Calcium Signalling and Reactive Oxygen Species in Non-Excitable Cells

Juan A. Rosado*, Pedro C. Redondo, Gines M. Salido and José A. Pariente

Department of Physiology, University of Extremadura, Caceres, Spain

Abstract: Reactive oxygen species can induce several biological processes by stimulating signal transduction components such as cytosolic free calcium concentration. The physiological significance of the role of biological oxidants in the regulation of calcium signalling pathway as well as the mechanisms of the oxidant-stimulation of signal transduction are discussed in this review.

INTRODUCTION

Reactive oxygen species (ROS), such as superoxide radical anion (O^-_2), singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2), hydroxyl radical (·OH), and hypochlorous acid (HOCl), are generated as by-products of oxidative metabolism in all aerobic organisms. Their production is enhanced during various cell dysfunctions; however, the mechanisms of ROS action at the cellular level are not well understood. In addition, ROS also arise in physiological processes. In fact, ROS have recently been recognised as intracellular messengers required for the activation of a larger number of signal transduction mechanisms and ROS have emerged as physiological mediators of cellular responses. In this context, ROS can be generated under physiological stimulation [1, 2], and they are known to be mediators in alterations of normal calcium homeostasis, which precede other morphological and functional alterations of the cell. Thus, the role of ROS on calcium homeostasis has been object of study during the past years.

A variety of mechanism have been proposed to explain the changes in calcium homeostasis induced by ROS, including (i) oxidation of sulphydryl groups located on the calcium transport proteins, (ii) peroxidation of membrane phospholipids, and (iii) inhibition of membrane-bound regulatory enzymes and modification of oxidative phosphorylation [3]. The metabolic pathways that are known to produce ROS include (i) the xanthine/xanthine oxidase (X/XO) system, (ii) the cyclooxygenase pathway of the arachidonic acid metabolism system, (iii) the electron transport system of mitochondria, (iv) the activated neutrophil system, and (v) the amyloid β protein system.

The superoxide radical anion O^-_2 is produced by the reduction of O_2 using an electron that can be supplied by superoxide-generating NADPH oxidase as follows:

$$2O_2 + NADPH \rightarrow 2O^-_2 + NADP^+ + H^+$$

In aqueous solution, the production of H_2O_2 is as follows:

$$2O^-_2 + 2H^+ \rightarrow H_2O_2 + Fe^{2+} + O_2$$ (dismutation reaction)

The Fe^{3+}-induced catalysis of ·OH production is shown in the Fenton reaction as follows:

$$Fe^{3+} + O^-_2 \rightarrow Fe^{2+} + O_2$$

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + \cdot OH$$

$$\text{Net: } O^-_2 + H_2O_2 \rightarrow O_2 + OH^- + \cdot OH$$

CALCIUM HOMEOSTASIS AND ROS

The calcium ion (Ca^{2+}) is an almost universal intracellular messenger controlling a diverse range of cellular processes such as contraction and secretion, gene transcription and cell growth [for review see 4]. In most cell types, Ca^{2+} has its major signaling function when it is elevated in the cytosol. Cytosolic free Ca^{2+} concentration ($[Ca^{2+}]_c$) is increased by the release of Ca^{2+} from intracellular stores or by Ca^{2+} entry through plasma membrane calcium channels. Removal of Ca^{2+} from the cytosol and the maintenance of low resting $[Ca^{2+}]_c$ is mainly mediated by pumping it into intracellular compartments, mainly endoplasmic reticulum (ER) and mitochondria, and by ejecting it out of the cell across the plasma membrane (PM). In this review, we have focused on the effects of ROS in the Ca^{2+} fluxes through cell membranes.

(A) Calcium Release From Intracellular Stores

In non-excitable cells the initial source for cytosolic Ca^{2+} signals are the intracellular Ca^{2+} pools. The binding of physiological agonists to specific receptors on the PM leads to activation of phospholipase C (PLC) and subsequent generation of inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol (DAG). IP_3 releases Ca^{2+} from intracellular pools, while DAG activates protein kinase C (PKC) [5, 6]. Although derived from a lipid, IP_3 is water-soluble and diffuses into the cell interior where it can encounter IP_3-receptors (IP_3R) on the ER. The binding of IP_3 changes the conformation of IP_3R such that an integral channel is opened, thus allowing the Ca^{2+} stored at high concentrations (0.1-1 mM) in the ER to enter the cytoplasm, in which resting $[Ca^{2+}]_c$ varies from 20 to 100 nM. In addition, the Ca^{2+} stored in ER can be also released through ryanodine receptors (RyR). The RyR are structurally and functionally analogous to IP3R, and can be activated by a rise in $[Ca^{2+}]_c$, which explains the Ca^{2+}-induced Ca^{2+} release process [7], and by the NAD^+ metabolite cyclic ADP-ribose (cADP-r) [8].

It is generally reported that ROS cause an increase in $[Ca^{2+}]_c$ of different cell types [9-17]. It seems that this

*Address correspondence to this author at the Department of Physiology, University of Extremadura, Apdo de Correos 643, 10071- Caceres, Spain; Tel: 34-927-257139; Fax: 34-927-257110; E-mail: jarosado@unex.es

1389-5575/06 $50.00+.00

increase can be due to both Ca^{2+} release from intracellular stores such as the ER, and to Ca^{2+} influx from the extracellular medium through the PM. However, the effect of ROS on Ca^{2+} signalling can vary from stimulative to repressive, depending on the type of oxidants, their concentrations, and the duration of exposure. For example, in human aortic endothelial cells low concentrations (1-10 μM) of H_2O_2 exhibit no effect on $[Ca^{2+}]_c$, while 100 μM H_2O_2 induced intracellular oscillations [18]. Previous studies in different cell types, including smooth [9, 10] and skeletal [11] muscle cells, mesangial cells [12], blood mononuclear cells [13], pancreatic β-cells [14], neuronal cells [15], cardiomyocytes [16] and renal tubular cells [17], have reported that hydroperoxides and other sulphydryl reagents can induce Ca^{2+} mobilisation. In pancreatic acinar cells, several workers have shown that the sulphydryl group oxidising agents thimerosal [19], vanadate [20] and phenylarsine oxide [21] are able to mobilise Ca^{2+} from intracellular stores, and this effect is reversible in the presence of the thiol-reducing agent dithiothreitol. Additionally, it has been shown that thimerosal is able to mobilise Ca^{2+} from intracellular stores in pancreatic acinar cells [19] and HeLa cells [22] by sensitising the IP_3R to the endogenous level of IP_3, whereas in skeletal muscle cells thimerosal was also able to produce Ca^{2+} release through RyR [23]. However, our results using the membrane-permeable IP_3R blocker, xestospongin C demonstrate that H_2O_2 releases Ca^{2+} from a non-mitochondrial and agonist-sensitive Ca^{2+} pool in mouse pancreatic acinar cells by an IP_3R independent mechanism [24], whereas in cardiac-derived fibroblasts pretreatment with xestospongin C reduced the Ca^{2+} release evoked by H_2O_2 [25]. In addition, H_2O_2 is able to release Ca^{2+} from mitochondria [24].

Alternatively, it has been proposed the existence of a redox sensor in the agonist-sensitive Ca^{2+} stores in human platelets [26]. The redox sensor in the agonist-releasable pool might consist of hyperreactive sulphydryl groups present in the IP_3R. These groups are highly sensitive of oxidation by agonist-generated ROS [1, 2] or when platelets are exposed to ROS. Consistent with this, we have previously shown that ROS induce concentration-dependent Ca^{2+} release from agonist-sensitive Ca^{2+} stores by oxidation of sulphydryl groups in IP_3R but independently of IP_3 generation. Blockade of either the IP_3 turnover by lithium or PLC by the specific inhibitor U-73122 is unable to prevent ROS-induced Ca^{2+} release from the agonist-sensitive pool [26]. Similarly, *tert*-butyl hydroperoxide-induced Ca^{2+} release [27] occur without any requirement for PLC activation in hepatocytes [28].

It has been also reported that ·OH generated by hypoxanthine/xanthine oxidase release Ca^{2+} from thapsigargin-insensitive [29] but ryanodine-sensitive Ca^{2+} stores [30, 31]. In fact, RyR show *in vitro* sensitisation in the presence of thimerosal [23]. In addition, it has been reported that the $[Ca^{2+}]_c$ increase evoked by H_2O_2 is followed by activation of the ryanodine-sensitive Ca^{2+} channel [11, 32].

On the other hand, ROS-induced Ca^{2+} signals show a similar spatial dynamic as agonist-evoked Ca^{2+} signals. In polarised cells, such as pancreatic acinar cells, Ca^{2+} mobilisation in response to the physiological agonists acetylcholine, bombesin and cholecystokinin results in an initial increase in $[Ca^{2+}]_c$ at the luminal cell and subsequent spreading of the Ca^{2+} signal towards the basolateral cell membrane. The xanthine oxidase-catalysed ROS generation induces a Ca^{2+} mobilisation, similar as agonist-induced Ca^{2+} signal, starting at the luminal cell pole and then spreading towards the basolateral side in form of a Ca^{2+} wave [33, 34]. The luminal pole is characterised as the trigger zone for agonist-induced Ca^{2+} signals [35], where the agonist-sensitive Ca^{2+} pools are sited, which supports further evidence that ROS affect the same trigger pools that those releasable by agonists.

(B) Calcium Entry Throughout the Plasma Membrane

The release of Ca^{2+} from finite intracellular Ca^{2+} compartments is often insufficient for full activation of cellular mechanisms, such as secretion or contraction, and many cellular functions, as well as the refilling of the intracellular stores, require a sustained increase in cytosolic free Ca^{2+}. Therefore, Ca^{2+} entry into the cell throughout the PM plays an important role in Ca^{2+} homeostasis.

In electrically excitable cells, such as neurons, muscle and some endocrine cells, Ca^{2+} entry generally occurs through voltage-operated Ca^{2+} channels (VOCs); however, in non-excitable cells, where VOCs are not present, Ca^{2+} influx is mainly mediated by receptor-operated channels, second messenger-operated channels or store-operated channels (SOCs).

Voltage-operated Ca^{2+} entry is conducted by VOCs, Ca^{2+} permeable channels that become briefly activated during action potentials [36]. These channels are mainly found in excitable cells, such as neurons, muscle and endocrine cells, where they open in response to membrane depolarizations to allow Ca^{2+} to enter the cell [37].

Second messenger-operated Ca^{2+} entry is activated by a diffusible intracellular molecule whose concentration was increased as a result of agonist-receptor binding [38]. Evidence for second messenger-operated Ca^{2+} entry in excitable cells is rather scarce; however, in non excitable cells, a Ca^{2+} permeable channel activated by Ca^{2+} and inositol 1,3,4,5-tetrakisphosphate has been found in endothelial cells [39]. In addition, in human platelets, thrombin induces the activation of a non-capacitative Ca^{2+} entry mediated via the activation of PKC [40] and the human homologue of the *Drosophila* transient receptor potential (TRP) channel, TRP3, stably expressed in human embryonic kidney (HEK293) cells, has been reported to be activated by DAG and its analogues [41]. At present no clear evidences have been presented reporting a role of ROS on the activation of voltage- or second messenger-operated Ca^{2+} entry.

Receptor-operated Ca^{2+} entry (ROCE) occurs through a series of functionally diverse channels that are especially relevant in secretory cells and neurones. ROCE is activated by a number of cellular agonists, usually neurotransmitters, including glutamate, acetylcholine, ATP and ADP. Activation of ROCE induces a rapid Ca^{2+} entry indicative of a direct opening of a Ca^{2+} permeable channel upon receptor occupation. The opening of receptor-operated Ca^{2+} channels might be activated directly by interaction of the agonist with

the subunits of a transmembrane protein that forms the channel or by some coupled system, perhaps involving a heterotrimeric G protein [38]. ROS have been shown to activate ROCE through TRPM2 in granulocytes by enhancing NAD concentration [42]. In contrast, oxidative stress inhibits ROCE in endothelial cells [43], suggesting that high concentrations of ROS might have adverse effects on ROCE.

The main mechanism for Ca^{2+} entry in non-excitable cells is store-mediated Ca^{2+} entry (SOCE) through SOCs. SOCE, which are also present in excitable cells [44], is regulated by the filling state of the intracellular Ca^{2+} stores, although at present it is not clear how depletion of the intracellular Ca^{2+} stores, either by physiological agonists or by pharmacological agents, is communicated to the PM. A number of hypotheses have been suggested to account for the activation of SOCE. These can be divided into those that propose a role for a diffusible messenger, those that propose a direct interaction between proteins in the ER and PM (conformational coupling) and finally the models that proposed the insertion of channels in the PM.

Diffusible messengers which might be involved in the activation of SOCE include small GTP-binding proteins, cyclic GMP, a product of cytochrome P450, a Ca^{2+} influx factor (CIF), a tyrosine phosphorylation-dependent step and a Ca^{2+}-calmodulin dependent step [see 45]. According to this model, store depletion results in the release of a diffusible molecule that gates a Ca^{2+} permeable channel in the PM.

The conformational coupling model suggests a permanent and physical interaction between a protein in the membrane of the ER and a Ca^{2+} channel in the PM to allow Ca^{2+} influx [46]. Classically, the protein located in the ER has been recognised as the IP_3R [47]. The conformational coupling has received support from studies that demonstrates that expressed exogenous TRPC1, TRPC3 and TRPC6 can be co-immunoprecipitated with IP_3Rs under resting conditions, and the finding that IP_3R fragments can modulate SOCE [48-50].

An alternative to the "classical" conformational coupling has recently been reported in platelets and other non excitable cells, where coupling between IP_3Rs and Ca^{2+} channels only occurs after store depletion and not at the resting conditions [51, 52]. The so called *de novo* conformational coupling [53] is based on the trafficking of portions of the ER towards the PM to enable a reversible coupling of the IP_3Rs in the ER and a Ca^{2+} channel in the PM, which requires actin cytoskeleton remodelling. In human platelets coupling between the IP_3R and endogenously expressed TRPC1 has been demonstrated [54, 55].

The *de novo* conformational coupling appears as an integrative model where components of the diffusible messenger and the conformational coupling might coexist. Consistent with this, small GTP-binding proteins and tyrosine kinases, initially considered part of the diffusible molecules hypotheses for the activation of SOCE, are essential for actin polymerisation induced by store depletion [56, 57]. In addition, reorganization of the cytoskeletal cortical barrier has been suggested to facilitate the activation of SOCE by a CIF [58].

The actin filament network might play two different roles in the *de novo* conformational coupling. A remarkable reorganisation of the cytosolic actin network has been shown to be required to provide a support for the transport of portions of the ER containing IP_3R to the PM [51, 52, 59]. In addition, a negative role for the cortical actin filament network in the activation of SOCE has been proposed, which needs to be depolymerised to allow the coupling between the IP_3R and the Ca^{2+} channel in the PM [51, 52, 59]. In fact, the cell-permeant peptide, jasplakinolide, which induces actin polymerisation and stabilizes actin filaments into a thick cortical layer, reduces SOCE in several cell types, including platelets [51] and pancreatic acinar cells [52]. Similar results were obtained with calyculin A, an inhibitor of protein phosphatases 1 and 2, which induces phosphorylation-dependent association of the actin filaments to the PM [51, 58]. These results provide compelling evidence against the activation of SOCE by a diffusible messenger in these cells, since a molecule would be expected to pass across the actin barrier and activate Ca^{2+} entry, in the same way that IP_3 generated by physiological agonists was found to be able to reach the ER and release stored Ca^{2+} in jasplakinolide treated cells [51].

The regulatory role of the cortical actin network is also shared by the hypothesis that proposes the translocation and insertion of preformed channels into the PM by vesicle fusion. The role of the actin cytoskeleton in the so called "secretion-like coupling model" parallels that reported in exocytosis, where the cortical actin network acts as a negative clamp [60, 61]. The idea of vesicle fusion during the activation of SOCE has received support from studies reporting an increased expression of stored TRPCs in the PM upon cell stimulation [62-64].

ROS, such as H_2O_2, play a concentration-dependent effect in the activation of SOCE. SOCE has been reported to be reduced by treatment with H_2O_2 at concentrations ≥ 100 μM through the activation of PKC, which leads to membrane depolarization and increased Ca^{2+} extrusion [65]. Our observations in human platelet indicate that exposure to low H_2O_2 concentrations favours Ca^{2+} release from intracellular stores and subsequently SOCE, showing a positive correlation between Ca^{2+} release and entry at 10μM and 100 μM H_2O_2 [66]. However, the ability of H_2O_2 to induce SOCE decreases at higher concentrations, so that induces a small amount of Ca^{2+} entry despite the extensive depletion of the intracellular Ca^{2+} stores. In addition, 1 mM H_2O_2 reduces both the activation and maintenance of SOCE stimulated by agonists [66].

The effect of H_2O_2 on SOCE is mediated by actin filament reorganisation. Low H_2O_2 concentrations (10 μM) induce a temporal actin network reorganisation similar to that induced by store depletion by thapsigargin plus ionomycin or by secretagogues in pancreatic acinar cells consistent with the *de novo* conformational coupling [53, 67]. Actin remodelling consists of an initial net depolymerization followed by a net increase in the actin filament content. In contrast, 1 mM H_2O_2 does not induce an initial net depolymerization and abolishes the typical actin reorganization pattern induced by agonists, which might explain the decrease in SOCE induced by high concentrations of H_2O_2 [66].

The role of other ROS, such as superoxide anion (O_2^-) on SOCE has also been described. In vascular endothelial cells incubation with the O_2^--generating system xanthine oxidase/hypoxanthine resulted in an increased intracellular Ca^{2+} release and SOCE in response to bradykinin and ATP in a time- and concentration-dependent manner [68]. In contrast, it has been reported that high O_2^- concentrations reduce SOCE in PLB-985 cell lines and neutrophilic granulocytes from peripheral blood [69], which further support that high concentrations of ROS impair the activation of SOCE.

The correlation between Ca^{2+} release and entry and the similar actin reorganization induced by low concentrations of H_2O_2 and physiological agonists suggests that endogenous H_2O_2 production might play a role in the activation of SOCE under physiological conditions. A physiological role of ROS on the activation of SOCE has been demonstrated in different cell types, such as mast cells, where inhibition of ROS production by diphenyleneiodonium impairs SOCE stimulated by FcεRI cross-linking [70]. In endothelial cells, enzymatically produced non-toxic H_2O_2, rather than O_2^- or ·OH induces Ca^{2+} release from thapsigargin sensitive stores and activates SOCE, at least partially by activating PLC [71]. In addition, in human platelets, Ca^{2+} store depletion, induced by physiological agonists or by pharmacological tools, stimulates the production of H_2O_2 in the micromolar range ($\leq$ 100 μM). Generated H_2O_2 stimulates actin filament reorganisation and subsequently the activation of $pp60^{src}$ by a PKC-dependent mechanism, which are required for the coupling between naturally expressed TRPC1 and IP_3R type II and the subsequent activation of SOCE in these cells [1]. The role of H_2O_2 as a messenger molecule release after store depletion was confirmed by the stimulation of Ca^{2+} entry by 10 μM H_2O_2 in the absence of Ca^{2+} release from the intracellular stores [1, 72]. Ca^{2+} mobilisation induced by low concentrations of H_2O_2 clearly differ from pathological "oxidative stress" associated with a progressive increase in $[Ca^{2+}]_c$.

(C) Removal of Cytosolic Calcium

Ca^{2+} removal from the cytosol, after reached a peak $[Ca^{2+}]_c$ elevation by stimulation of cells with agonists, is carried out by several Ca^{2+} pumps and exchangers that reintroduce Ca^{2+} into the internal stores or extrude it out of the cell.

Several Ca^{2+} stores have been described on different cell types, and those can be differentiated by the specific type of Ca^{2+} pump or exchanger that is placed on them. Released Ca^{2+} is returned to the ER by the sarcoendoplasmic reticulum Ca^{2+} ATPase (SERCA). Different SERCA isoforms can be found in other intracellular Ca^{2+} stores, encoded by three SERCA genes, named *SERCA-1* (*ATP2A1*), *SERCA-2* (*ATP2A2*), *SERCA-3* (*ATP2A3*) [73].

Several pharmacological tools, such as thapsigargin, 2,5-di(*tert*-butyl)-1,4-benzohydroquinone (TBHQ), curcumin and cyclopiazonic acid, have been developed to investigate the role of SERCA in Ca^{2+} signalling and identify where each SERCA isoform can be placed. Among them, the most widely used is thapsigargin, which binds to all SERCAs although with different affinity [74] and causes an irreversible inhibition of their activity by blocking the ATPase in the Ca^{2+}-free state [75]. A similar effect is induced with TBHQ, although with lower potency, and some isoforms seems to be insensitive to this inhibitor. TBHQ has been used to identify distinct intracellular Ca^{2+} stores [76]. The rest of inhibitors have not been extensively used due to their lack of specificity.

In addition, it has been proved the existence of additional types of ATPases that are regulated by H^+. Three different types have been identified: V, F and P H^+-ATPases [77]. H^+-ATPases generate a proton gradient inside different stores leading to the accumulation of Ca^{2+}. In fact, in platelets it has been recently described that acidic organelles, such as lysosomes can act as intracellular Ca^{2+} stores [78]. In the Golgi cisterns, a new Ca^{2+}-ATPase named PMR1/SPCA Ca^{2+}/Mn^{2+}, has been described as a Ca^{2+} transport that has a philogenic SERCA antecesor and belongs to the so called "secretory-pathway Ca^{2+}-ATPases" or SPCAs [79, 80]. The presence of these ATPases in the Golgi system provides its ability to act as Ca^{2+}-stores.

SERCA and other Ca^{2+}-ATPases can be modulated by several factors, the most common regulator factor is the $[Ca^{2+}]_c$ around them, which increases the efficacy to reuptake Ca^{2+} into the store and also SERCA expression [81]. SERCA has a high affinity for Ca^{2+} (0.1-0.4 μM), which suggests that SERCA is likely to be activated by an increase in $[Ca^{2+}]_c$ and inhibited by an increase in Ca^{2+} concentration into the stores ($[Ca^{2+}]_s$) [82]. Moreover, their activity can be also reduced by the activity of ERp57 protein, which belongs to a family of proteins regulated by calreticulin, a Ca^{2+} binding protein inside the ER sensitive to oxidative stress [83]. The ROS-regulated pathway can be essential for intracellular Ca^{2+} control, since in several studies SERCA activity has been shown to be regulated by ROS generated during ischemia and reperfusion of the tissue [84]. In this sense ROS, such as H_2O_2, can affect the activity of SERCA, however the mechanisms that underlie this process remain unclear. Several authors have described that metal-catalyzed oxidation result in SERCA inhibition by direct disulfide bonds oxidation, this is the case in platelets [26, 85], but in other cell types, such as skeletal-muscle cells [86] or myocardic H9c2 [83], SERCA inhibition is induced by an independent oxidative mechanism. Other ROS like hydroxyl radicals and peroxynitrite can also reduce the activity of SERCA [86, 87].

A different mechanism to reduce $[Ca^{2+}]_c$ is the extrusion of Ca^{2+} to the extracellular medium due the activity of the plasma membrane Ca^{2+}-ATPase (PMCA). The ability of PMCA to reduce $[Ca^{2+}]_c$ is higher than that of SERCA [88], and as well as SERCA, PMCA is activated by the $[Ca^{2+}]_c$ [89]. As other ion pumps, PMCA derive from a multigene family, which is encoded by 4 different genes named *PMCA*1, *PMCA*2, *PMCA*3 y *PMCA*4, and due to alternative splicing encode for at least 28 different isoforms.

A variety PMCA isoforms result in a complex regulatory system, in fact there are many factors that, as for SERCA, can regulate PMCA activity. The C-terminal part of Ca^{2+}-ATPase is a multifunctional regulatory region and contains an autoinhibitory domain with a high affinity calmodulin binding site, and sequences that are potent targets for phosphorylation by protein kinase [89]. The sequences

phosphorylated by protein kinases are not conserved in different PMCA isoform, which explain the different effect of phosphorylation on PMCA activity in different cell types. In fact, while PMCA1 is phosphorylated by PKA in the calmodulin binding region [90], PKC-mediated phosphorylation have been demonstrated in purified red blood cell Ca^{2+}-ATPase, in human neutrophils and platelets, cultured aortic endothelial cells, and rat neurons [91-93]. In contrast, reports about specific Ca^{2+}-ATPase dephosphorylation is rather scarce, and only in the membrane-inserted and purified erythrocyte enzyme the effect of PP2A on PMCA activity has been demonstrated [94]. Dephosphorylation of PMCA could be a complex process, because recently it has been reported that phosphatases PP1 and PP2A reversible inhibit PKCα activity and those phosphatases could directly or indirectly affect or modulate (via PKC) to Ca^{2+} pumps in same type cells [95].

ROS can also modify the activity of PMCA. A number of studies have shown that ROS, such as H_2O_2, produce an alteration on the ability of PMCA to extrude Ca^{2+} from the cytosol [84, 96], but the underlying mechanism still remain unclear. Different hypotheses include modulation by direct disulfide bounds oxidation or by the activity of an intermediate oxide-sensible proteins such as calmodulin [97, 98]. However, other oxidants, such as peroxynitrite ($ONOO^-$), induce loss of activity by direct changes on the PMCA structure in neurons and other cell types [97].

PMCA inhibition by ROS has very important physiological consequences and may be targets of oxidative stress in the aging brain. In fact, it has been shown that a reduction in PMCA activity may contribute to age-related alterations in neuronal $[Ca^{2+}]_c$ regulation [96], and could be the cause for other diseases relationship with Ca^{2+} homeostasis.

$[Ca^{2+}]_c$ is also regulated by the mitochondria. Localised in the vicinity of the Ca^{2+} channels, mitochondria sequester Ca^{2+} modulating the Ca^{2+} signals [98, 99]. Ca^{2+} enters the mitochondria by a high capacity and low affinity uniporter that requires local high $[Ca^{2+}]_c$ to function [100, 101]. Efflux of Ca^{2+} occurs by two different exchangers that countertransport Ca^{2+} for either Na^+ or H^+, or through a permeability transition pore that shows a reversible low conductance state and an irreversible high conductance state that collapses the mitochondrial membrane potential [101, 102].

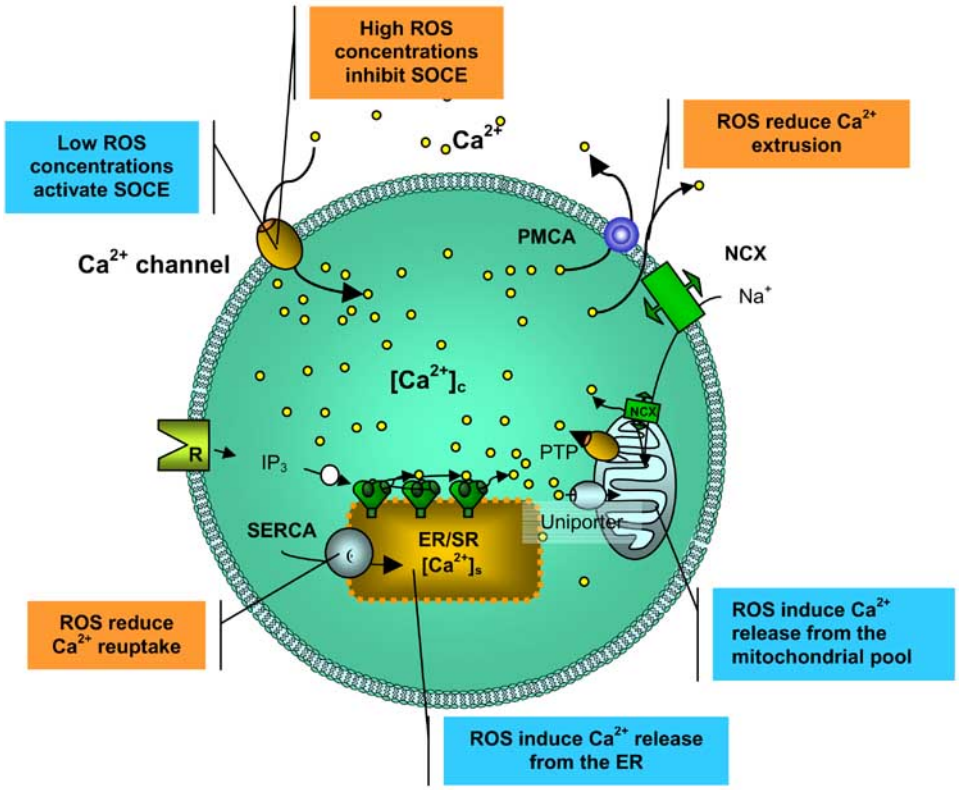

Fig. (1). Effect of ROS on the different cellular calcium transports. Membrane receptor occupation by agonists leads to the formation of second messengers that induce the release of Ca^{2+} from the endoplasmic/sarcoplasmic reticulum (ER/SR). Store depletion results in the activation of store-operated Ca^{2+} entry (SOCE). Ca^{2+} removal from the cytosol depends on the activity of various pumps and exchangers, and includes Ca^{2+} extrusion by the Na^+/Ca^{2+} exchanger (NCX) and the plasma membrane Ca^{2+} ATPase (PMCA) and Ca^{2+} reuptake into intracellular stores (ER/SR) by the sarcoendoplasmic reticulum Ca^{2+}-ATPase (SERCA). Mitochondria can also modulate the Ca^{2+} signals by sequestering Ca^{2+} through a uniporter, and Ca^{2+} release into the cytoplasm through the NCX or the permeability transition pore (PTP). ROS can elevate cytosolic Ca^{2+} concentration ($[Ca^{2+}]_c$) by either increasing Ca^{2+} release from the ER/SR or the mitochondrial pool or reducing Ca^{2+} removal from the cytosol. In addition, ROS show a concentration-dependent effect on Ca^{2+} entry across the plasma membrane, so that low concentrations favour SOCE while Ca^{2+} entry is impaired by high ROS concentrations.

The mechanisms of mitochondrial Ca^{2+} uptake and release can be modified by several ROS [103], but at the same time the $[Ca^{2+}]_c$ can increase the production of ROS when the activity of complex I is altered [104]. The relationship between mitochondrial Ca^{2+} ($[Ca^{2+}]_m$) and ROS production is not clear. An increase in $[Ca^{2+}]_m$ by uptake from the cytosol is able to induce ROS production on the electron mitochondrial transport chain. Oxidants can also affect the mitochondrial Ca^{2+} pool allowing the release of Ca^{2+} from this organelle, by inducing changes in the mitochondrial membrane potential (ψ_m) [103, 105]. It is not clear if ROS-induced changes in ψ_m are responsible for the opening of mitochondrial transition pore or if this opening is produced by direct changes in pore structure. However it is clear that in pancreatic acinar cells ROS, such as H_2O_2, produce changes in ψ_m, which have been proposed as the basis for free radicals injury to cells [105]. Additionally, it has been reported in pancreatic acinar cells that the Ca^{2+}-mobilizing agonist CCK induces increase in $[Ca^{2+}]_m$, depolarisation of ψ_m and increases in FAD autofluorescence. These changes in mitochondrial activity induced by CCK were completely blocked in the presence of H_2O_2 [106].

CONCLUSION

ROS and the redox state play an important role in intracellular Ca^{2+} homeostasis. A redox sensor in both the agonist-sensitive Ca^{2+} stores and mitochondria in human platelets regulates Ca^{2+} mobilisation from intracellular compartments (Fig. **1**). The redox sensor in the agonist-releasable pool might consist of hyperreactive sulphydryl groups present in the IP_3 receptors and SERCA. However, other mechanisms different from sulphydryl group oxidation are likely mediating the inhibitory effect of ROS on the ability of PMCA to extrude Ca^{2+} from the cytosol and mitochondrial Ca^{2+} mobilisation. In addition, ROS play a dual role in SOCE, both as a messenger molecule that induces store depletion and subsequent Ca^{2+} entry, and as injurious by-products of cellular metabolism, which lead to inhibition of SOCE. Considering the large number of cellular processes modulated by changes in $[Ca^{2+}]_c$, redox sensing might be of great relevance in cellular physiology.

ACKNOWLEDGEMENT

We would like to thank Mrs. Mercedes Gómez Blazquez for her technical support and MEC and Junta de Extremadura-FEDER for research grants (BFU2004-00165 and 2PR04A009 respectively).

REFERENCES

[1] Rosado, J.A.; Redondo, P.C.; Salido, G.M.; Gómez-Arteta, E.; Sage, S.O.; Pariente, J.A. *J. Biol. Chem.*, **2004**, *279*, 1665.
[2] Granados, M.P.; Salido, G.M.; Pariente, J.A.; González, A. *Mitochondrion*, **2004**, *3*, 285.
[3] Kourie, J.I. *Am. J. Physiol.-Cell Physiol.,* **1998**, *275*, C1.
[4] Berridge, M.J. *Nature Lond.*, **1993**, *361*, 315.
[5] Streb, H.; Irvine, R.F.; Berridge, M.J.; Schulz, I. *Nat. Lond.*, **1983**, *306*, 67.
[6] Berridge, M.J. *Annu. Rev. Biochem.*, **1987**, *56*, 159
[7] Endo, M.; Tanaka, M.; Ogawa, Y. *Nature*, **1970**, *228*, 34.
[8] Takasawa, S.; Nata, K.; Yonekura, H. *Science*, **1993**, *259*, 370.
[9] Roveri, A.; Coassin, M.; Maiorino, M.; Zamburlini, A.; Van Amsterdam, F.T.; Ratti, E.; Ursini, F. *Arch. Biochem. Biophys.*, **1992**, *297*, 265.
[10] Krippeit-Drews, P.; Haberland, C.; Fingerle, J.; Drews, G.; Lang, F. *Biochem. Biophys. Res. Commun.*, **1995**, *209*, 139.
[11] Favero, T.G.; Zable, A.C.; Abramson, J.J. *J. Biol. Chem.*, **1995**, *270*, 25557.
[12] Meyer, T.N.; Gloy, J.; Hug, M.J.; Greger, R.; Schollmeyer, P.; Pavenstadt, H. *Kidney Int.*, **1996**, *49*, 388.
[13] Korzets, A.; Chagnac, A.; Weinstein, T.; Ori, Y.; Malachi, T.; Gafter, U. *J. Lab. Clin. Med.*, **1999**, *133*, 362.
[14] Krippeit-Drews, P.; Krämer, C.; Welker, S.; Lang, F.; Ammon, H.P.T., Drews, G. *J. Physiol.*, **1999**, *514*, 471.
[15] Whittemore, E.R.; Loo, D.T.; Watt, J.A.; Cotman, C.W. *Neuroscience*, **1995**, *67*, 921.
[16] Wang, X.; Takeda, S.; Mochizuki, S.; Jindal, R.; Dhalla, N.S. *J. Cardiovasc. Pharmacol. Ther.*, **1999**, *4*, 41.
[17] Ueda, N.; Shah, S.V. *Am. J. Physiol.*, **1992**, *263*, F214.
[18] Hu, Q.; Corda, S.; Zweier, J.L.; Capogrossi, M.C.; Ziegelstein, R.C. *Circulation*, **1998**, *97*, 268.
[19] Thorn, P.; Brady, P.; Llopis, J.; Gallacher, D.V.; Petersen, O.H. *Pflügers Arch.*, **1992**, *422*, 173.
[20] Pariente, J.A.; Lajas, A.I.; Pozo, M.J.; Camello, P.J.; Salido, G.M. *Biochem. Pharmacol.*, **1999**, *58*, 77.
[21] Lajas, A.I.; Pozo, M.J.; Camello, P.J.; Salido, G.M.; Pariente, J.A. *Cell. Signal.*, **1999**, *11*, 727.
[22] Bootman, M.D.; Taylor, C.W.; Berridge, M.J. *J. Biol. Chem.*, **1992**, *267*, 25113.
[23] Abramson, J.J.; Zable, A.C.; Favero, T.C.; Salama, G. *J. Biol. Chem.*, **1995**, *270*, 29644.
[24] Pariente, J.A.; Camello, C.; Camello, P.J.; Salido, G.M. *J. Membr. Biol.*, **2001**, *179*, 27.
[25] Colston, J.T.; Chandrasekar, B.; Freeman, G.L. *J. Biol. Chem.*, **2002**, *277*, 23477.
[26] Redondo, P.C.; Salido, G.M.; Rosado, J.A.; Pariente, J.A. *Biochem. Pharmacol.*, **2004**, *67*, 491.
[27] Sakaida, I.; Thomas, A.P.; Farber, J.L. *J. Biol. Chem.*, **1991**, *266*, 717.
[28] Rooney, T.A.; Renard, D.C.; Sass, E.J.; Thomas, A.P. *J. Biol. Chem.*, **1991**, *266*, 12272.
[29] Bielefeldt, K.; Whiteis, C.A.; Sharma, R.V.; Abboud, F.M.; Conklin, J.L. *Am. J. Physiol.*, **1997**, *272*, G1479.
[30] Weber, H.; Roesner, J.P.; Nebe, B.; Rychly, J.; Werner, A.; Schröder, H.; Jonas, L.; Leitzmann, P.; Schneider, K.P.; Dummler, W. *Digestion*, **1998**, *59*, 175.
[31] Klonowski-Stumpe, H.; Schreiber, R.; Grolik, M.; Schulz, H.U.; Häussinger, D.; Niederau, C. *Am. J. Physiol.*, **1997**, *272*, G1489.
[32] Oba, T.; Ishikawa, T.; Yamaguchi, M. *Am. J. Physiol.*, **1998**, *274*, C914.
[33] González, A.; Camello, P.J.; Salido, G.M.; Pariente, J.A. *Biochem. Pharmacol.*, **2001**, *62*, 1621.
[34] González, A.; Schmid, A.; Salido, G.M.; Camello, P.J.; Pariente, J.A. *Cell. Signal.*, **2002**, *14*, 153.
[35] Kasai, H.; Li, Y.X.; Miyashita, Y. *Cell*, **1993**, *74*, 669.
[36] Tsien, R.W.; Lipscombe, D.; Madison, D.; Bley, K.; Fox, A. *Trends Neurosci.,* **1995,** *18,* 52.
[37] McCleskey, E.W. *Curr. Opinion Neurobiol.,* **1994,** *4,* 304.
[38] Sage, S. O. (1992). *Curr. Biol.,* **2,** 312-314.
[39] Lückhoff, A.; Clapham, D.E. *Nature,* **1992,** *335,* 356.
[40] Rosado, J.A.; Sage, S.O. *J. Physiol.,* **2000,** *529,* 159.
[41] Ma, H.-T.; Patterson, R.L.; Van Rossum, D.B.; Birbaumer, L.; Mikoshiba, K.; Gill D.L. *Science,* **2000,** *287,* 1647.
[42] Heiner, I.; Eisfeld, J.; Luckhoff, A. *Cell Calcium,* **2003,** *33,* 533.
[43] Elliott, S.J.; Doan, T.N.; Henschke, P.N. *Am. J. Physiol.,* **1995,** *268,* H278.
[44] Morales, S.; Camello, P.J.; Rosado, J.A.; Mawe, G.M.; Pozo, M.J. *Cell. Signal*., **2005,** *17,* 635.
[45] Parekh, A.B.; Penner, R. *Physiol. Rev*., **1997,** *77,* 901.
[46] Irvine, R.F. *FEBS Lett.,* **1990,** *263,* 5.
[47] Berridge, M.J. *Biochem. J.,* **1995,** *312,* 1.
[48] Boulay, G.; Brown, D.M.; Qin, N.; Jiang, M.; Dietrich, A.; Zhu, M.X.; Chen, Z.; Birnbaumer, M.; Mikoshiba, K.; Birnbaumer, L. *Proc. Natl. Acad. Sci. USA,* **1999,** *96,* 14955.
[49] Delmas, P.; Wanaverbecq, N.; Abogadie, F.C.; Mustry, M.; Brown, D.A. *Neuron,* **2002,** *14,* 209.
[50] Vazquez, G.; Wedel, B.J.; Aziz, O.; Trebak, M.; Putney, J.W. Jr. *Biochim. Biophys. Acta,* **2004,** *1742,* 21.

[51] Rosado, J.A.; Jenner, S.; Sage, S.O. *J. Biol. Chem.,* **2000,** *275,* 7527.
[52] Redondo, P.C.; Lajas, A.I.; Salido, G.M.; Gonzalez, A.; Rosado, J.A.; Pariente, J.A. *Biochem. J.,* **2003,** *370,* 255.
[53] Rosado, J.A.; Redondo, P.C.; Sage, S.O.; Pariente, J.A.; Salido, G.M. *J. Cell Physiol*., **2005,** (in press).
[54] Rosado, J.A.; Sage, S.O. *Biochem. J.,* **2000,** *350,* 631.
[55] Rosado, J.A.; Brownlow, S.L.; Sage, S.O. *J. Biol. Chem.,* **2002,** *277,* 42157.
[56] Rosado, J.A.; Sage, S.O. *Biochem. J.,* **2000,** *347,* 183.
[57] Rosado, J.A.; Graves, D.; Sage, S.O. *Biochem. J.,* **2000,** *351,* 429.
[58] Xie, Q.; Zhang, Y.; Zhai, C.; Bonanno J.A. *J. Biol. Chem.,* **2002,** *277,* 16559.
[59] Rosado, J.A.; Sage, S.O. *Biochem. J.* **2001,** *356,* 191.
[60] Muallem, S.; Kwiatkowska, K.; Xu, X., Yin, H.L. *J. Cell. Biol*., **1995,** *128,* 589.
[61] Patterson, R.L.; van Rossum, D.B.; Gill, D.L. *Cell,* **1999,** *98,* 487.
[62] Bezzerides, V.J.; Ramsey, I.S.; Kotecha, S.; Greka, A.; Clapham, D.E. *Nat. Cell. Biol*., **2004,** *6,* 709.
[63] Cayouette, S.; Lussier, M.P.; Mathieu, E.L.; Bousquet, S.M.; Boulay, G. *J. Biol. Chem.*, **2004,** *279,* 7241.
[64] Singh, B.B.; Lockwich, T.P.; Bandyopadhyay, B.C.; Liu, X.; Bollimuntha, S.; Brazer, S.C.; Combs, C.; Das, S.; Leenders, A.G.; Sheng, Z.H.; Knepper, M.A.; Ambudkar, S.V.; Ambudkar, I.S. *Mol. Cell,* **2004**, *15,* 635.
[65] Törnquist, K.; Vainio, P.J.; Björklund, S.; Titievsky, A.; Dugué, B.; Tuominen, R.K. *Biochem. J.,* **2000**, *351,* 47.
[66] Redondo, P.C.; Salido, G.M.; Pariente, J.A.; Rosado, J.A. *Biochem. Pharmacol*., **2004**, *67,* 1065.
[67] Rosado, J.A.; González, A.; Salido, G.M.; Pariente, J.A. *Cell. Signal*., **2002**, *14,* 547.
[68] Graier, W.F.; Hoebel, B.G.; Paltauf-Doburzynska, J.; Kostner, G.M. *Arterioscler. Thromb. Vasc. Biol.,* **1998**, *18,* 1470.
[69] Rada, B.K.; Geiszt, M.; Van Bruggen, R.; Nemet, K.; Roos, D.; Ligeti, E. *Clin. Exp. Immunol.,* **2003**, *132,* 53.
[70] Suzuki, Y.; Yoshimaru, T.; Matsui, T.; Inoue, T.; Niide, O.; Nunomura, S.; Ra, C. *J. Immunol*., **2003**, *171,* 6119.
[71] Volk, T.; Hensel, M.; Kox, W.J. *Mol. Cell. Biochem*., **1997**, *171,* 11.
[72] Redondo, P.C.; Jardín, I.; Hernández-Cruz, J.M.; Pariente, J.A.; Salido, G.M.; Rosado, J.A. *Biochem. Biophys. Res. Comun.,* **2005**, *333,* 794.
[73] Meldolesi, J.; Pozzan T. *Trends. Biochem. Sci.,* **1998,** *23*, 10.
[74] Bobe, R.; Bredoux, R.; Corvazier, E.; Andersen, J.P.; Clausen, J.D.; Dode, L.; Kovács, T.; Enouf, J. *J. Biol. Chem.,* **2004,** *279*, 24297.
[75] Wictome, M.; Henderson, I.; Lee, A.G.; East, J.M. *Biochem. J.*. **1992,** *283*, 525.
[76] Cavallini, L.; Coassin, M.; Alexandre, A. *Biochem. J.,* **1995,** *310*, 449.
[77] Yoshimori, T.; Yamamoto, A.; Moriyama, Y.; Futai, M.; Tashiro, Y. *J. Biol. Chem.,* **1991,** *266*, 17707.
[78] Lopez, J.J.; Camello-Almaraz, C.; Pariente, J.A.; Salido, G.M.; Rosado, J.A. *Biochem. J.,* **2005,** (in press).
[79] Wuytack, F.; Raeymaekers, L.; Missiaen, L. *Cell Calcium.,* **2002,** *32*, 279.
[80] Wuytack, F.; Raeymaekers, L.; Missiaen, L. *Pflugers. Arch.,* **2003,** *446*, 148.
[81] Wu K.D.; Bungard, D.; Lytton, J. *Am. J. Physiol. Cell. Physiol*., **2001,** *280,* 843.
[82] Carafoli, E. *Physiol. Rev.,* **1992,** *71*, 283.
[83] Ihara, Y.; Kageyama, K.; Kondo, T. *Biochem. Biohys. Res. Comm.*, **2005,** *329*, 134.
[84] Ermak, G.; Davies, K.J.A. *Mol. Immunol.,* **2002,** *38*, 713.
[85] Vats, J.; Klevets, M.; Fedirko, N. *J. Physiol.,* **2002,** *543*, S044.
[86] Moreau, V.H.; Castilho, R.F.; Ferreira, S.T.; Carvalho-Alves, P.C. *Free Radic. Biol. Med.,* **1998,** *25*, 554.
[87] Gutiérrez-Martín, Y.; Martín-Romero, F.J.; Iniesta-Vaquera, F.A.; Gutiérrez-Merino, C.; Henao, F. *Eur. J. Biochem.,* **2004,** 271, 2647.
[88] Juska, A.; Redondo, P.C.; Salido, G.M.; Rosado, J.A. *J. Physiol. Biochem.,* **2005,** *61*, 251.
[89] Stauffer, T.P.; Guerini, D.; Carafoli, E. *J. Biol. Chem.,* **1995,** *270*, 12184.
[90] James, P.H.; Pruschy, M.; Voherr, T.; Penninston, J.T.; Carafoli, E. *Biochemistry,* **1989,** *28*, 4253.
[91] Kosk-Kosicka, D.; Zylinska, L. *Mol. Cell. Biochem*., **1997,** *173*, 79.
[92] Grosman, N. *Immunopharmacology,* **1998,** *40*, 163.
[93] Usachev, Y.M.; DeMarco, S.J.; Campbell, C.; Strehler, E.E.; Thayer, S.A. *Neuron.,* **2002,** *33*, 113.
[94] Barford, D. *Biochem. Soc. Trans,* **1999,** *27*, 751.
[95] Ricciarelli, R.; Azzi, A. *Arch. Biochem. Biophys.,* **1998,** *355*, 197.
[96] Zaidi, A.; Michaelis, M.L. *Free Radic. Biol. Med.,* **1999,** *27*, 810.
[97] Chen, B.; Mayer, M.U.; Squier, T.C. *Biochemestry,* **2005,** *44*, 4737.
[98] Gonzalez, A.; Salido, G.M. *J. Physiol. Biochem*., **2001,** 57, 331.
[99] Parekh, A.B. *News Physiol. Sci*., **2003,** 18, 252.
[100] Pariente, J.A.; Redondo, P.C.; Granados, M.P., Lajas, A.I.; Gonzalez, A.; Rosado, J.A.; Salido, G.M. *E. C. Qua. L.,* **2003,** 1, 29.
[101] Berridge, M.J.; Lipp, P.; Bootman, M.D. *Nat. Rev*., **2000,** 1, 11.
[102] Ichas, F.; Jouaville, L.S.; Mazat, J.P. *Cell,* **1997,** 89, 1145.
[103] Gonzalez, A.; Granados, M.P.; Salido, G.M.; Pariente, J.A. *Mol. Cell. Biochem.,* **2005,** *269*, 165.
[104] Votyakova, T.V.; Reynolds, I.J. *J. Neurochem.,* **2005,** *93*, 526.
[105] Brooked, P.S.; Yoon, Y.; Robotham, J.L.; Anders, M.W., Sheu, S.S. *Am. J. Physiol. Cell Physiol.,* **2004,** *287*, 817.
[106] Granados, M.P., Salido, G.M., Pariente, J.A., Gonzalez, A. *Biol. Cell,* **2005,** (in press).

Received: July 08, 2005 **Revised: August 10, 2005** **Accepted: August 10, 2005**