A diversity of SERCA Ca$^{2+}$ pump inhibitors

Francesco Michelangeli and J. Malcolm East

Abstract

The SERCA (sarcoplasmic/endoplasmic reticulum Ca$^{2+}$-ATPase) is probably the most extensively studied membrane protein transporter. There is a vast array of diverse inhibitors for the Ca$^{2+}$ pump, and many have proved significant in helping to elucidate both the mechanism of transport and gaining conformational structures. Some SERCA inhibitors such as thapsigargin have been used extensively as pharmacological tools to probe the roles of Ca$^{2+}$ stores in Ca$^{2+}$ signalling processes. Furthermore, some inhibitors have been implicated in the cause of diseases associated with endocrine disruption by environmental pollutants, whereas others are being developed as potential anticancer agents. The present review therefore aims to highlight some of the wide range of chemically diverse inhibitors that are known, their mechanisms of action and their binding location on the Ca$^{2+}$ ATPase. Additionally, some ideas for the future development of more useful isof orm-specific inhibitors and anticancer drugs are presented.

Introduction

For more than 40 years, there has been considerable research into molecules that can affect the activity of one of the most extensively studied membrane protein transporters, the SERCA (sarcoplasmic/endoplasmic reticulum Ca$^{2+}$-ATPase). Originally identified in muscle in 1962 by Ebashi and Ebashi [1], this easy to isolate and study (at least in the skeletal muscle) membrane protein transporter has been the focus of considerable scientific attention over the intervening years. Initially, inhibitor studies were used to help to elucidate mechanistic and kinetic details of this Ca$^{2+}$-ATPase; however, more recently, the use of inhibitors has aided our determination of distinct conformational structures using X-ray crystallography of SERCA–inhibitor complexes.

Some inhibitors, such as thapsigargin, BHQ [2,5-di-(t-butyl)-4-hydroquinone] and CPA (cyclopiazonic acid), have also become widely employed as pharmacological tools in the cell signalling field. For instance, to date, more than 7000 scientific papers have been published that have used thapsigargin in their investigations of various Ca$^{2+}$ signalling properties in cells and tissues. More recently, inhibitors of SERCA pumps in Plasmodium falciparum, are also being tested as pharmaceutical drugs to combat malaria [2]. Focus has also been given to identifying isof orm-specific SERCA inhibitors, since it has been shown that some types of cancer cell appear to have altered expression of particular SERCA isoforms [3,4] and this could therefore lead to the development of novel anticancer drugs [5].

A diversity of Ca$^{2+}$-ATPase inhibitors

To date, many (probably hundreds) SERCA inhibitors with a variety of chemical structures have been identified. They range from small, but unrelated, hydrophobic molecules, which may contain hydroxy groups, to small charged anions such as orthovanadate or more complex peptide toxins such as mastoparan (Figure 1). Many of these diverse inhibitor molecules are able to inhibit the Ca$^{2+}$-ATPase in the low-micromolar to nanomolar concentration ranges, indicating high binding affinities. However, given such high-affinity binding, it is clear from the Ca$^{2+}$-ATPase structure that there are only a limited number of potential binding sites on the protein where these diverse molecules can bind.

Thapsigargin

As highlighted above, thapsigargin is by far the most widely used SERCA inhibitor. It was initially identified from an extract from the plant Thapsia garganica that could increase free cytosolic Ca$^{2+}$ levels in platelets [6], and this was later determined to be due to inhibition of the endoplasmic reticulum Ca$^{2+}$-ATPase [7]. Thapsigargin is a sesquiterpene lactone that can inhibit SERCA in the nanomolar concentration range [7,8]. It is also highly selective, since it does not appreciably inhibit other related Ca$^{2+}$-ATPases such as the PMCA (plasma membrane Ca$^{2+}$-ATPase) or the SPCA (secretory pathway Ca$^{2+}$-ATPase), at these very low concentrations [9,10]. Furthermore, it appears that thapsigargin also differentially inhibits SERCA isoforms, being 60 times more potent for SERCA1 than for SERCA3 (Ki values of 0.2, 1 and 12 nM for SERCA-isoforms 1, 2 and 3 respectively) [8]. Initial enzymology studies suggested that thapsigargin caused inhibition by both inhibiting Ca$^{2+}$ binding and phosphorylation [11]. Later studies additionally showed that it did so by stabilizing the E2 (low Ca$^{2+}$ affinity) conformational state causing it to be locked into an E2-type
A diverse range of SERCA inhibitors

Figure 1

The use of thapsigargin-insensitive mutant cells initially identified Phe256 within SERCA1 as an amino acid residue residing in the thapsigargin-binding site, since mutation of this residue to valine (F256V) resulted in a 200-fold decrease in thapsigargin sensitivity [8]. Interestingly, all other SERCA isoforms contained Phe256, which, when mutated, reduced their sensitivities to thapsigargin, albeit to lesser degrees than SERCA1 [8].

In 2002, the crystal structure of SERCA1 in the E2 state, in which thapsigargin was bound, identified the exact binding pocket that involved interactions with transmembrane helices M3, M5 and M7. Glu255, Phe256, Gln259, Leu260, Val263, Val269, Ile276, Phe334, Met338 and Tyr337 are particularly important in forming hydrogen bonds and other interactions with thapsigargin [13,14] (Figure 2). More recently, much attention has been given to the making and testing of structural analogues of thapsigargin in order to better understand the molecular interactions between thapsigargin and SERCA. These studies have shown that the acyl groups at positions O-3, O-8 and O-10 are particularly important, probably due to their appropriate hydrophobic interactions with SERCA [14]. It must be noted that most, if not all, thapsigargin analogues so far tested are weaker inhibitors than the parent compound. It has also been proposed that thapsigargin is only able to gain access to its buried hydrophobic binding site within the ATPase by first partitioning into the lipid membrane rather than gaining direct access from the aqueous phase [14].

In screening a number of natural products extracted from traditional Chinese medicinal herbs, in order to discover novel autophagy enhancers that could potentially be used as therapeutic agents, a compound called Alisol B was identified [15]. In addition to causing autophagy and cell death in a number of cancer cell lines, this compound was also shown to elevate intracellular Ca²⁺ levels via SERCA inhibition.
### Table 1 | Potencies and conformational effects of some SERCA inhibitors

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>$K_i$ or IC$_{50}$</th>
<th>E1-like or E2-like conformation</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thapsigargin</td>
<td>0.21–12 nM</td>
<td>E2</td>
<td>[8]</td>
</tr>
<tr>
<td>Cyclopiazonic acid</td>
<td>90–2500 nM</td>
<td>E2</td>
<td>[8]</td>
</tr>
<tr>
<td>BHQ</td>
<td>2–7 μM</td>
<td>E2</td>
<td>[8]</td>
</tr>
<tr>
<td>Nonylphenol</td>
<td>6 μM</td>
<td>E2</td>
<td>[22,31]</td>
</tr>
<tr>
<td>Bisphenol</td>
<td>2 μM</td>
<td>E2</td>
<td>[33]</td>
</tr>
<tr>
<td>Bisphenol A</td>
<td>233 μM</td>
<td>E2</td>
<td>[23]</td>
</tr>
<tr>
<td>TBPA</td>
<td>0.5–2.3 μM</td>
<td>E2</td>
<td>[30]</td>
</tr>
<tr>
<td>4-Chloro-m-cresol</td>
<td>2.8 mM</td>
<td>?</td>
<td>[54]</td>
</tr>
<tr>
<td>Orthovanadate</td>
<td>10–100 μM</td>
<td>E2</td>
<td>[55]</td>
</tr>
<tr>
<td>Quercitin</td>
<td>9 μM</td>
<td>E1</td>
<td>[40]</td>
</tr>
<tr>
<td>3,6-Dihydroxyflavone</td>
<td>6 μM</td>
<td>E1</td>
<td>[40]</td>
</tr>
<tr>
<td>Galangin</td>
<td>9 μM</td>
<td>E1</td>
<td>[40]</td>
</tr>
<tr>
<td>2APB</td>
<td>70–700 μM</td>
<td>E1</td>
<td>[43]</td>
</tr>
<tr>
<td>Curcumin</td>
<td>7–15 μM</td>
<td>E1</td>
<td>[35]</td>
</tr>
<tr>
<td>Paxilline</td>
<td>5 μM</td>
<td>E1</td>
<td>[56]</td>
</tr>
<tr>
<td>Alisol B</td>
<td>27 μM</td>
<td>E2</td>
<td>[15]</td>
</tr>
<tr>
<td>Mastoparan</td>
<td>1 μM</td>
<td>E1</td>
<td>[57,58]</td>
</tr>
<tr>
<td>Peptide M391</td>
<td>0.3 μM</td>
<td>E1?</td>
<td>[57,58]</td>
</tr>
<tr>
<td>Ivermectin</td>
<td>15 μM</td>
<td>E1</td>
<td>[59]</td>
</tr>
<tr>
<td>Cyclosporin A</td>
<td>62 μM</td>
<td>?</td>
<td>[59]</td>
</tr>
<tr>
<td>Rapamycin</td>
<td>77 μM</td>
<td>?</td>
<td>[59]</td>
</tr>
<tr>
<td>Chlorpromazine</td>
<td>23 μM</td>
<td>E1</td>
<td>[60]</td>
</tr>
<tr>
<td>Calmidazolium</td>
<td>0.5 μM</td>
<td>E1</td>
<td>[60]</td>
</tr>
<tr>
<td>Fluphenazine</td>
<td>15 μM</td>
<td>E1</td>
<td>[60]</td>
</tr>
<tr>
<td>DES</td>
<td>20 μM</td>
<td>?</td>
<td>[23]</td>
</tr>
<tr>
<td>3,3-Dibromo-2,4,6-tris(methylisothiouronium)benzene (Br$_2$-TITU)</td>
<td>29 μM</td>
<td>E1</td>
<td>[32]</td>
</tr>
<tr>
<td>sHA 14-1</td>
<td>23 μM</td>
<td>?</td>
<td>[61]</td>
</tr>
</tbody>
</table>

The structure of Alisol B is steroid in nature with a ketone group at the C-3 position and a branched acyl hydroxylated epoxide side chain at the C-17 position (Figure 1) [15]. Energy-minimized molecular docking analysis showed that Alisol B was likely to bind best to the transmembrane domain at the same site occupied by thapsigargin. The best binding pose was achieved when the steroid ring was in close contact with Phe$^{256}$, Val$^{265}$, Ile$^{765}$ and Val$^{769}$, all very similar to the interactions seen with thapsigargin [14,15].

Early studies with cholesterol and androstenol showed that they do not appear to affect the activity of SERCA1 when added to it in its native-like phospholipid membrane [15,17]. They do, however, bind directly to SERCA, as demonstrated by fluorescence quenching studies, where the intrinsic typtophan fluorescence of the Ca$^{2+}$-ATPase is quenched by contact with brominated sterol analogues [16,17]. These sites were also shown not be at the lipid–protein interface where annular lipids bind, since additional quenching was observed when the ATPase was first reconstituted in brominated phospholipids. From these studies, the existence of hydrophobic ‘non-annular’ binding sites on the Ca$^{2+}$-ATPase was first proposed [16]. When such sites are occupied by these sterols in SERCA which has been reconstituted in suboptimal fatty acyl chain length phospholipids, a dramatic enhancement in activity is observed [16, 17]. Therefore these sterol-binding sites, which have also been observed in other ion-translocating ATPases [18], may be the evolutionary remnants of some lost regulatory mechanism. Detailed structural analysis of the thapsigargin-binding site has led to the suggestion that this site and the ‘non-annular’ sites could be one and the same [14]; however, this has yet to be proven.

### Inhibitors that stabilize the E2 conformation

Table 1 lists some of the vast array of diverse compounds that are able to act as SERCA inhibitors and included in this Table are inhibition constants (or IC$_{50}$) values for these inhibitors. Some of these molecules, such as bisphenol A, nonylphenol and DES (diethylstilbestrol), have been in the limelight recently owing to their endocrine-disrupting properties which are linked with male infertility, and which are believed to be due to their ability to bind to oestrogen
However, it cannot be discounted that some of these chemicals which pervade our environment could also be acting as endocrine disrupters by dysregulating Ca\(^{2+}\) signalling events through their action on SERCA [21–23]. Furthermore, of note are BHQ and CPA, which, like thapsigargin, have been used extensively as pharmacological tools by scientists to mobilize SERCA-loaded Ca\(^{2+}\) stores [24]. Both inhibitors were shown previously to inhibit the Ca\(^{2+}\)-ATPase by stabilizing it in one of the E2 conformational states [12,25] and, to date, many other inhibitors of this class have also been shown to cause this same effect. It was originally proposed that molecules such as CPA and BHQ bound to the same site as thapsigargin [26]; however, this was challenged by inhibitor competition studies [27], and then confirmed by crystal structures clearly showing them binding to different sites [28,29]. One crystal structure was produced containing both bound thapsigargin and BHQ (PDB code 2AGV) which clearly shows these two sites on opposite sides of the transmembrane bundle (Figure 3). From these structures, it appears that in the E2-like conformational state a buried hydrophobic groove is created between transmembrane helices M1, M2, M3 and M4, owing to M1 adopting a ‘kinked’ conformation driven by movement of the actuator domain. This allows access of BHQ or CPA to bind to this site [28,29]. Amino acids important in inhibitor binding to this site include a number of polar ones such as Gln56, Asp59 and Asn101, which presumably interact with the hydroxy groups on these inhibitors [28,29]. Fluorescence-quenching studies using the brominated hydrophobic inhibitor TBBPA (tetrabromobisphenol A) causes quenching of the SERCA tryptophan fluorescence and can be displaced by BHQ, but not by thapsigargin. This therefore indicates that TBBPA also binds to the BHQ-binding site [30]. Detailed mechanistic studies have shown that TBBPA also stabilizes the enzyme in an E2 state [30]. Additionally, nonylphenol which also stabilizes the E2 state [31], can displace TBBPA and reverse fluorescence quenching [30], and it therefore appears likely that this and a number of other small hydroxylated hydrophobic inhibitors (which lock the enzyme in an E2 state) will work in a similar fashion. One can envisage these small molecules acting as a ‘doorstop’, wedging open the gap between the helix M1 and helices M2, M3 and M4, keeping it ajar and thus making it unable to go back into the E1 conformation. The region where TBBPA binds is also postulated to be very close to Ca\(^{2+}\)-binding site II, which could also explain how TBBPA reduces the Ca\(^{2+}\)-binding affinity more than 20-fold [30].

The use of brominated inhibitors such as TBBPA have also been useful in determining whether this class of inhibitor can either partition directly from the aqueous phase into the Ca\(^{2+}\)-ATPase or indirectly by first partitioning into the lipid phase. By measuring the rate constants for TBBPA binding to the Ca\(^{2+}\)-ATPase (by following its ability to quench protein fluorescence), it was found that the rates were approximately 10-fold higher than for TBBPA binding to phospholipid bilayers alone, indicating that this inhibitor is able to bind directly to SERCA [30].
was curcumin [1,7-bis(4-hydroxy-3-methoxyphenol)-1,6-heptadiene-3,5-dione] [35]. Curcumin is derived from the spice tumeric, which has been used as an anti-inflammatory agent and is currently being investigated as an anticancer drug [36]. Curcumin inhibits SERCA1 activity with a $K_i$ of 15 $\mu$M. This inhibition is non-competitive with respect to Ca$^{2+}$ and competitive with respect to ATP, as determined by a reduction in both ATP binding and ATP-dependent phosphoenzyme formation [35]. In addition, experiments with FITC-labelled ATPase show that it stabilizes the E1 conformation. FITC is known to label the Ca$^{2+}$-ATPase at Lys$^{515}$ within the ATP-binding site. Labelling this position of the ATPase with FITC is known to inhibit ATP binding.

The fact that curcumin still alters FITC–ATPase fluorescence must indicate that it does not bind to the nucleotide-binding site directly, but rather to another site within the ATPase that then induces a conformational change to prevent ATP binding. These findings have been interpreted as curcumin stabilizing the interaction between the nucleotide-binding and phosphorylation domains inhibiting ATP from binding. In order for phosphorylation to occur the nucleotide-binding domain (with ATP bound) and the phosphorylation domain need to come into close contact. Both domains are highly mobile and are known to move together and undergo major rearrangements in going from E1 to E2 conformations [13,37]. Additionally, in the E1 conformations, these two domains can also come into contact with each other by simple thermal fluctuations [37]. As these two domains are linked together via a ‘hinge’ region, we have speculated that curcumin could affect this region of the ATPase, locking the two domains together and thereby occluding the ATP-binding site. Alternatively, curcumin may stabilize the association between the nucleotide and phosphorylation domains by binding to the interface.

Flavonoids are commonly found in fruit and vegetables and have been shown to reach concentrations of several micromolar on human blood plasma [38]. Flavonoids are heterocyclic compounds consisting of three linked rings, of which two are aromatic (see Figure 1). These compounds are also believed to have cancer chemoprotective properties, possibly by triggering apoptosis via the Ca$^{2+}$-dependent mitochondrial pathway [39]. One mechanism by which this can occur is via exaggerated increases in cytosolic [Ca$^{2+}$], which could be due to the fact that some flavonoids are able to potently inhibit SERCA [40]. Of an extensive range of flavonoids tested, the most potent inhibitors were 3,6-dihydroxyflavone, quercetin and galangin (Table 1) [40]. A quantitative structure–activity relationship study indicated that polyhydroxylation was important, with hydroxylation at positions 3 and 6 (on rings C and A respectively). A detailed study also showed that the mechanism by which 3,6-dihydroxyflavone and galangin inhibit SERCA1A appears to be by altering the ATP affinity and the associated ATP-dependent phosphorylation step, in addition to stabilizing the enzyme in an E1 conformational state. Again, using energy-minimization molecular modelling programs, it was shown that these flavonoids appear to bind to the cytosolic region of the Ca$^{2+}$-ATPase between the ATP-binding and phosphorylation domains (Figure 4). Thus these flavonoids could prevent ATP from binding, in a similar manner to that postulated for curcumin, rather than directly occupying the nucleotide-binding site.

2APB (2-aminoethoxydiphenyl borate) has been used as both an InsP$_3$ receptor inhibitor [41] and an activator of store-operated Ca$^{2+}$ entry [42]. However, our research has also shown it to be a pH-sensitive SERCA inhibitor with an IC$_{50}$ of between 70 $\mu$M at pH 6 and 700 $\mu$M at pH 7 [43]. Although a relatively weak inhibitor, it was shown to inhibit Ca$^{2+}$ binding 20-fold, reducing the association rate of binding and increasing its rate of dissociation [43]. Furthermore, 2APB reduced phosphoryl transfer without inhibiting ATP binding, but still stabilizing the E1 conformational state [43]. Activity studies using a mutant form of SERCA1 where Tyr$^{397}$ was replaced by phenylalanine showed that it became insensitive to inhibition with 2APB. Mutation of another amino acid which is in close proximity to this residue (F834A), however, had little effect on 2-APB’s ability to inhibit SERCA1 [43]. Molecular modelling studies identified two potential binding sites for 2APB close to this residue near to transmembrane helices M3, M4, M5 and M7 and close to the cytoplasmic loop between M6 and M7 (L6–L7) (Figure 5). Taking into account that our studies showed that 2APB inhibited Ca$^{2+}$ binding, with the proposal that the L6–L7 loop has been implicated in
the Ca$^{2+}$ entry pathway/route by which Ca$^{2+}$ can enter their Ca$^{2+}$-binding sites [44,45], led us to suggest that, when 2APB binds to either of these sites, it acts as a ‘plug’ blocking access to Ca$^{2+}$-binding site I [43].

Can inhibitor studies tell us about the Ca$^{2+}$-entry pathways?
There have so far been several proposed Ca$^{2+}$-entry pathways by which the two Ca$^{2+}$ ions gain access to their respective binding sites [37,43,46]; however, there is no clear consensus. Our studies have so far identified two inhibitors (2APB and TBBPA) which can both inhibit Ca$^{2+}$ binding via different mechanisms involving different conformational states (E1 for 2APB and E2 for TBBPA). Furthermore, activity, mutagenesis and modelling studies have predicted that each bind to different sites at opposite sides of the transmembrane bundle, close to Ca$^{2+}$-binding site I in the case of 2APB and Ca$^{2+}$-binding site II for TBBPA. These observations have led us to speculate that the two Ca$^{2+}$ ions bind to their respective binding sites by different routes located at adjacent sides of the transmembrane bundle [30]. If such a possibility exists, then one would predict that, under some circumstances, the stoichiometry for Ca$^{2+}$ binding can go from 2 to 1 if one pathway was blocked independently. In support of this, we have already shown that when the Ca$^{2+}$-ATPase is reconstituted into short-chain phospholipid bilayers, the stoichiometry for Ca$^{2+}$ binding is reduced from 2 to 1 [47]. However, to confirm this hypothesis, more detailed Ca$^{2+}$-binding, mutagenesis and structural studies need to be undertaken.

Future directions
Isoform-specific inhibitors
Three isoforms of SERCA are known to exist in mammals, with approximately 75% sequence homology between one another and each isoform also existing in a variety of splice variant forms [48]. The expression profiles of the SERCA isoforms are known to vary both in different tissues and during development and disease [3,4,48]. Most tissue/cell types appear to express more than one SERCA isoform or splice-variant type, with each potentially associated with physiologically distinct Ca$^{2+}$ stores [49]. It would therefore be highly desirable to be able to distinguish and study these SERCA-isoform-specific Ca$^{2+}$ stores using isoform-specific inhibitors. A comparative study of the effects of a number of SERCA inhibitors was undertaken using cells overexpressing each isoform subtype. As highlighted above, thapsigargin is the most potent of all inhibitors, and its potency is different for the three isoforms, being most potent for SERCA1 ($K_i = 0.2\text{ nM}$) and relatively least potent for SERCA3 ($K_i = 12\text{ nM}$). Modelling of the three-dimensional structures of SERCA2 and SERCA3 suggested that there were minor differences in the thapsigargin-binding sites within the three isoforms which could account for these differences in potency [8]. CPA also showed some isoform selectivity, again being most potent for SERCA1 ($K_i = 90\text{ nM}$), least potent for SERCA2b ($K_i = 2.5\text{ \mu M}$) and intermediate for SERCA3 ($K_i = 600\text{ nM}$). All of the other inhibitors tested in this study showed only minor differences [8], except for curcumin, which was 6-fold less effective in inhibiting SERCA3a compared with SERCA2b. As most non-muscle tissues express either SERCA2b alone or in combination with SERCA 3, it would be ideal if one could identify better inhibitors which could be highly selective between these two isoforms. Therefore investigating the selective potencies of curcumin analogues may well prove useful.

Inhibitors that can differentiate between SERCA, PMCA and SPCA
It is clear that some SERCA inhibitors such as vanadate can also inhibit PMCA and SPCA located on the Golgi membrane. In order to selectively distinguish between these different types of Ca$^{2+}$-ATPases, thapsigargin has been the pharmacological tool of choice. It can be used in the 10–1000 nM range on cells to selectively inhibit SERCA without unduly affecting PMCA and SPCA, which requires several micromolar to achieve any degree of inhibition.

An approach involving screening a random peptide library for the binding of peptides to PMCA extracellular domains has recently led to the discovery of novel peptide PMCA inhibitors called caloxins which are highly selective towards PMCA over SERCA [50]. One such inhibitor, caloxin 1c2 (TAWSEVLDDLRRGGSK-amide), has a $K_i$ of 2.3 $\text{\mu M}$ for PMCA4. Thus these or related peptides may well prove to be useful in differentiating the roles that PMCA and SERCA play in cellular Ca$^{2+}$ homeostasis.
As yet, there are no inhibitors which can selectively inhibit SPCA. However, such an inhibitor would prove very useful in investigating the role SPCA and SPCA-loaded Ca\(^{2+}\) stores play in protein processing and protein trafficking through the Golgi.

**Inhibitors as cancer therapeutic agents**

Owing to the major role SERCA plays in maintaining intracellular Ca\(^{2+}\) levels within acceptable limits in order to avoid Ca\(^{2+}\)-mediated autophagy and apoptosis, there is some interest in its role in diseases such as cancer [3–5]. It has been observed in some transformed cells such as colon epithelial cells that there is a down-regulation of expression of SERCA3 when the cells become transformed during carcinogenesis [3, 51]. It also appears that the decreased expression of SERCA3 is more pronounced the more undifferentiated the cells become [3]. Conversely, colon cancer cells such as CaCO-2 can be induced to differentiate and show signs of more normal phenotypic behaviour if allowed to become highly confluent in culture. Under such conditions, they also increase their SERCA3 expression levels, proportionately [3]. Decreased expression of SERCA3 was also noted in transformed gastric cells and lymphocytic cells; however, little or no changes in SERCA2 expression levels were noted in these three cell types in transformed and differentiated states [3]. Furthermore, mutations in SERCA3 have also recently been found to occur in some head and neck squamous cell carcinomas [52]. Cancer cell SERCA isoform expression profiles also appear to be cell-type-specific, since when comparing SERCA2b expression in normal and transformed thyroid cells, a dramatic down-regulation in the expression of this particular isoform was shown to occur [4].

The fact that SERCA is decreased in some transformed cells and that some cancers cells appear to be more prone to Ca\(^{2+}\)-mediated apoptosis has led to an approach of developing thapsigargin-based anticancer drugs. Thapsigargin analogues have been developed into prodrugs by coupling them to a targeting peptide to produce an inactive precursor that only becomes activated once the specific peptide sequence is cleaved by a tissue-specific protease. Currently, a thapsigargin prodrug with a peptide that is targeted by prostate-specific proteases is being evaluated as a potential cancer chemotherapeutic agent for the treatment of prostate cancer [5,53].

As highlighted above, a number of flavonoids and curcumin are also being evaluated as anticancer agents owing to their anti-proliferative or apoptotic properties [36,40]. It would now appear that these compounds could also be exerting their effects on cancer cells by triggering apoptosis through a Ca\(^{2+}\)-mediated pathway involving SERCA inhibition [40], in addition to any effects on other pathways.

**Concluding remarks**

Inhibitor studies on SERCA pumps were of paramount importance in helping to elucidate the mechanism by which this transporter works. More recently, inhibitors have been useful in helping to determine the tertiary structure of specific conformational states.

Over the years, it has become apparent that SERCAs play an important role in Ca\(^{2+}\) homoeostasis in all mammalian cells and that their inhibition can lead to cell death. In identifying potent SERCA inhibitors, we now have a novel strategy for targeting certain types of cancer cells. It is hoped that, in the future, more potent and specifically targeted inhibitors can be developed to combat this disease.

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