World Journal of Biological Chemistry

Online Submissions: http://www.wjgnet.com/1949-8454office
wjbc@wjgnet.com
doi:10.4331/wjbc.v1.i7.221

World J Biol Chem 2010 July 26; 1(7): 221-228
ISSN 1949-8454 (online)

TOPIC HIGHLIGHT

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Plasma membrane calcium pump regulation by metabolic stress

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Author contributions: Bruce JIE solely contributed to this paper.
Supported by A New Investigator Award from the BBSRC
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Received: May 22, 2010 Revised: June 30, 2010
Accepted: July 7, 2010
Published online: July 26, 2010

Abstract

The plasma membrane Ca^{2+}-ATPase (PMCA) is an ATP-driven pump that is critical for the maintenance of low resting $[Ca^{2+}]_i$ in all eukaryotic cells. Metabolic stress, either due to inhibition of mitochondrial or glycolytic metabolism, has the capacity to cause ATP depletion and thus inhibit PMCA activity. This has potentially fatal consequences, particularly for non-excitable cells in which the PMCA is the major Ca^{2+} efflux pathway. This is because inhibition of the PMCA inevitably leads to cytosolic Ca^{2+} overload and the consequent cell death. However, the relationship between metabolic stress, ATP depletion and inhibition of the PMCA is not as simple as one would have originally predicted. There is increasing evidence that metabolic stress can lead to the inhibition of PMCA activity independent of ATP or prior to substantial ATP depletion. In particular, there is evidence that the PMCA has its own glycolytic ATP supply that can fuel the PMCA in the face of impaired mitochondrial function. Moreover, membrane phospholipids, mitochondrial membrane potential, caspase/calpain cleavage and oxidative stress have all been implicated in metabolic stress-induced inhibition of the PMCA. The major focus of this review is to challenge the conventional view of ATP-dependent regulation of the PMCA and bring together some of the alternative or additional mechanisms by which metabolic stress impairs PMCA activity resulting in cytosolic Ca^{2+} overload and cytotoxicity.

Key words: Plasma membrane Ca^{2+}-ATPase; Calcium overload; Metabolic stress; Calcium pump; Mitochondria

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Bruce JIE. Plasma membrane calcium pump regulation by metabolic stress. *World J Biol Chem* 2010; 1(7): 221-228 Available from: URL: http://www.wjgnet.com/1949-8454/full/v1/i7/221.htm DOI: http://dx.doi.org/10.4331/wjbc.v1.i7.221

INTRODUCTION

The ubiquitously expressed plasma membrane Ca^{2+}-ATPase (PMCA) is an ATP-driven Ca^{2+} pump that removes Ca^{2+} from the cytosol, thereby maintaining the low resting Ca^{2+} concentration ($[Ca^{2+}]_i$) in all eukaryotic cells[1]. In the face of cellular stress, when many other Ca^{2+} transport pathways are inhibited or impaired, the PMCA may be the last gatekeeper for the control of resting $[Ca^{2+}]_i$. This is particularly true for many non-excitable cells that do not abundantly express the Na^+-Ca^{2+} exchanger, such as pancreatic acinar cells[2], where the PMCA is the major Ca^{2+} efflux pathway. In these cells, if the PMCA is inhibited, for example during oxidative or metabolic stress, Ca^{2+} essentially has nowhere else to go and thus, over prolonged periods, this will lead to the eventual cytosolic Ca^{2+} overload and the inevitable cell death[1,3-5]. The mechanism of cell death (i.e. necrosis *vs* apoptosis) will largely depend on the extent of the metabolic stress. ATP depletion during extensive metabolic stress has been suggested to be the switch from apoptosis to necrosis[6-8],

largely because ATP is required for many of the apoptotic processes, but not for necrosis[8]. Intuitively, one might predict that ATP depletion would cause inhibition of the PMCA leading to necrotic cell death and indeed there is compelling evidence in support of this idea[9-11]. However, this is likely to be an oversimplification and the sequence of these events is not necessarily as straightforward as one might predict. The focus of this review is to challenge this rather simplistic and dogmatic view of ATP-dependent regulation of the PMCA and describe some alternative or additional mechanisms by which metabolism, and in particular impaired metabolism, may differentially regulate the PMCA.

ATP DEPENDENCY OF PLASMA MEMBRANE Ca^{2+}-ATPase

PMCA is an ATP-driven pump and so by definition PMCA activity is critically dependent on ATP. Early studies showed that PMCA has a high affinity catalytic site (Km, 3 μmol/L) and a low affinity regulatory site (Km, 145 μmol/L)[12]. However, the absolute ATP-dependency of the PMCA is controversial and has recently been suggested to be more complex than proposed in earlier studies[13]. Nevertheless, this raises two related but critical questions. First, by how much would ATP levels have to drop to inhibit PMCA activity when metabolism is inhibited? Presumably, this will depend on whether mitochondria or glycolysis is inhibited, the overall metabolic activity of the cell and thus how quickly ATP is consumed within the cell. Secondly, would inhibition of mitochondrial metabolism alone be enough to deplete ATP levels sufficiently to inhibit the PMCA activity, especially if glycolytic ATP production remains active? Presumably, this will depend on the relative contribution of mitochondrial metabolism versus glycolytic metabolism to global ATP production. The classic textbook view is that mitochondrial metabolism contributes approximately 95% of ATP (i.e. 32 molecules of ATP per glucose molecule), whereas glycolysis provides only 5% (i.e. 2 molecules of ATP per glucose molecule). Although this is likely to be true for many cells under optimum, normoxic conditions, it is likely to be an oversimplification and many cells exhibit metabolic plasticity and an incredible ability to adapt to their environment, for example during hypoxia. Cancer cells are an extreme example of this and often undergo a dramatic switch from mitochondrial metabolism to glycolytic metabolism due to mutations of key mitochondrial enzymes and a profound overexpression of glycolytic enzymes[14,15].

Moreover, it has been suggested that the PMCA has its own localised glycolytic ATP supply that may render it largely insensitive to inhibition of mitochondrial metabolism[16-18]. Specifically, these studies showed that in isolated inside-out plasma membrane vesicles from pig stomach smooth muscle enriched with PMCA, an endogenous membrane-bound glycolytic system provided ATP to fuel the PMCA-dependent Ca^{2+} uptake[16,17]. Moreover, providing the glycolytic substrates were present the Ca^{2+} uptake (PMCA activity) persisted in the absence of an exogenously applied ATP regenerating system and the presence of a hexokinase-based ATP trap[16,17].

An important *caveat* when interpreting experiments to determine the ATP-dependency of the PMCA is that most of these studies have utilized cell-free *in vitro* assays, such as ATPase phosphorylation assays using purified PMCA protein, or Ca^{2+}-uptake assays using PMCA-enriched membrane vesicles, whereby the ATP-dependency may be influenced by multiple factors such as [Mg^{2+}], [Ca^{2+}], calmodulin (CaM) and the phospholipid composition of the membrane[12,16,17,19,20]. This makes extrapolation to experiments using intact cells difficult, especially when one considers the numerous cytosolic factors, metabolic factors and plasma membrane interactions that might also influence PMCA activity. On the other hand, the benefit of using *in vitro* assays to determine the ATP-dependency of the PMCA activity is that one has full control over the absolute ATP concentration. Although measuring PMCA activity *in situ* (i.e. within intact cells or tissues) clearly represents a more physiological system in which the PMCA is more likely to behave "normally", it is more difficult to accurately predict the ATP concentration and almost all methods have their limitations.

MEASUREMENT OF ATP TO DETERMINE ATP-DEPENDENCY OF PLASMA MEMBRANE Ca^{2+}-ATPase ACTIVITY

In vitro chemiluminescence of firefly luciferase

By far the most commonly used method for measuring ATP concentration is the use of fire-fly luciferase-catalysed ATP-dependent oxidation of luciferin, which emits luminescence[21,22]. This usually requires either lysing, homogenising or snap freezing the cells to extract the total ATP, which is invasive, artefact-prone and insufficient to measure dynamic changes of ATP on a moment-to-moment basis. Moreover, these methods also represent a measure of total cellular ATP and do not take into account differences of ATP concentration in different organelles.

In vivo bioluminescence of expressed luciferase

An extension of the above method is to overexpress luciferase in intact cells, which circumvents some of the problems described above and is regarded by many as the method of choice for measuring ATP in a variety of cells[9,23-26]. However, the drawback of this technique is that the expression of luciferase needs to be relatively high in order to detect the low levels of luminescence and thus usually requires adenoviral transduction methods. This is particularly useful for cultured cell lines or primary cultured cells but is more problematic for terminally differentiated acutely isolated cells, such as pancreatic acinar cells. Nevertheless, Barrow *et al*[9] and Voronina *et al*[26] have perfected this technique with relative ease in pancreatic acinar cells. However, one potential problem is that, while functional responses (e.g. signalling and secretory responses) appear reasonably well-preserved following adenoviral transduction

and short-term culture[9,26,27], the metabolic capacity of these cells may be severely compromised. Several studies using expressed luciferase have shown that metabolic inhibition can induce relatively rapid and severe ATP depletion within just a few minutes[9,23-26], broadly in line with *in vitro* methods.

Nuclear magnetic resonance spectroscopy is a non-invasive technique for accurately measuring ATP levels (and metabolites), primarily based on the ^{31}P NMR spectrum, in intact perfused organs and tissues, such as heart[28], salivary gland[29] and different smooth muscle tissues (vascular, uterine and gastrointestinal)[30]. These techniques have generally demonstrated that severe metabolic inhibition, for example using cyanide or ischaemia/anoxia, takes much longer (several tens of minutes) to fully deplete ATP and sometimes this is not complete[28,30].

The Mg^{2+}-sensitive fluorescent dyes, such as magfura or magnesium green (MgGreen) have been used extensively as an indirect measure of cytosolic ATP concentration[10,31,32]. This is based on the principle that almost all cytosolic ATP exists as MgATP, such that ATP depletion causes an increase in free Mg^{2+} concentration ($[Mg^{2+}]_i$). MgGreen has a Kd for Mg^{2+} of approximately 0.9mmol/L, close to the resting $[Mg^{2+}]_i$, thus making it useful for sensing ATP concentration changes around the physiological range[32]. In cardiac myocytes, estimates of ATP concentration using MgGreen are comparable to those obtained using NMR methods[33].

DOES ATP DEPLETION INHIBIT PLASMA MEMBRANE Ca^{2+}-ATPase ACTIVITY DURING METABOLIC INHIBITION?

The answer to this question will depend on the "extent" of metabolic stress and whether mitochondria or glycolysis is inhibited. It will also depend on the method of choice for measuring ATP depletion, how quickly ATP is depleted and whether this is sufficient to inhibit the PMCA. We have previously reported that oxidative stress, induced by H_2O_2, which is known to inhibit both mitochondrial and glycolytic metabolism[34], induced an irreversible Ca^{2+} overload and marked inhibition of the PMCA in pancreatic acinar cells[31,35]. This H_2O_2-induced PMCA inhibition could occur without mitochondrial Ca^{2+} handling, coincided with mitochondrial depolarization and was sensitive to inhibitors of the mitochondrial permeability transition pore (mPTP)[31]. However, based on our MgGreen measurements of ATP, inhibition of the PMCA could occur independent or prior to any substantial ATP depletion.

Using MgGreen fluorescence to measure ATP levels we found that maximum ATP depletion could only be achieved with an "ATP-depletion" cocktail designed to maximise ATP consumption and simultaneously inhibit all ATP production. This consisted of CCCP, a mitochondrial uncoupler and protonophore that dissipates the mitochondrial membrane potential; oligomycin, to inhibit the ATP synthase; iodoacetate, to inhibit glycolysis; and carbachol to maximally stimulate the cells and thus maximally consume ATP. In addition, even using this ATP depletion cocktail, the rise in MgGreen fluorescence (fall in ATP concentration) took up to 40 min to reach a maximum with a latency of approximately 8 min. In addition when applied separately, three mechanistically distinct mitochondrial inhibitors (CCCP, antimycin-A and oligomycin) depleted ATP levels by similar amounts (approximately 20%), consistent with inhibition of mitochondrial metabolism. However, oligomycin had no effect on PMCA activity, while CCCP and antimycin both inhibited PMCA by approximately 50%[31]. The high concentration of 500 μmol/L H_2O_2, which inhibited PMCA by approximately 80%, caused only approximately 55% ATP depletion, which was greater than the mitochondrial inhibitors alone but much less than the ATP depletion cocktail. This suggested that high concentrations of H_2O_2 inhibit both mitochondria and glycolysis, consistent with previous studies[34,36]. Interestingly, however, the lower concentration of 50 μmol/L H_2O_2 had no effect on ATP depletion and yet significantly inhibited PMCA activity by approximately 50%. It is also worth noting that H_2O_2 can directly oxidize the PMCA[37] and reduce the PMCA expression at the plasma membrane[38] (see later) suggesting that inhibition of the PMCA by H_2O_2 can occur independent of metabolic inhibition and ATP depletion. Furthermore, the PMCA was inhibited within 5-10 min of treatment with mitochondrial inhibitors (CCCP or antimycin), which was well before any substantial ATP depletion was observed even with the ATP depletion cocktail[31].

This is not an unprecedented phenomena and using NMR to measure ATP, inhibition of mitochondria with cyanide caused a partial ATP depletion in smooth muscle[30]. This was suggested to be due to compartmentalised glycolytic metabolism that was sufficient to maintain ATP to fuel plasma membrane pumps[30], consistent with the idea that the PMCA has its own glycolytic ATP supply[16,17].

In studies using a luciferase assay to measure ATP in HeLa cells, a high concentration of H_2O_2 (32 mmol/L) caused ATP depletion to undetectable levels within 15 min[39]. Similarly, using the same technique, a variety of classical glycolytic inhibitors (iodoacetate), mitochondrial inhibitors (oligomycin, rotenone) and pancreatitis-inducing agents (bile acids, ethanol metabolites, fatty acids) that disrupt mitochondrial function[10,40], all induced substantial ATP depletion within 5-20 min[9,26]. Indeed, many of these reagents also inhibited PMCA activity[9].

There remains compelling evidence that severe ATP depletion can inhibit the PMCA under certain conditions[9-11]. PMCA activity was shown to decrease approximately 5 fold within 800 s of "complete" ATP depletion, for example following the combined treatment with oligomycin and iodoacetate in pancreatic acinar cells[9]. In another study by the same group, fatty acid ethyl esters (FAEE) induced a Ca^{2+} overload response, mitochondrial depolarisation and ATP depletion[10]. Moreover, this FAEE-induced Ca^{2+} overload response was largely abrogated by replenishment of ATP *via* a patch pipette, strongly suggesting that this response was due to ATP depletion, which the authors partly attributed to inhibition of the PMCA[10].

However, PMCA activity was not directly measured in this study[10].

In relation to this, studies in HeLa cells demonstrated that high [necrosis-inducing] concentrations of H_2O_2 (32 mmol/L) for prolonged periods (> 20 min) caused a secondary Ca^{2+} overload response that was due to Na^+ influx and inhibition of the PMCA activity[11]. This was suggested to be due to ATP depletion[39], which preferentially inhibited PMCA activity over the Na^+-K^+-ATPase, despite having similar ATP dependencies[11]. The authors concluded that this was due to a thermodynamic shift of free energies of the pumps in favour of the Na^+-K^+-ATPase[11]. It was argued that the H_2O_2-induced Na^+ influx reduced the free energy for the Na^+ pump, due to the dissipation of the ion gradients, without any appreciable effect on the free energy for the PMCA (Ca^{2+} gradient maintained). After prolonged periods (> 20 min), ATP reaches a critical concentration where it becomes more energetically favourable to pump Na^+ than Ca^{2+} and so the PMCA is inhibited in favour of a fully functional Na^+-K^+-ATPase. In other words, the Na^+-pump "steals" ATP from the PMCA[11]. The key evidence in support of this idea came from experiments using an *in situ* $[Ca^{2+}]_i$ clearance assay, similar to our previous studies[31,35,41], in which PMCA activity was functionally isolated in intact cells. In these experiments, H_2O_2-inhibited $[Ca^{2+}]_i$ clearance (PMCA activity) and induced a Ca^{2+} overload response, both of which were reversed by ouabain, suggesting that inhibition of the PMCA requires functional Na-K-ATPase activity[11]. This study suggests that the PMCA may be inhibited by submaximal ATP depletion making this a potentially desirable mechanism to help reconcile some of the conflicting observations and interpretations. However, in the above study[11], the H_2O_2-evoked Ca^{2+} overload response was biphasic and it was the large, delayed increase in $[Ca^{2+}]_i$ that was attributed to inhibition of the PMCA, which occured following 20-60 min of H_2O_2 treatment.

ALTERNATIVE MECHANISMS FOR PLASMA MEMBRANE Ca^{2+}-ATPase INHIBITION DURING METABOLIC STRESS

Regulation of the plasma membrane Ca^{2+}-ATPase by acidic phospholipids

There is an important *caveat* to the idea that submaximal/partial ATP depletion is insufficient to inhibit PMCA activity, as observed in our own previous study[31]. Acidic phospholipids, such as phosphatidylinositol (PI) and phosphatidylserine (PS) increase the ATP sensitivity of the PMCA and mimic regulation by CaM[19,20]. In particular, removal of PS (or PI) from the lipid environment of the PMCA decreased the affinity for ATP at the low affinity regulatory site (Km, 5-10 mmol/L)[42,43]. This therefore suggests that depletion of PS (or PI) from the membrane may be sufficient to render the PMCA highly sensitive to ATP depletion, or even partially inactive at resting cytosolic ATP concentration. However, the evidence is based on *in vitro* experiments whereby PS/PI was either absent or present in an artifical membrane, which makes it difficult to extrapolate to intact cells. For example, it is unclear from these studies what the critical concentration of PS is to maintain "normal" ATP-sensitivity of the PMCA and if any other factors in the membrane or cytosol might affect this relationship. In particular, PS is known to line the inner leaflet of the plasma membrane and a proportion is thought to flip to the extracellular side of the membrane during apoptosis[44]. This is believed to make the cell surface "sticky" allowing macrophages to detect dying cells so that they can be phagocytosed and removed from the tissue[44]. Furthermore, aminophospholipid translocase (flippase), the enzyme responsible for this PS asymmetry within the plasma membrane[45], is dependent on millimolar ATP[46,47] and can also be inhibited by oxidative stress[48,49]. It is unclear whether externalisation of PS from the internal phospholipid membrane would be sufficient to affect the ATP sensitivity of the PMCA. However, there is evidence from Jurkat T lymphocytes, that H_2O_2 inhibits PS synthesis[50]. Whether either of these phenomena would be sufficient to affect the ATP sensitivity of the PMCA such that only mild ATP depletion would cause marked inhibition of the PMCA remains to be determined. Nevertheless, this is an interesting possibility that could help to reconcile some of the competing arguments described above.

Effect of mitochondrially-derived reactive oxygen species

Severe mitochondrial stress, whatever the mechanism, often leads to the generation of reactive oxygen species (ROS)[51]. ROS are generated by incomplete reduction of oxygen during the process of oxidative metabolism. In fact, approximately 1%-5% of electrons "escape" the electron transport chain to generate superoxide ($O_2^{\cdot-}$)[51]. The principal source of superoxide is complex Ⅲ[52] and complex I [53] which in turn can be converted to H_2O_2 by superoxide dismutase and released by mitochondria. There is also good evidence that oxidants (H_2O_2) can directly oxidise the PMCA and also oxidise CaM, which is the main activator of the PMCA[37]. Hence, metabolically derived ROS may have a profound inhibitory effect on the PMCA activity.

In addition, H_2O_2 has been reported to reduce the functional expression of PMCA at the plasma membrane of culture hippocampal neurons within 1-2 h[38]. Such rapid changes in functional expression of PMCA at the plasma membrane could lead to reduced Ca^{2+} efflux during metabolic stress even in the presence of continued high ATP levels.

Effect of mitochondrial membrane potential on plasma membrane Ca^{2+}-ATPase activity

Notwithstanding the various *caveats*, our data do suggest that under conditions of metabolic or oxidant stress, when ATP depletion is only partial, non-ATP dependent mechanisms can also inhibit PMCA activity, thus revealing a potentially novel mechanism[31]. Inhibition of the PMCA was independent of mitochondrial Ca^{2+} handling and coincided with depolarisation of the mitochondrial

membrane potential (Ψm). Interestingly, all the reagents tested that inhibited the PMCA also caused mitochondrial depolarisation, regardless of whether they caused ATP depletion (H_2O_2, CCCP, antimycin-A, but not oligomycin). This suggested that collapse of the mitochondrial Ψm may be responsible, at least in part, for the inhibition of the PMCA activity or that both phenomena share a common cause. Similar studies in pancreatic acinar cells[10,54] have also shown that oxidants, CCCP and antimycin-A all cause mitochondrial Ψm depolarisation and in adrenal chromaffin cells[55] oligomycin has no effect. Moreover, the oxidant-induced mitochondrial Ψm depolarisation and inhibition of the PMCA were both attenuated by inhibitors of the mitochondrial permeability transition pore (bongkrekic acid and cyclosporin-A)[31], suggesting that inhibition of the PMCA was partly mediated by the opening of the mPTP.

Opening of the mPTP is likely to cause dissipation of the mitochondrial H^+ gradient, which could also conceivably inhibit the PMCA as Ca^{2+} efflux is coupled to H^+ influx[56]. This may be particularly important in regions where mitochondria are in close proximity to the plasma membrane[57] and thus PMCA, whereby local pH changes may have a profound effect. However, previous studies in pancreatic acinar cells have shown that CCCP has no appreciable effect on bulk cytosolic pH[58] and, since any local pH changes are likely to be rapidly buffered, it is unlikely that local pH changes are responsible for a prolonged and sustained inhibition of the PMCA.

Release of mitochondrial factors?

Another possibility is that opening of the mPTP causes the release of some mitochondrial factor, such as a protein (e.g. cytochrome C[59,60]) or lipid (cardiolipin[61]), that normally resides within the matrix, intermembrane space or associated with either the inner or outer mitochondrial membrane. This phenomenon has been well characterised and is strongly implicated as one of the early events during apoptosis[59,62]. The mechanism remains controversial but some studies suggest that, under certain conditions, the mPTP becomes permeable to small proteins or may facilitate mitochondrial swelling that ultimately leads to rupture and the release of much larger proteins[63]. Although speculative, such a putative mPTP-dependent, mitochondrially released factor may inhibit the PMCA, either by direct binding or most likely by regulation of some other signalling pathway. It is interesting to note that the time-frame over which cytochrome C release can occur (> 2 min)[54] concides with the time the PMCA can be observed to be inhibited, and well before ATP depletion was observed[31].

Although cytochrome C is perhaps the best characterised factor to be released from the mitochondria during apoptosis, several other proteins have also been characterised, including Smac/DIABLO (second mitochondria-derived activator of caspase/direct IAP-binding protein with low pl), HtrA2 (high temperature requirement protease A2), Endo G and AIF (apoptosis-inducing factor)[59,60]. Binding and/or regulation of Ca^{2+} transport proteins by mitochondrially-released proteins is not an unprecedented phenomenon. For example, cytochrome C[64], Bax/Bak[65] and Bcl-2/Bcl-xL[66-68], have all been shown to bind to and regulate inositol 1,4,5-trisphosphate receptors, whereas Bcl-2 can regulate SERCA[69]. However, this is highly speculative and, to the authors' knowledge, none of the above mentioned, putative mitochondrially-derived, PMCA inhibitory factors have been shown to bind directly to the PMCA.

However, the release of cytochrome C and the subsequent activation of caspases and calpain[70] have both been reported to cleave and eventually lead to the inactivation of the PMCA[5,71,72]. In fact, the initial cleavage by caspase and calpain actually activates the pump, but through the subsequent internalisation and degradation of the PMCA the protease effect is manifested as pump inhibition[73,74]. Specific caspase-3 cleavage of PMCA4b produces a 120 kDa fragment that is constitutively activated, due to the removal of the autoinhibitory domain[3,4,75]. It is also interesting to note that calpain can also be directly activated by H_2O_2 and Ca^{2+}[76].

It is also worth mentioning that, in addition to the release of a mitochondrially-derived protein, the mitochondria can also release the mitochondrial-specific lipid cardiolipin during apoptosis[61]. Under normal situations, cardiolipin resides on the inner mitochondrial membrane, where it couples to cytochrome C and maintains normal electron transport. However, following treatment with apoptosis-inducing agents (e.g. Fas stimulation of the death receptor), cardiolipin can translocate from the mitochondria to other organelles, in particular to the plasma membrane[77,78]. This is particularly important because cardiolipin is another of the acidic phospholipids reported to modulate the PMCA activity in the same way as phophatidylserine and phosphatidylinositol[19]. Therefore, the translocation of cardiolipin to the plasma membrane during cell stress or apoptosis might be expected to increase PMCA activity or increase the affinity for ATP, thereby making the PMCA less sensitive to ATP depletion. This seems counterintuitive but may represent a protective mechanism to circumvent the effect of phophatidylserine externalisation on the PMCA that occurs during apoptosis. These are interesting possibilities, although the direct effects of phosphatidylserine externalisation and plasma membrane cardiolipin on PMCA activity within intact cells during apoptosis are unknown and warrant further investigation.

In addition, in the context of cell death and cytoprotection, the impairment of PMCA function and subsequent dysregulation of cytosolic Ca^{2+} homeostasis can, in some cases, be cytoprotective[79]. During oxidative stress or tumour necrosis factor (TNF)-induced cell death, the accumulation and damage of lysosomes has been suggested to be important[80,81]. In TNF-resistant cell lines, in which PMCA4 is mutated, the resulting enhanced Ca^{2+} signalling has been shown to promote the exocytotic loss of lysosomes resulting in protection against TNF-induced cell death[79]. This therefore suggests, somewhat counterintuitively, that PMCA4 promotes TNF-induced cell death.

CONCLUSION

The PMCA is critical for maintaining low resting cytosolic Ca^{2+} and for preventing cytotoxic Ca^{2+} overload and cell death. Since the PMCA is an ATP-driven pump, by definition, severe metabolic stress and ATP depletion should lead to inhibition of the PMCA and thus Ca^{2+} overload and cell death. However, this relationship is likely an oversimplification and there is now increasing evidence that there are additional mechanisms that link metabolic stress and regulation of PMCA activity. There is good evidence that the PMCA has its own glycolytic supply of ATP that fuels the PMCA, which, in the context of PMCA function, makes the mitochondria somewhat redundant as a source of ATP. However, the functional relationship between the mitochondria and the PMCA has become increasingly more intimate and complex and is not as simple as the provision of ATP. In addition to mitochondrial-derived ATP depletion, impaired mitochondrial function can regulate the PMCA as a consequence of oxidative stress, which can directly oxidise the PMCA, alter the membrane expression of the PMCA, and induce the release of mitochondrial factors. This in turn can lead to the subsequent caspase/calpain cleavage and the regulation of membrane phospholipids, phosphatidylserine or cardiolipin. Protection of the PMCA *via* either of these mechanisms may represent important therapeutic strategies for the treatment of a variety of diseases in which cytosolic Ca^{2+} overload induces cell death, such as pancreatitis, ischaemia-reperfusion injury and myocardial infarction.

ACKNOWLEDGMENTS

The author would like to that thank all contributors to the original work described within this review but particularly Erin Baggaley, Parini Mankad, Omar Masood, Emily Burgess, Ajith Siriwardena and Austin Elliott.

REFERENCES

1 **Brini M**, Carafoli E. Calcium pumps in health and disease. *Physiol Rev* 2009; **89**: 1341-1378
2 **Muallem S**, Beeker T, Pandol SJ. Role of Na+/Ca2+ exchange and the plasma membrane Ca2+ pump in hormone-mediated Ca2+ efflux from pancreatic acini. *J Membr Biol* 1988; **102**: 153-162
3 **Pászty K**, Antalffy G, Hegedüs L, Padányi R, Penheiter AR, Filoteo AG, Penniston JT, Enyedi A. Cleavage of the plasma membrane Ca+ATPase during apoptosis. *Ann N Y Acad Sci* 2007; **1099**: 440-450
4 **Pászty K**, Verma AK, Padányi R, Filoteo AG, Penniston JT, Enyedi A. Plasma membrane Ca2+ATPase isoform 4b is cleaved and activated by caspase-3 during the early phase of apoptosis. *J Biol Chem* 2002; **277**: 6822-6829
5 **Schwab BL**, Guerini D, Didszun C, Bano D, Ferrando-May E, Fava E, Tam J, Xu D, Xanthoudakis S, Nicholson DW, Carafoli E, Nicotera P. Cleavage of plasma membrane calcium pumps by caspases: a link between apoptosis and necrosis. *Cell Death Differ* 2002; **9**: 818-831
6 **Criddle DN**, Gerasimenko JV, Baumgartner HK, Jaffar M, Voronina S, Sutton R, Petersen OH, Gerasimenko OV. Calcium signalling and pancreatic cell death: apoptosis or necrosis? *Cell Death Differ* 2007; **14**: 1285-1294
7 **Kim JS**, Qian T, Lemasters JJ. Mitochondrial permeability transition in the switch from necrotic to apoptotic cell death in ischemic rat hepatocytes. *Gastroenterology* 2003; **124**: 494-503
8 **Leist M**, Single B, Castoldi AF, Kühnle S, Nicotera P. Intracellular adenosine triphosphate (ATP) concentration: a switch in the decision between apoptosis and necrosis. *J Exp Med* 1997; **185**: 1481-1486
9 **Barrow SL**, Voronina SG, da Silva Xavier G, Chvanov MA, Longbottom RE, Gerasimenko OV, Petersen OH, Rutter GA, Tepikin AV. ATP depletion inhibits Ca2+ release, influx and extrusion in pancreatic acinar cells but not pathological Ca2+ responses induced by bile. *Pflugers Arch* 2008; **455**: 1025-1039
10 **Criddle DN**, Murphy J, Fistetto G, Barrow S, Tepikin AV, Neoptolemos JP, Sutton R, Petersen OH. Fatty acid ethyl esters cause pancreatic calcium toxicity via inositol trisphosphate receptors and loss of ATP synthesis. *Gastroenterology* 2006; **130**: 781-793
11 **Castro J**, Ruminot I, Porras OH, Flores CM, Hermosilla T, Verdugo E, Venegas F, Härtel S, Michea L, Barros LF. ATP steal between cation pumps: a mechanism linking Na+ influx to the onset of necrotic Ca2+ overload. *Cell Death Differ* 2006; **13**: 1675-1685
12 **Richards DE**, Rega AF, Garrahan PJ. Two classes of site for ATP in the Ca2+-ATPase from human red cell membranes. *Biochim Biophys Acta* 1978; **511**: 194-201
13 **Echarte MM**, Rossi RC, Rossi JP. Phosphorylation of the plasma membrane calcium pump at high ATP concentration. On the mechanism of ATP hydrolysis. *Biochemistry* 2007; **46**: 1034-1041
14 **Pedersen PL**. Warburg, me and Hexokinase 2: Multiple discoveries of key molecular events underlying one of cancers' most common phenotypes, the "Warburg Effect", i.e., elevated glycolysis in the presence of oxygen. *J Bioenerg Biomembr* 2007; **39**: 211-222
15 **Pedersen PL**. The cancer cell's "power plants" as promising therapeutic targets: an overview. *J Bioenerg Biomembr* 2007; **39**: 1-12
16 **Hardin CD**, Raeymaekers L, Paul RJ. Comparison of endogenous and exogenous sources of ATP in fueling Ca2+ uptake in smooth muscle plasma membrane vesicles. *J Gen Physiol* 1992; **99**: 21-40
17 **Hardin CD**, Zhang C, Kranias EG, Steenaart NA, Raeymaekers L, Paul RJ. Regulation of glycolytically fueled Ca2+ uptake in smooth muscle plasmalemmal vesicles by phosphorylation. *Am J Physiol* 1993; **265**: H1326-H1333
18 **Paul RJ**, Hardin CD, Raeymaekers L, Wuytack F, Casteels R. Preferential support of Ca2+ uptake in smooth muscle plasma membrane vesicles by an endogenous glycolytic cascade. *FASEB J* 1989; **3**: 2298-2301
19 **Niggli V**, Adunyah ES, Carafoli E. Acidic phospholipids, unsaturated fatty acids, and limited proteolysis mimic the effect of calmodulin on the purified erythrocyte Ca2+ - ATPase. *J Biol Chem* 1981; **256**: 8588-8592
20 **Niggli V**, Adunyah ES, Penniston JT, Carafoli E. Purified (Ca2+-Mg2+)-ATPase of the erythrocyte membrane. Reconstitution and effect of calmodulin and phospholipids. *J Biol Chem* 1981; **256**: 395-401
21 **Bell CJ**, Manfredi G, Griffiths EJ, Rutter GA. Luciferase expression for ATP imaging: application to cardiac myocytes. *Methods Cell Biol* 2007; **80**: 341-352
22 **Stanley PE**. A review of bioluminescent ATP techniques in rapid microbiology. *J Biolumin Chemilumin* 1989; **4**: 375-380
23 **Jouaville LS**, Pinton P, Bastianutto C, Rutter GA, Rizzuto R. Regulation of mitochondrial ATP synthesis by calcium: evidence for a long-term metabolic priming. *Proc Natl Acad Sci USA* 1999; **96**: 13807-13812
24 **Kennedy HJ**, Pouli AE, Ainscow EK, Jouaville LS, Rizzuto R, Rutter GA. Glucose generates sub-plasma membrane ATP microdomains in single islet beta-cells. Potential role for

strategically located mitochondria. *J Biol Chem* 1999; **274**: 13281-13291
25 **Koop A**, Cobbold PH. Continuous bioluminescent monitoring of cytoplasmic ATP in single isolated rat hepatocytes during metabolic poisoning. *Biochem J* 1993; **295** (Pt 1): 165-170
26 **Voronina SG**, Barrow SL, Simpson AW, Gerasimenko OV, da Silva Xavier G, Rutter GA, Petersen OH, Tepikin AV. Dynamic changes in cytosolic and mitochondrial ATP levels in pancreatic acinar cells. *Gastroenterology* 2010; **138**: 1976-1987
27 **Padfield PJ**, Elliott AC, Baldassare JJ. Adenovirus-mediated gene expression in isolated rat pancreatic acini and individual pancreatic acinar cells. *Pflugers Arch* 1998; **436**: 782-787
28 **Elliott AC**, Smith GL, Allen DG. Simultaneous measurements of action potential duration and intracellular ATP in isolated ferret hearts exposed to cyanide. *Circ Res* 1989; **64**: 583-591
29 **Murakami M**, Seo Y. Energy consumption associated with fluid and electrolyte transport in the mandibular and parotid glands. *Eur J Morphol* 1998; **36** Suppl: 128-131
30 **Nakayama S**, Clark JF. Smooth muscle and NMR review: an overview of smooth muscle metabolism. *Mol Cell Biochem* 2003; **244**: 17-30
31 **Baggaley EM**, Elliott AC, Bruce JI. Oxidant-induced inhibition of the plasma membrane Ca2+-ATPase in pancreatic acinar cells: role of the mitochondria. *Am J Physiol Cell Physiol* 2008; **295**: C1247-C1260
32 **Leyssens A**, Nowicky AV, Patterson L, Crompton M, Duchen MR. The relationship between mitochondrial state, ATP hydrolysis, [Mg2+]i and [Ca2+]i studied in isolated rat cardiomyocytes. *J Physiol* 1996; **496** (Pt 1): 111-128
33 **Murphy E**, Steenbergen C, Levy LA, Raju B, London RE. Cytosolic free magnesium levels in ischemic rat heart. *J Biol Chem* 1989; **264**: 5622-5627
34 **Hyslop PA**, Hinshaw DB, Halsey WA Jr, Schraufstätter IU, Sauerheber RD, Spragg RG, Jackson JH, Cochrane CG. Mechanisms of oxidant-mediated cell injury. The glycolytic and mitochondrial pathways of ADP phosphorylation are major intracellular targets inactivated by hydrogen peroxide. *J Biol Chem* 1988; **263**: 1665-1675
35 **Bruce JI**, Elliott AC. Oxidant-impaired intracellular Ca2+ signaling in pancreatic acinar cells: role of the plasma membrane Ca2+-ATPase. *Am J Physiol Cell Physiol* 2007; **293**: C938-C950
36 **Colussi C**, Albertini MC, Coppola S, Rovidati S, Galli F, Ghibelli L. H2O2-induced block of glycolysis as an active ADP-ribosylation reaction protecting cells from apoptosis. *FASEB J* 2000; **14**: 2266-2276
37 **Zaidi A**, Michaelis ML. Effects of reactive oxygen species on brain synaptic plasma membrane Ca(2+)-ATPase. *Free Radic Biol Med* 1999; **27**: 810-821
38 **Kip SN**, Strehler EE. Rapid downregulation of NCX and PMCA in hippocampal neurons following H2O2 oxidative stress. *Ann N Y Acad Sci* 2007; **1099**: 436-439
39 **Barros LF**, Kanaseki T, Sabirov R, Morishima S, Castro J, Bittner CX, Maeno E, Ando-Akatsuka Y, Okada Y. Apoptotic and necrotic blebs in epithelial cells display similar neck diameters but different kinase dependency. *Cell Death Differ* 2003; **10**: 687-697
40 **Voronina SG**, Barrow SL, Gerasimenko OV, Petersen OH, Tepikin AV. Effects of secretagogues and bile acids on mitochondrial membrane potential of pancreatic acinar cells: comparison of different modes of evaluating DeltaPsim. *J Biol Chem* 2004; **279**: 27327-27338
41 **Baggaley E**, McLarnon S, Demeter I, Varga G, Bruce JI. Differential regulation of the apical plasma membrane Ca(2+)-ATPase by protein kinase A in parotid acinar cells. *J Biol Chem* 2007; **282**: 37678-37693
42 **Lehotsky J**, Raeymaekers L, Missiaen L, Wuytack F, De Smedt H, Casteels R. Stimulation of the catalytic cycle of the Ca2+ pump of porcine plasma-membranes by negatively charged phospholipids. *Biochim Biophys Acta* 1992; **1105**: 118-124
43 **Rossi JP**, Rega AF. A study to see whether phosphatidylserine, partial proteolysis and EGTA substitute for calmodulin during activation of the Ca2+-ATPase from red cell membranes by ATP. *Biochim Biophys Acta* 1989; **996**: 153-159
44 **Fadok VA**, Voelker DR, Campbell PA, Cohen JJ, Bratton DL, Henson PM. Exposure of phosphatidylserine on the surface of apoptotic lymphocytes triggers specific recognition and removal by macrophages. *J Immunol* 1992; **148**: 2207-2216
45 **Daleke DL**. Phospholipid flippases. *J Biol Chem* 2007; **282**: 821-825
46 **Seigneuret M**, Devaux PF. ATP-dependent asymmetric distribution of spin-labeled phospholipids in the erythrocyte membrane: relation to shape changes. *Proc Natl Acad Sci USA* 1984; **81**: 3751-3755
47 **Smriti**, Nemergut EC, Daleke DL. ATP-dependent transport of phosphatidylserine analogues in human erythrocytes. *Biochemistry* 2007; **46**: 2249-2259
48 **Brunauer LS**, Moxness MS, Huestis WH. Hydrogen peroxide oxidation induces the transfer of phospholipids from the membrane into the cytosol of human erythrocytes. *Biochemistry* 1994; **33**: 4527-4532
49 **Herrmann A**, Devaux PF. Alteration of the aminophospholipid translocase activity during in vivo and artificial aging of human erythrocytes. *Biochim Biophys Acta* 1990; **1027**: 41-46
50 **Pelassy C**, Breittmayer JP, Aussel C. Inhibition of phosphatidylserine synthesis in Jurkat T cells by hydrogen peroxide. *Biochim Biophys Acta* 2001; **1539**: 256-264
51 **Ricci JE**, Waterhouse N, Green DR. Mitochondrial functions during cell death, a complex (I-V) dilemma. *Cell Death Differ* 2003; **10**: 488-492
52 **Korshunov SS**, Skulachev VP, Starkov AA. High protonic potential actuates a mechanism of production of reactive oxygen species in mitochondria. *FEBS Lett* 1997; **416**: 15-18
53 **Cadenas E**, Boveris A, Ragan CI, Stoppani AO. Production of superoxide radicals and hydrogen peroxide by NADH-ubiquinone reductase and ubiquinol-cytochrome c reductase from beef-heart mitochondria. *Arch Biochem Biophys* 1977; **180**: 248-257
54 **Gerasimenko JV**, Gerasimenko OV, Palejwala A, Tepikin AV, Petersen OH, Watson AJ. Menadione-induced apoptosis: roles of cytosolic Ca(2+) elevations and the mitochondrial permeability transition pore. *J Cell Sci* 2002; **115**: 485-497
55 **Inoue M**, Fujishiro N, Imanaga I, Sakamoto Y. Role of ATP decrease in secretion induced by mitochondrial dysfunction in guinea-pig adrenal chromaffin cells. *J Physiol* 2002; **539**: 145-155
56 **Carafoli E**. Calcium pump of the plasma membrane. *Physiol Rev* 1991; **71**: 129-153
57 **Johnson PR**, Dolman NJ, Pope M, Vaillant C, Petersen OH, Tepikin AV, Erdemli G. Non-uniform distribution of mitochondria in pancreatic acinar cells. *Cell Tissue Res* 2003; **313**: 37-45
58 **Tinel H**, Cancela JM, Mogami H, Gerasimenko JV, Gerasimenko OV, Tepikin AV, Petersen OH. Active mitochondria surrounding the pancreatic acinar granule region prevent spreading of inositol trisphosphate-evoked local cytosolic Ca(2+) signals. *EMBO J* 1999; **18**: 4999-5008
59 **Bhatia M**. Apoptosis versus necrosis in acute pancreatitis. *Am J Physiol Gastrointest Liver Physiol* 2004; **286**: G189-G196
60 **Garrido C**, Galluzzi L, Brunet M, Puig PE, Didelot C, Kroemer G. Mechanisms of cytochrome c release from mitochondria. *Cell Death Differ* 2006; **13**: 1423-1433
61 **Esposti MD**. Lipids, cardiolipin and apoptosis: a greasy licence to kill. *Cell Death Differ* 2002; **9**: 234-236
62 **Orrenius S**, Zhivotovsky B, Nicotera P. Regulation of cell death: the calcium-apoptosis link. *Nat Rev Mol Cell Biol* 2003; **4**: 552-565
63 **Halestrap AP**. Calcium, mitochondria and reperfusion injury: a pore way to die. *Biochem Soc Trans* 2006; **34**: 232-237
64 **Boehning D**, Patterson RL, Sedaghat L, Glebova NO, Kurosaki T, Snyder SH. Cytochrome c binds to inositol (1,4,5) trisphosphate receptors, amplifying calcium-dependent apoptosis. *Nat*

Cell Biol 2003; **5**: 1051-1061
65 **Oakes SA**, Scorrano L, Opferman JT, Bassik MC, Nishino M, Pozzan T, Korsmeyer SJ. Proapoptotic BAX and BAK regulate the type 1 inositol trisphosphate receptor and calcium leak from the endoplasmic reticulum. *Proc Natl Acad Sci USA* 2005; **102**: 105-110
66 **White C**, Li C, Yang J, Petrenko NB, Madesh M, Thompson CB, Foskett JK. The endoplasmic reticulum gateway to apoptosis by Bcl-X(L) modulation of the InsP3R. *Nat Cell Biol* 2005; **7**: 1021-1028
67 **Li C**, Wang X, Vais H, Thompson CB, Foskett JK, White C. Apoptosis regulation by Bcl-x(L) modulation of mammalian inositol 1,4,5-trisphosphate receptor channel isoform gating. *Proc Natl Acad Sci USA* 2007; **104**: 12565-12570
68 **Chen R**, Valencia I, Zhong F, McColl KS, Roderick HL, Bootman MD, Berridge MJ, Conway SJ, Holmes AB, Mignery GA, Velez P, Distelhorst CW. Bcl-2 functionally interacts with inositol 1,4,5-trisphosphate receptors to regulate calcium release from the ER in response to inositol 1,4,5-trisphosphate. *J Cell Biol* 2004; **166**: 193-203
69 **Kuo TH**, Kim HR, Zhu L, Yu Y, Lin HM, Tsang W. Modulation of endoplasmic reticulum calcium pump by Bcl-2. *Oncogene* 1998; **17**: 1903-1910
70 **Harwood SM**, Yaqoob MM, Allen DA. Caspase and calpain function in cell death: bridging the gap between apoptosis and necrosis. *Ann Clin Biochem* 2005; **42**: 415-431
71 **Brown CS**, Dean WL. Regulation of plasma membrane Ca2+-ATPase in human platelets by calpain. *Platelets* 2007; **18**: 207-211
72 **Guerini D**, Pan B, Carafoli E. Expression, purification, and characterization of isoform 1 of the plasma membrane Ca2+ pump: focus on calpain sensitivity. *J Biol Chem* 2003; **278**: 38141-38148
73 **Chami M**, Ferrari D, Nicotera P, Paterlini-Bréchot P, Rizzuto R. Caspase-dependent alterations of Ca2+ signaling in the induction of apoptosis by hepatitis B virus X protein. *J Biol Chem* 2003; **278**: 31745-31755
74 **Pottorf WJ 2nd**, Johanns TM, Derrington SM, Strehler EE, Enyedi A, Thayer SA. Glutamate-induced protease-mediated loss of plasma membrane Ca2+ pump activity in rat hippocampal neurons. *J Neurochem* 2006; **98**: 1646-1656
75 **Pászty K**, Antalffy G, Penheiter AR, Homolya L, Padányi R, Iliás A, Filoteo AG, Penniston JT, Enyedi A. The caspase-3 cleavage product of the plasma membrane Ca2+-ATPase 4b is activated and appropriately targeted. *Biochem J* 2005; **391**: 687-692
76 **Weber H**, Hühns S, Lüthen F, Jonas L, Schuff-Werner P. Calpain activation contributes to oxidative stress-induced pancreatic acinar cell injury. *Biochem Pharmacol* 2005; **70**: 1241-1252
77 **Sorice M**, Circella A, Cristea IM, Garofalo T, Di Renzo L, Alessandri C, Valesini G, Esposti MD. Cardiolipin and its metabolites move from mitochondria to other cellular membranes during death receptor-mediated apoptosis. *Cell Death Differ* 2004; **11**: 1133-1145
78 **Sorice M**, Circella A, Misasi R, Pittoni V, Garofalo T, Cirelli A, Pavan A, Pontieri GM, Valesini G. Cardiolipin on the surface of apoptotic cells as a possible trigger for antiphospholipids antibodies. *Clin Exp Immunol* 2000; **122**: 277-284
79 **Ono K**, Wang X, Han J. Resistance to tumor necrosis factor-induced cell death mediated by PMCA4 deficiency. *Mol Cell Biol* 2001; **21**: 8276-8288
80 **Brunk UT**, Svensson I. Oxidative stress, growth factor starvation and Fas activation may all cause apoptosis through lysosomal leak. *Redox Rep* 1999; **4**: 3-11
81 **Ollinger K**, Brunk UT. Cellular injury induced by oxidative stress is mediated through lysosomal damage. *Free Radic Biol Med* 1995; **19**: 565-574

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