genes have been discovered for both types of ATPases which, upon alternative splicing, give rise to various mRNA transcripts and protein isoforms which are tissue specific.

It has been shown that the calmodulin-sensitive plasma membrane Ca$^{2+}$-ATPase is not a single enzyme but, rather, consists of a family of enzymes encoded by multiple genes. The structure of two distinct isoforms from rat brain (PMCA 1 and PMCA 2) have been determined by molecular cloning procedures (Strehler et al., 1990). PMCA 1 has a molecular weight of 129,500 and 1,176 amino acid residues while PMCA 2 has a molecular weight of 132,600 and 1,198 amino acid residues. The two proteins exhibit 82% amino acid similarity to each other and are encoded by separate genes. Analysis of the nucleotide sequence
of the cDNA encoding PMCA 1 suggests the possibility that alternative splicing of the primary transcript might yield mRNAs encoding enzyme variants which differ in their regulatory properties. A cDNA encoding one of the PMCA 1 variants (now termed PMCA 1b) was isolated from a human teratoma library and shown to encode a protein containing 1,220 amino acids with a M, of 134,700 (Verma et al., 1988). It contains an alternative calmodulin-binding domain and a CAMP-dependent phosphorylation site that might also be encoded by an alternatively spliced exon.

Greeb and Shull (1989) have reported the molecular cloning of a third distinct isoform of the plasma membrane Ca\(^{2+}\) pump (PMCA 3). Amino acid similarity comparison of this isoform indicate that it does not correspond to the erythrocyte enzyme or to the enzyme encoded by the bovine brain cDNA. This suggests that there is a high degree of diversity among plasma membrane Ca\(^{2+}\)-ATPases due to the existence of multiple genes and alternative processing of the primary transcripts.

The control of cytoplasmic Ca\(^{2+}\) concentrations is also mediated, in part, by the uptake or release of Ca\(^{2+}\) from the intracellular organelles. The major storage organelle in non-muscle cells appears to be the endoplasmic reticulum or the recently discovered Ca\(^{2+}\) accumulating structure which has been named calciosome. The ATP-dependent pumps responsible for sequestering Ca\(^{2+}\) in these storage vesicles belong to the E1-E2 family of ATPases which are also referred to as P-type or aspartyl-phosphate ATPases. They cross-react with antibodies directed against the sarcoplasmic reticulum Ca\(^{2+}\)-ATPase (Papp et al., 1992). They also form phosphorylated intermediates during their reaction cycle and are sensitive to vanadate, properties which they share with other members of the E1-E2 family of enzymes such as the Na\(^+\), K\(^+\)-ATPase, the gastric H\(^+\), K\(^+\)-ATPase and the plasma membrane Ca\(^{2+}\)-ATPase.

So far, three different mammalian SERCA genes have been reported: SERCA 1, SERCA 2 and SERCA 3 (Burk et al., 1989; Verboomen et al., 1992). Alternative splicing of the SERCA 2 gene transcript gives rise to two distinct protein isoforms: SERCA 2a, which is expressed in slow skeletal muscle, heart and, to a limited extent, in smooth muscle; SERCA 2b which is expressed in smooth muscle and non-muscle tissues. The last four amino acids of the expressed SERCA 2a ATPase are replaced by an extended tail of 49 amino acids in the SERCA 2b isoform.

**Modulation of Ca\(^{2+}\)-ATPase activity by phospholamban**

Sequestration of Ca\(^{2+}\) by the calcium pump of sarcoplasmic reticulum (SR) membranes accounts for the major portion of the Ca\(^{2+}\) removed from the cytoplasm during cardiac muscle relaxation (Negretti et al., 1993). Therefore, factors that affect SR calcium pump activity are expected to have marked effects on the mechanical properties of the heart. This has been found to be the case with phospholamban, the natural protein regulator of the cardiac SR calcium pump (Tada 1992). The phosphorylation of phospholamban is catalyzed by the cyclic AMP-dependent protein kinase A (PKA). This leads to increased calcium pump activity and plays a role in modulating the effects of catecholamines on the myocardium.

Despite major progress in our understanding of the physiological role of phospholamban, the molecular mechanism of its regulation of the SR calcium pump is not completely understood. Unphosphorylated phospholamban is believed to function as a calcium pump inhibitor that becomes inactive upon phosphorylation by PKA (Inui et al., 1986; Kirchberger et al., 1986; Suzuki and Wang, 1986). Removal of this inhibitory influence is also produced *in vitro* by proteolytic cleavage of the cytoplasmic domain of phospholamban (Kirchberger et al., 1986) or by incubation of SR membranes in the presence of certain polyanions (Xu and Kirchberger, 1989) or monoclonal antibodies against phospholamban (Suzuki and Wang, 1986).

The major elementary steps in the catalytic cycle of the cardiac Ca\(^{2+}\)-ATPase are shown in Fig. 1, which represents a modification of the models given by Gould et al., 1986 and by Cable and Briggs, 1988. The enzyme is believed to exist in two major functional states, E\(_1\) and E\(_2\), whose equilibrium is shifted toward E\(_1\) in the presence of either ATP or Ca\(^{2+}\). ATP, in the presence of Mg\(^{2+}\), binds to E\(_1\) with high affinity and accelerates the conversion of E\(_2\) to E\(_1\) and the conformational change involved in the binding of Ca\(^{2+}\), which also binds to E\(_1\) with high affinity (step 2) (Gould et al., 1986; Guillaud et al., 1981; Champeil et al., 1986). ATP may also bind to E\(_2\) with reduced affinity (step 1a) and accelerate the conversion of E\(_2\) to E\(_1\), followed by high affinity binding of Ca\(^{2+}\) to the latter (Wakabayashi and Shigekawa, 1990) (step 2a). After hydrolysis of the ATP and occlusion of Ca\(^{2+}\) (step 3), ADP is released on the cytoplasmic side of the SR membrane (step 4), and Ca\(^{2+}\) is vectorially transferred across the membrane (step 5) and released on its lumenal side (step 6). Finally, E\(_2\)-P decomposition and release of inorganic phosphate on the cytoplasmic side of the membrane return the enzyme to the ligand-free E\(_2\) state (step 7). However, ATP may bind with...
reduced affinity to the enzyme already at steps 3, 5, and 7 of the cycle and produce forward acceleration of these transitions (Cable and Briggs, 1985; Champeil et al., 1986).

Antipenko et al. (1997) have compared the effects of phospholamban phosphorylation or trypsin treatment of purified cardiac microsomes on some of the properties of the calcium pump of SR membranes with those produced by the addition of jasmone. Jasmone, a minor component of peppermint oil, has been shown to accelerate $E_2$P decomposition (step 7) in fast skeletal muscle SR, causing a decrease in the equilibrium constant for phosphorylation of $E_2$ by $P_i$ (step 7) (Starling et al., 1994). This effect was attributed to a specific interaction of jasmone with the fast skeletal muscle Ca$^{2+}$-ATPase rather than to general perturbation of the phospholipid bilayer of the SR membrane, although an interaction with the Ca$^{2+}$-ATPase that involves its relationship to phospholipids cannot be ruled out. Using our newly developed preparation of ruthenium red-insensitive cardiac microsomes enriched in SR membranes, we demonstrate an increase in the rate of $E_2$P decomposition of the Ca$^{2+}$-ATPase as a result of treatment of these membranes with jasmone or trypsin. This increase may account for or contribute to an observed increase in the $V_{\text{max}}(\text{Ca})$ of calcium uptake that is observed with similar treatment or as a result of PKA-catalyzed phosphorylation of the microsomes.

![Catalytic cycle of cardiac sarcoplasmic reticulum Ca$^{2+}$-ATPase](image)

Fig. 1: The catalytic cycle of cardiac sarcoplasmic reticulum Ca$^{2+}$-ATPase

**Modulation of Ca$^{2+}$-ATPase activity by inhibitors**

A number of investigators have reported that thapsigargin (TG), a plant-derived sesquiterpene lactone, is a potent and specific inhibitor of calcium transport (and Ca$^{2+}$-ATPase) by the SERCA family of Ca$^{2+}$ pumps (Thastrup et al., 1990; Lytton et al., 1991; Sagara and Inesi, 1991; Kijima et al., 1991; Papp et al., 1991) but does not inhibit the plasma membrane Ca$^{2+}$ pumps (PMCA) or other ion-motive ATPases (Papp et al., 1991; Bewaji and Dawson, 1995).

Two other inhibitors have been reported to elevate Ca$^{2+}$ in many cells by causing Ca$^{2+}$ to be released from intracellular stores. These are 2,5-di(tert-butyl)-1,4-hydroquinone (DBHQ) (Robinson and Burgoyne, 1991) and cyclopiazonic acid (CPA) (Demaurex et al., 1992). These inhibitors have become useful tools in characterizing the Ca$^{2+}$ mobilization process from intracellular Ca$^{2+}$ stores. In rat liver endoplasmic reticulum, the Ins(1,4,5)P$_3$-stimulated Ca$^{2+}$ release is greatly enhanced by low concentrations of GTP, in the presence or absence of polyethylene glycol (Dawson, 1985; Dawson et al., 1986). Dawson et al. (1987) have shown that GTP mediates a slow but extensive Ca$^{2+}$ mobilization which is not observed in the absence of potassium as counterions. Bewaji (1995) investigated the ionic requirement for Ins(1,4,5)P$_3$ and thapsigargin-induced Ca$^{2+}$ release from rat liver endoplasmic reticulum and showed that while the Ins(1,4,5)P$_3$-induced Ca$^{2+}$ release was dependent on the presence of K$^+$ in the external medium, thapsigargin released Ca$^{2+}$ from the vesicles irrespective of the ionic composition of the external medium (Figs. 2 – 4). This suggests that a specific ion channel is not involved in the release of Ca$^{2+}$ from the ER vesicles by thapsigargin.
Fig. 2: Effects of potassium and chloride ions on Ca$^{2+}$ uptake and release in rat liver microsomal vesicles. Microsomes (1.5 mg/ml) were incubated in (a) the basic reaction medium containing 150 mM sucrose, 50 mM KCl, 10 mM Hepes/KOH (pH 7.0), 5% (w/v) polyethylene glycol (PEG), 1 mM dithiothreitol, 5 mM ATP, 2 mM MgCl$_2$, 10mM Phosphocreatine and Fluo 3, (b) a solution in which KCl and MgCl$_2$ were replaced with K-gluconate (50 mM) and Mg-gluconate (2 mM) respectively, (c) a solution in which MgCl$_2$ in the basic medium was replaced with Mg-gluconate (2 mM) and KCl was omitted without replacement. Arrows indicate where the following were added: M, microsomes; G, GTP (50µM); IP$_3$, Ins(1,4,5)P$_3$ (2µM); Ca$^{2+}$, calcium ions (10 nmoles). Traces were obtained from data generated by Shimadzu RF 5000 software which were subsequently fed into Microsoft Excel software. [From Bewaji, 1995].
Fig. 3: Effects of sodium ions on Ca\(^{2+}\) uptake and release in rat liver microsomal vesicles. Microsomes (1.5 mg/ml) were incubated in (a) a solution in which KCl in the basic reaction medium described in the legend to Fig. 2a was replaced with NaCl (50 mM), (b) a solution in which KCl in the basic medium was replaced with choline chloride (50 mM), (c) a solution in which KCl in the basic medium was replaced with Na-gluconate (2 mM) and KCl was omitted without replacement. Arrows indicate where the following were added: M, microsomes; G, GTP (50µM); IP\(_3\), Ins(1,4,5)P\(_3\) (2µM); Ca\(^{2+}\), calcium ions (10 nmoles). Traces were obtained from data generated by Shimadzu RF 5000 software which were subsequently fed into Microsoft Excel software. [From Bewaji, 1995].
Fig. 4: Ionic requirement for thapsigargin-induced Ca\(^{2+}\) release from rat liver microsomal vesicles. Microsomes (1.5 mg/ml) were incubated in (a) the basic reaction medium described in the legend to Fig. 2a, (b) a solution in which KCl and MgCl\(_2\) in the basic medium were replaced with K-gluconate (50 mM) and Mg-gluconate (2 mM) respectively, (c) a solution in which MgCl\(_2\) in the basic medium was replaced with Mg-gluconate (2 mM) and KCl was omitted without replacement. Arrows indicate where the following were added: M, microsomes; G, GTP (50 µM); IP\(_3\), Ins(1,4,5)P\(_3\) (2 µM); Ca\(^{2+}\), calcium ions (10 nmoles). Traces were obtained from data generated by Shimadzu RF 5000 software which were subsequently fed into Microsoft Excel software. [From Bewaji, 1995].
Fig. 5: Drug-induced Ca\(^{2+}\) release from rat liver endoplasmic reticulum.

Microsomes (1.5 mg/ml) were incubated in the basic medium containing 150 mM sucrose, 50 mM KCl, 10 mM Hepes/KOH (pH 7.0), 5% (w/v) polyethylene glycol (PEG), 1 mM dithiothreitol, 5 mM ATP, 2 mM MgCl\(_2\), 10mM Phosphocreatine and Fluo 3,. Arrows indicate where the following were added: M, microsomes; TG, thapsigargin (100 nM); DBHQ, 2,5-di-(tert-butyl)-1,4-hydroquinone (2µM); CPA, cyclopiazonic acid (2µM); Ca\(^{2+}\), calcium ions (10 nmoles). Traces were obtained from data generated by Simadzu RF 5000 software which were subsequently fed into Microsoft Excel software.
The effects of the three inhibitors mentioned above on calcium release from rat liver endoplasmic reticulum were also compared (Bewaji, 1996). The inhibitors released Ca\(^{2+}\) to various extents from the ER. Thapsigargin caused a slow but extensive release of Ca\(^{2+}\) from the ER, releasing almost all the Ca\(^{2+}\) accumulated by the vesicles during the uptake phase. However, DBHQ and CPA did not release all the Ca\(^{2+}\) accumulated during the uptake phase. Furthermore, CPA did not prevent the re-uptake of the Ca\(^{2+}\) added for calibration purposes after the release process (Fig. 5). This suggests that there may be some intracellular stores that are insensitive to DBHQ and CPA.

The role of jasmonates in apoptosis and suppression of cell proliferation

Jasmonates belong to a group of plant stress hormones (Sembdner and Parthier, 1993). Upon exposure to stress (e.g., wounding by chewing insects or some pathogens), jasmonates are produced in plants and cause the induction of a proteinase inhibitor (Farmer and Ryan, 1990). A coordinated activation of programmed cell death and defense mechanisms often accompany the antimicrobial response of plants (Mitler and Lam, 1996).

Professor Enitan Bababunmi in 2002 suggested to scientists at the University of Nevada Cancer Research Center and Chemistry Department that jasmonates should be investigated for anti-cancer properties (see Yeruva et al., 2006). Indeed, jasmonates have been shown to induce apoptosis and cell cycle arrest in non-small cell lung cancer cell lines. It has previously been shown that Ca\(^{2+}\) homeostasis is altered in small and non-small cancer cell lines (Yeruva et al., 2006, 2008). The effect of alteration in Ca\(^{2+}\) homeostasis on apoptosis has also been confirmed. When introduced in anticancer molecules, the cyclopentenone pharmacophore can increase their potency (Flescher, 2005).

It has recently been reported that jasmonates can suppress the proliferation of human cancer cells and induce their death. Methyl jasmonate induced death in breast and prostate carcinoma cells, as well as in melanoma, lymphoma, and leukemia cells (Fingrut and Flescher, 2002). Furthermore, it has been shown that jasmonates are capable of killing cancer cells in a manner independent of cellular mRNA transcription, protein translation (Rotem et al., 2003), and p53 expression. Finally, methyl jasmonate significantly increased the life span of lymphoma-bearing mice (Fingrut and Flescher, 2002).

Apoptotic cell death can be mediated via several pathways. One pathway involves the engagement of so-called death receptors belonging to the tumor necrosis factor receptor superfamily. A cascade of proteolytic digestion occurs, involving caspases, resulting in cell death. However, caspase-8 may digest the Bid protein to yield a truncated form that can induce mitochondrial damage, eventually leading to cell death. In addition, many agents (including chemotherapeutic drugs) induce cellular stress, which may also lead to mitochondrial perturbation and, finally, cell death. A mechanism causing the mitochondrial dysfunction mentioned above has been proposed, consisting of mitochondrial membrane permeability transition, dissipation of the inner membrane potential, osmotic swelling of the matrix, rupture of the outer mitochondrial membrane, release of cytochrome c and other apoptogenic proteins from the mitochondria, and formation of the caspase-3 activation complex, the apoptosome (Debatin et al., 2002; Newmeyer and ferguson-Miller, 2003). Permeability transition involves the opening of a channel named permeability transition pore complex (PTPC). The main components of this pore are adenine nucleotide translocator and cyclophilin D in the inner membrane of the mitochondria, and voltage-dependent anion channel and peripheral benzodiazepine receptor in the outer mitochondrial membrane. The PTPC is formed in regions of contact between the inner and outer mitochondrial membranes. Prolonged opening of the PTPC leads to the above-mentioned effects, exposes the cytosol to the contents of the mitochondria, and culminates in cell death (Debatin et al., 2002; Newmeyer and ferguson-Miller, 2003). Interestingly, mitochondrial permeability transition can lead to both apoptosis as well as necrosis (Lemasters et al., 1998).
Since it has been shown that jasmonates exert their cytotoxic effects independent of cellular transcription, translation, and p53 expression it was suggested that these compounds may act directly on mitochondria (Rotem et al., 2005). These findings suggest that jasmonates are mitochondriotoxic toward human cancer cell lines, in a PTPC-mediated mechanism. In addition, they perturb mitochondria isolated from leukemic cells derived from patients with chronic lymphocytic leukemia (CLL) but not those isolated from normal blood lymphocytes.

Jasmonates have been found to be selectively cytotoxic toward transformed cells (Fingrut and Flescher, 2002). Because methyl jasmonate induced cytochrome c release from mitochondria isolated from cancer cells but not from normal lymphocytes and induced swelling only in mitochondria isolated from cancer cells but not from nontransformed fibroblasts and normal lymphocytes, it most probably exploits mitochondrial characteristics that are differentially expressed in cancer cells. Moreover, the mere fact that cells are proliferating is not sufficient to render them susceptible to methyl jasmonate because mitochondria isolated from an immortal nontransformed cell line, or from proliferating normal lymphoblasts, do not swell upon exposure to methyl jasmonate. Several reports in the literature suggest that the composition and function of mitochondria in cancer cells and normal cells differ. These include a higher mitochondrial membrane potential, possible modulation of the expression of PTPC components, and enhanced rates of ATP generation through glycolysis rather than through oxidative phosphorylation (the Warburg effect) in cancer cells (Debatin et al., 2002; Chen, 1988; Dang and Semenza, 1999). Recent studies of samples freshly taken from human tumors also suggest that these cancers bear a distinct signature of mitochondrial energy generation. Liver carcinomas exhibit depletion of cellular mitochondrial contents. Tumors originating from the colon, kidney, breast, stomach, esophagus, and lung have significantly reduced expression of the $\beta$-catalytic subunit of the mitochondrial $\mathrm{H}^+$-ATP synthase (Cuezva et al., 2002; Isidoro et al., 2004). Thus, even if jasmonates reach normal cells as well, their effects on mitochondria from cancer cells may be different. The difference may be related to PTPC components that jasmonates may directly
interact with. Another possibility is that the defective ability of mitochondria in cancer cells to generate ATP may turn these organelles into the weak point of the cells.

From the foregoing, there is ample evidence that jasmonates constitute a novel group of selective anticancer agents, structurally different from any known cancer chemotherapeutics whose target organelles are the mitochondria. These compounds exhibit activity against leukemic cells from patients with CLL and are thus promising candidates for the treatment of that and other types of cancer.

**Jasmonates and calcium homeostasis**

Biochemical investigations have recently shown that jasmonate, menthone, menthol, and methyl jasmonate are highly selective stimulators of the SR Ca\(^{2+}\)-ATPase in mammalian skeletal muscle, whereas they have no effect on the properties of the phospholipid bilayer (Starling et al., 1994). Surprisingly, no study has investigated the effects of these substances on skeletal muscle contraction. Several studies have shown that two compounds of the lipoxygenase pathway, i.e., jasmonic acid and methyl jasmonate, regulate wound response in plants (Veronesi et al., 1996). Methyl jasmonate, a linolenic acid-derived cyclopentanone-base, has also been shown to trigger defense reactions in various plants and to be produced in response to wounds and elicitor treatment (Rickauer et al., 1997). Because methyl jasmonate can stimulate ATP-dependent Ca\(^{2+}\) activity of the purified Ca\(^{2+}\)-ATPase from rabbit fast-twitch skeletal muscle (Starling et al., 1994), it seemed of interest to estimate the specificity of methyl jasmonate effects on Ca\(^{2+}\) loading by the SR. Chemically skinned slow muscle fibers (soleus) were chosen for these experiments, as sarcolemmal functions can be eliminated and Ca\(^{2+}\) mobilization greatly simplified. Other experiments investigated whether methyl jasmonate in intact soleus fibers acts at different steps in the excitation-contraction coupling process.

Joumaa et al. (2002) were the first to describe the effects of methyl jasmonate on the contractile responses of intact mammalian skeletal muscle fibers. Soleus muscle was used for their experiment because a stimulating effect of SR Ca\(^{2+}\)-ATPase was likely to be observed more easily on a slow- than a fast-twitch muscle. Moreover, it has been shown that SR Ca\(^{2+}\)-ATPase plays a rate-limiting role in the twitch relaxation of single slow skeletal muscle. (Chua and Dulhunty, 1988). These investigators demonstrated that methyl jasmonate concentrations of up to 100 µM reduced the time required to load the SR with calcium, whereas this effect was less marked at higher concentrations. These results are similar to those previously reported on the ATPase activity of the purified Ca\(^{2+}\)-ATPase from rabbit fast skeletal muscle (Starling et al., 1994), except that in the experiments of Joumaa et al. (2002) methyl jasmonate concentrations were 100-fold lower. The fact that comparable effects were obtained for calcium loading in both types of muscle suggests that the application of methyl jasmonate resulted in an increase of ATPase activity in slow muscle, with a maximum response at 100 µM.

They observed that the characteristics of the twitch generated by short electrical stimulation were modified by methyl jasmonate, and their results indicated that methyl jasmonate had distinct dose-dependent effects on the contraction of skeletal soleus muscle cells.

Although the excitation-contraction coupling mechanism was not affected by methyl jasmonate, the shift in the activation curve to more positive values, resulting from exposure to methyl jasmonate, could be related to changes in membrane potential and/or the depolarization rate and/or Ca\(^{2+}\) sensitivity for contractile proteins.

It is now well established that cyclopiazonic acid (CPA) is a specific inhibitor of sarcoplasmic Ca\(^{2+}\)-ATPase in skeletal muscle (Seidler et al., 1989; Mason et al., 1991; Demaurex et al., 1992; Bewaji, 1996). Thus, the fact that the effect of methyl jasmonate on the activation curve was opposite to that of CPA suggests that methyl jasmonate is a stimulator of sarcoplasmic Ca\(^{2+}\)-ATPase. Methyl jasmonate, therefore, appears to be a tool for selective activation of SR Ca\(^{2+}\)-ATPase in slow-twitch skeletal muscle and a regulator of calcium homeostasis in this tissue.

**Concluding Remarks**

The role of the various calcium transporting systems in intracellular calcium homeostasis seems rather complex. The regulation of intracellular calcium is the direct result of the cooperation between these systems. With the unravelling of the primary structures of the various isoforms of the enzymes involved in calcium pumping, comparison of the structures, functions and properties of the plasma membrane and intracellular Ca\(^{2+}\)-ATPases has become possible. This brings us nearer to the goal of understanding how a
Ca\textsuperscript{2+} pumping event is achieved. It will also enable us to understand the differences in the response of the various ATPases to agents like jasmonate, thapsigargin, cyclopiazonic acid, phospholamban etc, or even differences in the stoichiometries of calcium pumping. It has already been suggested, for example, that the extended C-terminal tail of the SERCA 2b Ca\textsuperscript{2+} pump may be responsible for its higher Ca\textsuperscript{2+} affinity when compared with the truncated SERCA 2a variant (Verboomen et al., 1992). Such observed structural differences may also be used to rationalise functional differences between the PMCA and SERCA types Ca\textsuperscript{2+}-ATPases.

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