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Review

Oxidative stress and arachidonic acid mobilization

María A. Balboa, Jesús Balsinde *

Institute of Molecular Biology and Genetics Spanish Research Council and University of Valladolid School of Medicine, Calle Sanz y Forés s/n, 47003 Valladolid, Spain

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Abstract

Reactive oxygen species are known to contribute to tissue damage during injury and inflammation. However, these species can also be sensed by the cells and trigger intracellular signaling cascades. This review examines recent evidence on the involvement of reactive oxygen species in lipid signaling. Attention is focused on activation of phospholipase A_2s, enzymes whose action on membrane phospholipids can also render molecules with opposite effects on cells. The participation of Ca^{2+}-dependent and Ca^{2+}-independent phospholipase A_2s in arachidonic acid mobilization from phospholipids is discussed, with particular attention to the interplay between cytosolic and secreted Ca^{2+}-dependent forms. The involvement of alternative routes for arachidonic acid mobilization under oxidative stress is also considered.

Keywords: Oxidative stress; Arachidonic acid; Lysophosphatidylcholine; Phospholipase A_2; Eicosanoids

1. Introduction

Oxidative damage of tissues and cells has been widely recognized as a key factor for the development of many human diseases [1–4]. Production of reactive oxygen species (ROS) is an inevitable consequence of normal oxidative metabolism. ROS generated at low concentrations by the cells may play roles as signaling molecules within the signal transduction cascades triggered by growth factors, cytokines, and hormones, or may act themselves as direct cellular stimuli, mimicking in this manner the action of these receptor-directed stimuli. However, high ROS levels can be lethal [5]. The cells are therefore endowed with a number of defensive mechanisms against oxidative stress. Occasionally, these antioxidant mechanisms fail and oxidative stress occurs. High ROS levels have a number of direct and indirect consequences on cell signaling pathways that often result in the induction of apoptosis or necrosis [6].

One of the best described effects of ROS on cells is the oxidative modification of fatty acids within membrane phospholipids, i.e., lipoperoxidation. Oxidative modification of membrane phospholipids is a harmful process, altering membrane fluidity, protein structure and cell signaling. The most efficient manner to repair these phospholipids is the selective cleavage of the peroxidized fatty acid residues and their subsequent replacement by native fatty acids [7]. Since most unsaturated and polyunsaturated fatty acids are esterified in the sn-2 position of phospholipids, lipid peroxidation predominantly occurs at this position, which is the one targeted by phospholipase A_2 (PLA_2). For this reason, PLA_2 enzymes may be envisioned to play protective roles against oxidant injury under certain conditions [7]. In contrast with this view, however, oxidative stress and peroxidation of membrane phospholipids have been shown to enhance PLA_2 activity in a number of systems, and the associated activation of PLA_2 may constitute a critical factor in injury [8]. Increased PLA_2 activity in cells responding to oxidants may not only act to help repair damaged membranes but can also give rise to a number of biologically active mediators such as arachidonic acid (AA)-

Abbreviations: AA, arachidonic acid; PLA_2, phospholipase A_2; $iPLA_2$, calcium-independent phospholipase A_2 activity (undefined molecular identity); $iPLA_2$-VIA, Group VIA phospholipase A_2; $cPLA_2\alpha$, cytosolic Group IVA phospholipase A_2; $cPLA_2\gamma$, Group IVC phospholipase A_2; $sPLA_2$, secreted phospholipase A_2; BEL, bromoenol lactone; MAFP, methyl arachidonyl fluorophosphonate; lysoPC, lysophosphatidylcholine; ROS, reactive oxygen species

* Corresponding author. Tel.: +34 983 423 062; fax: +34 983 184 800.
E-mail address: jbalsinde@ibgm.uva.es (J. Balsinde).

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derived metabolites and lysophosphatidylcholine (lysoPC), with potential protective or deleterious roles on their own.

The PLA_2 superfamily of enzymes is comprised of at least 22 distinct mammalian proteins, all of which hydrolyze membrane phospholipids at the sn-2 position of the glycerol backbone, releasing a free fatty acid and a lysophospholipid [9–11]. The PLA_2 enzymes have systematically been classified into several group types according to their primary structure [12–14]. From a mechanistic point of view, the PLA_2s can be grouped into two major families, namely the low-molecular mass enzymes (<20 kDa), which utilize a catalytic histidine, and the high molecular mass enzymes (>40 kDa), which utilize a catalytic serine [9,10]. For detailed information on the structural features, biochemical characteristics, and cellular functions of the PLA_2 enzymes, the reader is kindly referred to recent reviews on the subject [9,10,14–16].

Although the physiological and pathological functions of the PLA_2 enzymes appear to overlap in some cases and differ in others, the involvement of particular PLA_2 forms in AA mobilization under oxidative stress conditions remains a matter of debate. Nonetheless, a consensus is emerging that PLA_2 responsiveness during oxidative stress is a complex process involving multiple enzymes and multiple pathways, and these may vary from one cell to another. To further recognize the role of PLA_2 in oxidant-mediated injury and to clarify the different routes for AA mobilization during oxidative stress, we have focused in this review on recent evidence dealing with the role of PLA_2-dependent increases in AA release from phospholipids and eicosanoid production during oxidative stress. For the purposes of this review we have treated the Ca^{2+}-dependent PLA_2s separately from the Ca^{2+}-independent ones, as the signaling mechanisms involved and general pathophysiological significance appears to clearly differ. While AA mobilization mediated by Ca^{2+}-dependent enzymes generally appears to involve the activation of kinase cascades and intracellular calcium movements, AA mobilization by Ca^{2+}-independent enzymes appears to require neither of these. This may suggest that Ca^{2+}-dependent PLA_2s, when involved in the response to oxidants, participate as signalers on their own. On the contrary, involvement of Ca^{2+}-independent phospholipase A_2s may potentiate the damage in cells undergoing oxidative stress in the absence of clear regulated signaling.

In addition to PLA_2, it is important to note that free AA could also be generated by alternative, PLA_2-independent pathways which may be significant in some cases. A particularly relevant one with regard to oxidative stress is the inhibition of fatty acid reacylation. Evidence has been provided that in some systems, oxidants may increase free AA availability by decreasing reacylation of lysoPC with the fatty acid. This will be discussed further below.

2. Ca^{2+}-dependent phospholipase A_2s

Two different kinds of Ca^{2+}-dependent PLA_2 have been shown to be involved in AA mobilization in response to a variety of receptor-directed and soluble stimuli. These are (i) the Group IVA cytosolic $PLA_2\alpha$ ($cPLA_2\alpha$), and (ii) certain members of the secreted PLA_2 ($sPLA_2$) family, particularly but not exclusively, Groups IIA, V, and X [10,15,17]. Stimulation of the cells by receptor agonists results in the activation of $cPLA_2\alpha$. Under stimulation conditions, the rate of AA release exceeds that of reincorporation into phospholipids, hence net accumulation of AA occurs that is followed by its conversion into different oxygenated compounds, collectively called the eicosanoids. During long-term AA mobilization responses, i.e., those that are typical from immunoinflammatory cells such as macrophages or mast cells, the inducible $sPLA_2$ may also participate in the process, thereby creating an amplification loop that results in a greatly enhanced release of AA for eicosanoid synthesis. There seems to be cross-talk between these two kinds of PLA_2s during cellular activation [18], although the precise molecular details are not yet understood.

To study the interplay between oxidative stress and eicosanoids, Bonventre and colleagues studied the effect of expression of various PLA_2s on H_2O_2-induced AA mobilization in murine mesangial cells [19]. These authors had previously generated a mouse line with the $cPLA_2\alpha$ gene mutated [20]. The mouse strains (C57b/6 and SV/129) used to construct the $cPLA_2^{-/-}$ strain have spontaneous null mutations in the gene encoding for Group IIA PLA_2 [21], and murine mesangial cells do not express Group V $sPLA_2$ under resting or activation conditions [19]. Bonventre and co-workers employed an adenoviral infection technique to stably express either Group IIA or Group V $sPLA_2$ into the cells [19]. Using this approach, it was possible to study the cross-talk between $cPLA_2\alpha$ and either of the $sPLA_2$s during oxidative stress. These are well-defined settings that avoid incomplete PLA_2 inhibitions by the use of either pharmacological inhibitors or antisense approaches. $cPLA_2\alpha$ was found to be responsible for AA release in response to H_2O_2 via a mechanism involving activation of protein kinase C and extracellular-signal regulated kinases, and intracellular Ca^{2+} movements (Fig. 1). Thus, in this system, H_2O_2 mimics the action of a classical receptor-directed agonist in terms of $cPLA_2\alpha$ activation and AA release. Importantly, when present, both Groups IIA and V $sPLA_2$s amplified the $cPLA_2\alpha$-mediated response, this resulting in an increased AA release response [19]. Collectively, these data support the possible existence of cross-talk between $cPLA_2$ and $sPLA_2$ in eliciting a full AA release response. In addition to initiating phospholipid hydrolysis and the subsequent mobilization of AA, $cPLA_2\alpha$ might also provide signals that allow $sPLA_2$ to amplify AA release by one of two possible mechanisms: (i) $sPLA_2$ acts itself on AA-containing phospholipids to provide a more or less significant portion of the total AA liberated and/or (ii) $sPLA_2$ provides further stimulatory signals that allow sustained $cPLA_2\alpha$ activation, which is responsible for the AA release (Fig. 1).

While the central involvement of $cPLA_2\alpha$ in receptor-linked lipid mediator production is demonstrated in a conclusive manner under most pathophysiological conditions [22], the relative contribution of $sPLA_2$ enzymes to lipid mediator production is still controversial. In spite of this, $sPLA_2$s, particularly those belonging to Groups IIA, V, and X, can augment the release of AA and other fatty acids in cell culture studies under different experimental settings [23–27], and pharmacological inhibition of $sPLA_2$s can often attenuate inflammation in experimental animal

models [28–30]. Importantly, a recent gene targeting study of Group V $sPLA_2$ has provided unequivocal evidence for the role of this enzyme in eicosanoid production by macrophages in vivo [31]. Group V $sPLA_2$ might potentially release AA after secretion through two pathways, one involving re-internalization via caveolae [32,33], and the other involving direct interaction with the phosphatidylcholine-rich membranes to directly release AA from various cellular membranes [32,34,35]. Either of these mechanisms would require the $sPLA_2$ to exit the cell as a consequence of the activation process and to subsequently re-associate and/or undergo re-internalization for action in an autocrine or paracrine manner. In contrast, a recent study has uncovered a third mechanism for $sPLA_2$ involvement in AA release in which the de novo synthesized $sPLA_2$s can act intracellularly without the requirement for prior secretion [36].

In the studies of Bonventre and associates [19], a scenario in which $sPLA_2$ enhances the activity of $cPLA_2\alpha$, which then acts to release AA was favored on the basis of experiments demonstrating preferential release of AA over oleic acid in response to H_2O_2 in cells expressing both kinds of PLA_2s. While more direct evidence was not provided, the authors also noted that a correlation exists between the expression level of $cPLA_2\alpha$ and the magnitude of AA release. Such a correlation did not appear to occur between the expression level of $sPLA_2$ and the extent of AA release. Moreover, cells expressing $sPLA_2$ but not $cPLA_2\alpha$ fail to mobilize AA, whereas cells expressing $cPLA_2\alpha$ but not $sPLA_2$ still produce a robust AA release response. These data indicate that $cPLA_2\alpha$ alone is a major enzyme for H_2O_2-induced AA release in mesangial cells even without enhancement from $sPLA_2$.

In agreement with the above scenario, Cho and associates have shown that $sPLA_2$s, acting on the outer membrane of human neutrophils, release fatty acids and lysoPC, which, in turn can act on the cell to increase cytosolic free calcium levels and phosphorylation of $cPLA_2\alpha$, resulting in $cPLA_2\alpha$-dependent leukotriene biosynthesis [37]. Nonetheless, $sPLA_2$s may also act to release AA in a $cPLA_2\alpha$-independent manner, as demonstrated

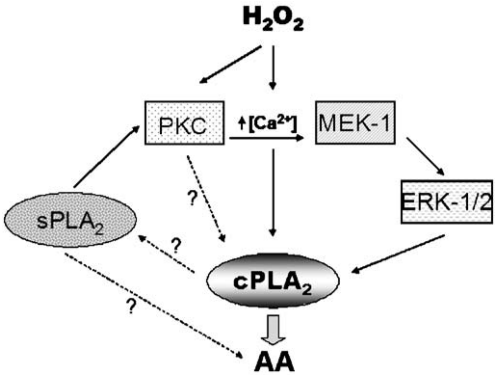

Fig. 1. Role of Ca^{2+}-dependent PLA_2s in oxidative stress. H_2O_2 exposure of the cells results in the activation of intracellular kinase cascades and Ca^{2+} mobilization. Both of these signals converge at the $cPLA_2\alpha$, which is the dominant enzyme in AA mobilization. When present, $sPLA_2$ may amplify AA release by potentiating $cPLA_2$ activation, perhaps via PKC. Alternatively, $cPLA_2\alpha$ may modulate the action of $sPLA_2$, and the latter may also directly effect AA release by directly hydrolyzing membrane phospholipids. PKC, protein kinase C; ERK1/2, extracellular signal-regulated kinases 1/2; MEK, ERK kinase.

by studies in cells from mice lacking $cPLA_2\alpha$ by genetic disruption [38]. Likewise, products of the 12/15-lipoxygenase pathway generated downstream of $cPLA_2\alpha$ mediate the enhanced cellular responses to Groups V and IIA $sPLA_2$s [39,40]. Collectively, these instances, taken from very recent literature, illustrate quite well some of the different possibilities of interplay between $cPLA_2\alpha$ and $sPLA_2$ when present within the same cell.

Similar to the results of Han et al. [19] in murine mesangial cells reported above, in rat mesangial cells, H_2O_2 in combination with platelet-derived growth factor activates the extracellular-signal regulated kinases and p38 mitogen-activated protein kinase, which in turn phosphorylate $cPLA_2\alpha$ and lead to enhanced AA release [41]. Although rat mesangial cells are known to express Groups IIA and V $sPLA_2$s in response to stimulation [42], the possible involvement of either of these in H_2O_2-triggered AA release was not investigated. Importantly however, earlier studies by the same authors in platelets treated with different oxidants had demonstrated an increase in AA release and lysoPC production that was attributed to the enhanced activity of Group IIA $sPLA_2$ [43]. It should be indicated that these studies were performed in rabbit platelets subjected to strong oxidizing conditions, and thus, severe modification of membrane phospholipids by oxidizing reagents occurred, which likely increased accessibility of the membrane to platelet Group IIA $sPLA_2$ [43]. Under most conditions, platelet AA release does not involve participation of Group IIA $sPLA_2$, and this has been correlated with the enzyme's inability to attack the outer platelet surface if drastic membrane rearrangements do not occur first [44,45].

Expression of $sPLA_2$ proteins is typically low in resting cells. Therefore, before $sPLA_2$s can significantly participate in phospholipid hydrolysis and AA mobilization under activation conditions, their content must be increased by induction of the corresponding gene(s). Induction of various $sPLA_2$s in response to a number of proinflammatory stimuli has been widely documented [42,46,47]. Recently, induction of the AA-releasing $sPLA_2$s, Group IIA and Group V, has also been reported under oxidative stress conditions [48,49]. Ishikawa et al. [48] found that Group V $sPLA_2$ is induced during the acute phase of infarction in human heart. These authors hypothesized that, under these settings, Group V $sPLA_2$ may play a dual role, acting both to remove degraded cell membrane in necrotic areas and to produce bioactive lipid metabolites through cooperation with cyclooxygenase-2. A biphasic increase in the expression of Group IIA $sPLA_2$ was reported by Lin et al. [49] after cerebral ischemia in rats. Initially, there was an increase in the ischemic cortex at 30 min, and this was followed by a secondary increase in the penumbral area at 1 day after ischemia–reperfusion. Interestingly, in this study $cPLA_2\alpha$ expression was studied as well, but no changes could be detected under any condition [49].

Studies in Her14 fibroblasts have shown that H_2O_2 and other oxidants such as cumene hydroperoxide induce the transient activation of $cPLA_2\alpha$ in a concentration-dependent manner [50]. $cPLA_2\alpha$ is activated in part by a mechanism involving phosphorylation via the extracellular-regulated kinase pathway, although a phosphorylation-independent mechanism was identified as well. The latter was proposed to be due to free radical

mediated lipid peroxidation, resulting in increased substrate availability because of the presence of oxidized lipids in the membrane. This is reminiscent of the mechanism described for $sPLA_2$ in platelets exposed to various oxidants described above.

In cardiomyocytes, oxidative stress is known to cause either cell death or hypertrophy, and the abundance of antioxidant species within the cell appears to constitute a major factor in determining whether the cell commits to one fate or the other. There is discrepancy as to which signaling pathways are activated under hypertrophy-inducing conditions versus cell-death-related conditions, but one of the biochemical events that appears to take place in both cases is the release of AA from its phospholipid storage sites. Utilizing H9c2 cardiomyocyte-like cells, Winstead et al. [51] investigated the pathways responsible for AA release by these cells in response to H_2O_2. AA release was blunted by the dual $cPLA_2/iPLA_2$ inhibitor methyl arachidonyl fluorophosphonate (MAFP) and the specific $cPLA_2$ inhibitor pyrrophenone, but not by the $iPLA_2$ inhibitor bromoenol lactone (BEL). This suggests the involvement of $cPLA_2\alpha$ in the response [51]. Moreover, MAFP and pyrrophenone also prevent alterations in cell morphology induced by H_2O_2, thus highlighting the role of $cPLA_2\alpha$ in mediating the damage of H9c2 cells undergoing oxidative stress.

3. Ca^{2+}-independent phospholipase A_2s

Involvement of Ca^{2+}-independent PLA_2 enzymes in the selective release of AA following cellular stimulation with receptor or soluble agonists has remained as a debatable issue because most of the earlier evidence was obtained with the use of BEL, an inhibitor whose selectivity in vivo is unclear. Recently, more selective techniques such as antisense oligonucleotide or RNA interference have been utilized to demonstrate that under certain circumstances, Group VIA calcium-independent PLA_2 ($iPLA_2$-VIA) may catalyze membrane hydrolysis leading to increased AA mobilization [52].

The first indications for the involvement of $iPLA_2$ in phospholipid catabolism and fatty acid release during oxidative stress conditions were provided by Gross and colleagues in the 1980s in studies on ischemia–reperfusion injury, although the molecular identity of the $iPLA_2$ activity involved was not ascertained [53]. More recently, two independent studies in phagocytes have shown that micromolar concentrations of H_2O_2 induce substantial release of AA and attendant eicosanoid production by a mechanism that, unexpectedly, does not involve $cPLA_2\alpha$ but rather $iPLA_2$-VIA [54,55]. Longer incubation times with the oxidant results in the cells committing to apoptotic cell death [56], suggesting that in these studies, the oxidant is acting as a cellular toxin.

Incubation of mouse peritoneal macrophages and RAW 264.7 macrophage-like cells with H_2O_2 causes a significant release of AA that is completely abrogated by the selective $iPLA_2$ inhibitor BEL but not by antisense oligonucleotide inhibition of $cPLA_2\alpha$. Moreover, neither protein kinase C inhibitors nor calcium chelators prevented the release of AA [54]. Similar results were reported in U937 phagocytes where, in addition, the effect of an $iPLA_2$-VIA antisense oligonucleotide was tested as well and found to blunt the oxidant-induced AA release [55]. In the latter study, abundant release of oleic acid was also observed, indicating that the $iPLA_2$-VIA effect is not AA-specific.

The mechanism for H_2O_2-induced, $iPLA_2$-mediated fatty acid mobilization was studied in detail in the U937 cell system [55]. H_2O_2 treatment of these cells does not increase the $iPLA_2$ specific activity of the cells, as measured by different in vitro assays, indicating that a stable activation of the $iPLA_2$ (e.g., phosphorylation) is not the mechanism for H_2O_2-mediated AA release in U937 cells. When membranes from H_2O_2-treated cells are used in the assay, the $iPLA_2$ activity measured is significantly higher than that found in membranes from otherwise unstimulated cells. Therefore, treating the cells with H_2O_2 results in facilitated $iPLA_2$ attack on membrane phospholipids. Since membranes from H_2O_2-treated cells were found to contain higher amounts of lipid peroxides than membranes from untreated cells [55], these findings suggest that lipid hydrolysis by $iPLA_2$ occurs more readily in H_2O_2-treated cells because of changes in the physical state of membrane substrates, which may result, at least in part, from lipid peroxide accumulation.

Taken together, the above findings suggest a model for $iPLA_2$-mediated fatty acid mobilization in H_2O_2-treated cells whereby the oxidant induces lipid oxidation, which results in accumulation of lipid peroxides at the membrane. These lipid peroxides destabilize the membrane and render it more susceptible to $iPLA_2$ attack, which then results in increased liberation of fatty acids and formation of prostaglandins (Fig. 2). An important aspect of the above model is that this fatty acid release appears to occur in the absence of $cPLA_2\alpha$ activation, which underscores the apparent lack of a regulated signaling component in the process. Still, a mechanism such as this one may be very relevant under oxidative stress, where increased $iPLA_2$ activity may account for a significant phospholipid hydrolysis before cellular homeostasis is re-established.

$iPLA_2$-dependent AA mobilization in response to oxidant exposure has also been documented in cells not of phagocytic origin like rat uterine stromal cells [57] and murine astrocytes

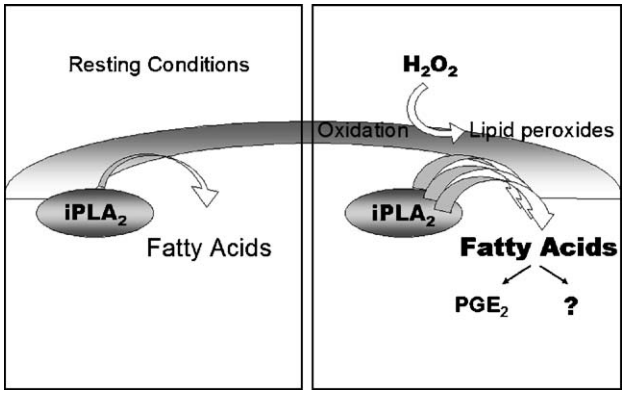

Fig. 2. Role of $iPLA_2$ in oxidative stress. The continuing action of $iPLA_2$ membrane phospholipids under resting conditions helps maintaining the steady-state levels of lysoPC and results the liberation of low levels of free fatty acids such as AA (left panel). When the cells are exposed to H_2O_2, the hydrolytic activity of the $iPLA_2$ increases as result of lipoperoxidation, this leading to increased levels of free AA. Free AA under these conditions may be utilized for prostaglandin synthesis or be used for other cellular functions (right panel).

[58]. In the latter study, a possible parallel role for cPLA$_2\alpha$ was suggested as well on the basis of partial dependence with Ca^{2+} and the complete abrogation of the response by MAFP but incomplete with BEL [58]. However, the response of astrocytes to H_2O_2 appears to also proceed independently of protein kinase cascades, which is in agreement with the results in macrophages discussed above [55].

Group IVC PLA$_2$, also known as cPLA$_2\gamma$, is a Ca^{2+}-independent paralogue of cPLA$_2\alpha$. In contrast to cPLA$_2\alpha$, the information on cPLA$_2\gamma$ is very limited. It has been shown that cPLA$_2\gamma$ can be activated in vivo by serum [59]. However, its fatty acid selectivity is controversial. When human embryonic kidney 293 overexpressing cPLA$_2\gamma$ are exposed to H_2O_2 and other peroxides, significant release of AA is observed [60]. Inhibitors of tyrosine kinases blunt the effect but immunoprecipitation and immunoblotting assays fail to detect direct tyrosine phosphorylation of cPLA$_2\gamma$, which suggests the existence of accessory proteins that may help regulate cPLA$_2\gamma$ during situations of oxidative stress [60]. Thus, the proposal is made that cPLA$_2\gamma$ is constitutively expressed in the endoplasmic reticulum and Golgi complex, and plays important roles in remodeling and maintaining membrane phospholipids under oxidative stress, both by removing peroxidized fatty acyl chains and enhancing free AA levels [52].

4. PLA$_2$-independent mobilization of AA

AA is a participant of a deacylation/reacylation cycle of membrane phospholipids, the so-called Lands cycle, in which a fatty acid is hydrolyzed from phospholipids by PLA$_2$ and another fatty acid is incorporated by the concerted action of fatty acyl-CoA synthetase and lysophospholipid acyltransferase [61]. It is believed that the Lands pathway constitutes the major route for incorporation of AA and other polyunsaturated fatty acids in the phospholipids of a variety of cells, most notably inflammatory cells [61]. Since under unstimulated cells, the reacylation pathway dominates over the phospholipolytic step, cellular free AA is kept at a very low level compared to that present in esterified form in phospholipids. However, given this dominance of AA reacylation over AA deacylation in cells, accumulation of free fatty acid may also occur if the reacylation is inhibited, and this is exactly what has been shown to occur in rat alveolar macrophages [62] and vascular smooth muscle cells [63] exposed to H_2O_2. In both of these studies, a reciprocal correlation was found between inhibition of phospholipid fatty acid acylation and H_2O_2-stimulated accumulation of free AA. An effect of H_2O_2 on the enzymes of the reacylation pathway, arachidonoy-CoA synthetase and lysophospholipid: arachidonoyl-CoA acyltransferase was discarded by in vitro assay studies. Importantly, a good correlation was observed between decreases in AA acylation and cellular ATP content. Since ATP is required for synthesis of the acylation intermediate arachidonoyl-CoA, the suggestion was made that this was the means by which H_2O_2 inhibited acylation and hence increased AA release in rat alveolar macrophages [62] and vascular smooth muscle cells [63].

5. Conclusion

Oxidant stress is implicated in numerous proinflammatory responses in mammalian cells. H_2O_2 is known to trigger AA release and metabolism in various cell types, but the mechanisms involved appear to profoundly vary from one cell to another. Thus, AA mobilization in response to an oxidative stress appears to be a very complex process, potentially involving multiple enzymes and pathways.

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