PART I

OXIDATIVE STRESS IN VERTEBRATES

1

GENERATION OF REACTIVE OXYGEN SPECIES IN THE BRAIN: SIGNALING FOR NEURAL CELL SURVIVAL OR SUICIDE

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1.1 INTRODUCTION

Oxidative stress is a redox-sensitive process that occurs in the cell when antioxidant mechanisms are overwhelmed by the generation of reactive oxygen species (ROS), leading to oxidation of lipids, proteins, and DNA in ways that impair cellular function [1]. Thus oxidative stress is a threshold phenomenon characterized by a major increase in the amount of oxidized cellular components. ROS include superoxide anions, hydroxyl, alkoxyl, and peroxyl radicals, and hydrogen peroxide, which are generated as by-products of oxidative metabolism, in which energy activation and electron reduction are involved. The chemical reactivity of ROS varies from the very toxic hydroxyl ($^{\bullet}OH^{-}$) to the less reactive superoxide radical ($O_2^{\bullet-}$). The initial product, $O_2^{\bullet-}$, results from the addition of a single electron to molecular oxygen. $O_2^{\bullet-}$, is rapidly dismutated by superoxide dismutase (SOD), yielding H_2O_2 and O_2, which can be reused to generate superoxide radical. In the presence of reduced transition metals, H_2O_2, although less reactive than $O_2^{\bullet-}$, and highly diffusible, can be converted into the highly reactive hydroxyl radical $HO^{\bullet}$. The tight regulation of ROS generation and removal makes fluctuations in their levels transient, a feature that is characteristic of second messengers. ROS may also act as an intracellular "rheostat," closely modulating the activity of a discrete set of biochemical reactions, which contribute to cell proliferation, migration, and survival [2]. ROS not only inactivate membrane proteins and DNA but also promote peroxidation of neural membrane polyunsaturated fatty acids associated with glycerophospholipids, enhance levels of ceramide, and facilitate the formation of hydroxyl/ketocholesterol levels (Fig. 1.1). These processes promote neurodegeneration through apoptosis [3–5]. The polyunsaturated fatty acids, which are located at the *sn*-2 position of glycerol moiety in the glycerophospholipid, are most susceptible to free radical attack at the α-methylene carbon in the alkyl chain of the fatty acid that is adjacent to the carbon-carbon double bond. Under aerobic conditions a polyunsaturated fatty acid with an unpaired electron undergoes a molecular rearrangement by reaction with O_2 to generate a peroxyl radical. The peroxyl radical captures hydrogen atoms from the adjacent fatty acids to form a lipid hydroperoxide. The lipid hydroperoxides thus formed are not completely stable in vivo and, in the presence of iron, can further break down to radicals that can propagate the chain reactions started by an initial free radical attack. The major sources of ROS are the mitochondrial respiratory chain, where $O_2^{\bullet-}$, is generated by electron leakage from complexes I and III of the electron transport chain (Fig. 1.2) [6, 7]. Microsomes and peroxisomes are also sources of ROS, primarily H_2O_2, whereas immune cells such as neutrophils and macrophages possess oxygen-dependent mechanisms to fight against invading microorganisms. Enzymes, such as xanthine/xanthine oxidase, myeloperoxidase, cytochrome *P*450 in cell cytoplasm,

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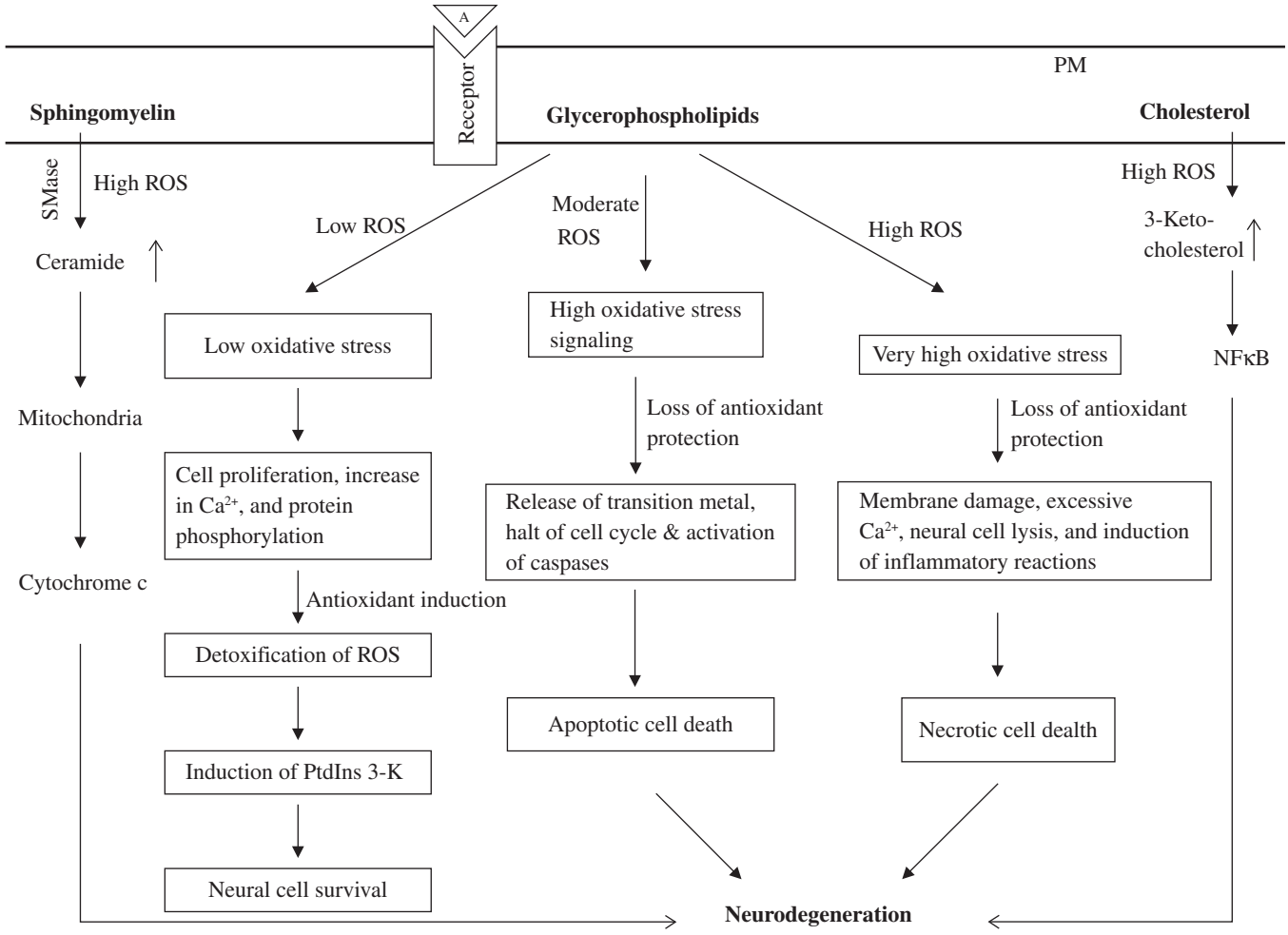

Fig. 1.1 Effect of reactive oxygen species (ROS) on lipid constituents (glycerophospholipid, sphingolipid, and cholesterol) of neural membranes. Low ROS levels promote neural cell survival, whereas high ROS levels promote neurodegeneration through apoptotic and necrotic cell death. PM, plasma membrane; NF-κB, nuclear factor-κB; SMase, sphingomyelinases.

COX, LOX, nitric oxide synthase, and NADPH oxidase contribute to ROS production in plasma membranes and mitochondria (Fig. 1.2). The presence of redox-active metals, such as iron and copper, also contributes to ROS generation. In the presence of Fe^{2+} and Fe^{3+}, $HO^{\bullet}$ can be generated through the Fenton reaction or the Haber–Weiss reaction [7].

1.2 ROLE OF REACTIVE OXYGEN SPECIES IN NEURAL CELLS

As stated above, in brain ROS are generated during oxidative metabolism. ROS-mediated damage to neural membranes is accompanied by (a) changes in physicochemical properties of neural membranes (microviscosity and fluidity) not only resulting in exchange of phospholipids between the two halves of the lipid bilayer but also altering the orientation of optimal domains of receptors, enzymes, and ion channels; (b) changes in the number of receptors and their affinity for neurotransmitters; and (c) inhibition of ion pump operation and entry of K^+ and Ca^{2+} into neural cells resulting in changes in ion homeostasis. The presence of peroxidized glycerophospholipids in neural membranes may also induce a membrane-packing defect, making the *sn*-2 ester bond more accessible to the action of phospholipase A_2 (PLA_2) and the release of free arachidonic acid (ARA) or docosahexaenoic acid (DHA). ARA and DHA act as substrates for the synthesis of eicosanoids and docosanoids, respectively [3]. Lyso-phospholipid, the other product of PLA_2-catalyzed reaction, not only induces detergent-like effects leading to further disorganization of neural membranes but also acts as substrate for platelet-activating factor (PAF) [8]. Emerging evidence suggests that enzymic and nonenzymic oxidation of polyunsaturated fatty acids

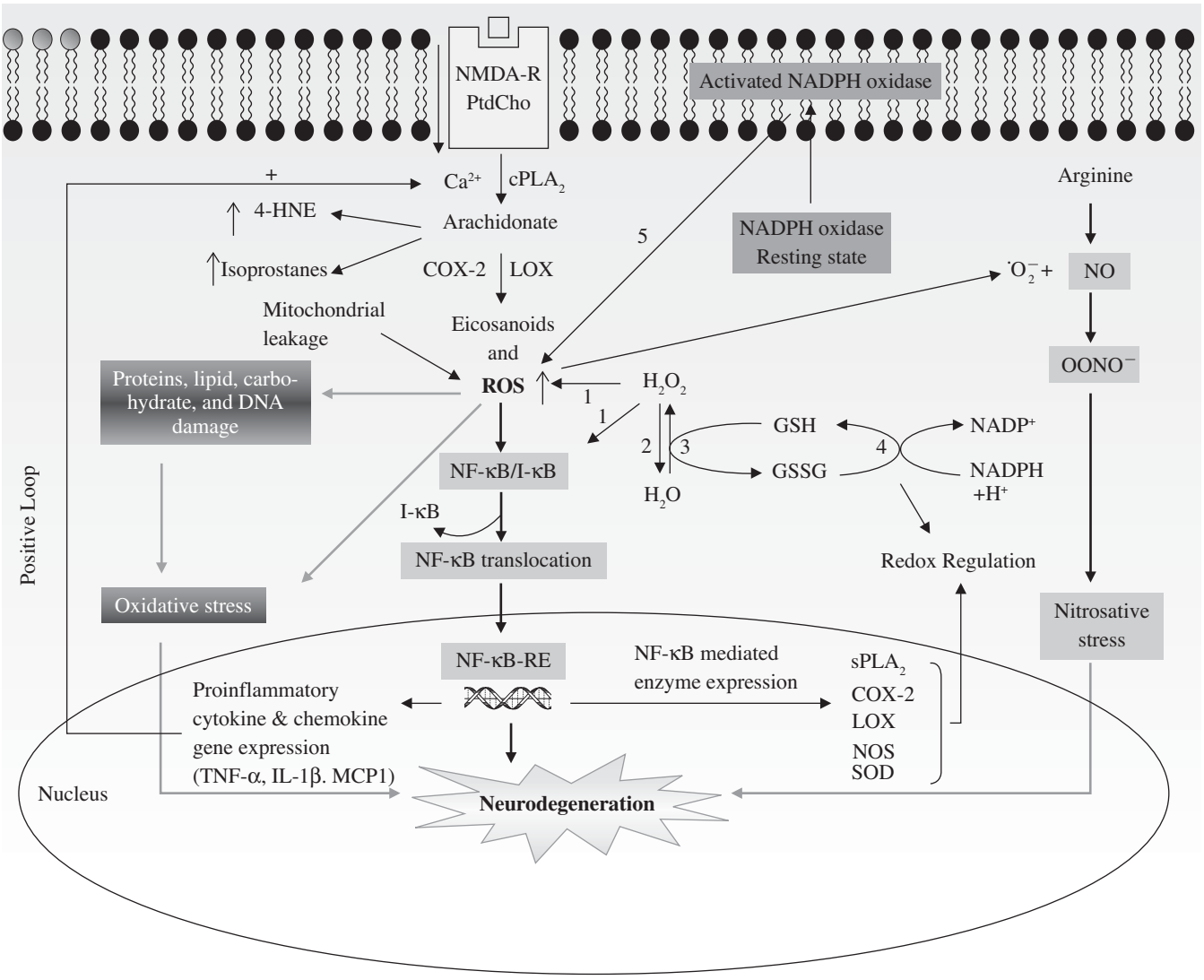

Fig. 1.2 Generation of reactive oxygen species (ROS) and enzymic and nonenzymic markers for oxidative stress. 1, Superoxide dismutase (SOD); 2, catalase; 3, glutathione peroxidase; 4, glutathione reductase; 5, NADPH oxidase. $cPLA_2$, cytosolic phospholipase A_2; $sPLA_2$, secretory phospholipase A_2; COX-2, cyclooxygenase-2; LOX, lipoxygenase; NOS, nitric oxide synthase; GSH, reduced glutathione; GSSG, oxidized glutathione; H_2O_2, hydrogen peroxide; 4-HNE, 4-hydroxynonenal; NO, nitric oxide; $OONO^-$, peroxynitrite. Activation of NF-κB by ROS leads to its translocation to the nucleus, where it facilitates the transcription of proinflammatory enzymes ($sPLA_2$, COX-2, NOS, and SOD) and proinflammatory cytokines (TNF-α and IL-1β). These cytokines upregulate activities of $cPLA_2$ and $sPLA_2$ through a positive loop mechanism in cytoplasm and neural membranes. Upward arrows indicate increase in levels of metabolites. *(See color insert.)*

leads to the formation and accumulation of ARA-derived eicosanoids, 4-hydroxy-2-nonenal (4-HNE), isoprostanes, isofurans, and isoketals and DHA-derived docosanoids, 4-hydroxyhexanal, neuroprostanes, neurofurans, and neuroketals that induce specific cellular dysfunction [3, 5, 9]. In addition, lipid peroxidation also leads to the generation of lipid hydroperoxides, which inhibit the reacylation of phospholipids in neuronal membranes [10]. The detoxification of glycerophospholipid hydroperoxides is accomplished through the combined enzymic activity of PLA_2 and reduction of the released fatty acid hydroperoxides with phospholipid hydroperoxide glutathione peroxidase [11–13]. The latter enzyme not only acts on membranes and reduces glycerophospholipid hydroperoxides to the nontoxic hydroxyl derivatives [14, 15] but reduces H_2O_2 to water to limit its harmful effects. Phospholipid hydroperoxide glutathione peroxidase is different from the classic glutathione

peroxidase, which mainly reduces H_2O_2. The restoration of neural membrane integrity by the reaction catalyzed by phospholipid hydroperoxide glutathione peroxidase is achieved by the reinsertion of nonoxidized fatty acyl groups through the involvement of the deacylation/reacylation cycle [16]. Nonenzymically, ROS buildup can be prevented by vitamins E and C. These vitamins terminate lipid chain reactions involving peroxyl radicals. In addition to being a cofactor of various antioxidant enzymes, GSH, which is the most abundant peptide in cells, performs many functions. The thioredoxin system is another important thiol antioxidant system consisting of thioredoxin (Trx) and thioredoxin reductase. Trx is a multifunctional selenoprotein containing two redox-active cysteines and a conserved active site (Cys-Gly-Pro-Cys) [17, 18]. Although many ROS are quenched by GSH, other thiol-containing proteins also participate in neutralizing ROS [19].

1.2.1 Modulation of Enzyme Activities, Transcription Factors, and Genes by ROS

ROS regulate activities of several enzymes in neural cells. Thus ROS not only modulate activities of protein tyrosine kinases, protein phosphatases, and mitogen-activated protein kinases [extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK)/stress-activated protein kinase, and p38 pathway] (Fig. 1.3) but also play an important role in regulating intracellular Ca^{2+} homeostasis and RhoA/Rho kinase signaling [20]. Low and moderate levels of ROS activate PtdIns 3-kinase signaling and promote cell survival. PtdIns 3-kinase/protein kinase B (Akt) transduces the signal for cell survival mainly through phosphorylation of target molecules by Akt. This results in the inactivation of proapoptotic proteins and activation of transcription factors that target the expression of antiapoptotic proteins. ROS increase vascular $[Ca^{2+}]_i$ by stimulating inositol trisphosphate-mediated Ca^{2+} mobilization, by increasing cytosolic Ca^{2+} accumulation through sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase inhibition, and by stimulating Ca^{2+} influx through Ca^{2+} channels. Increased ROS production enhances Ca^{2+} signaling and upregulates RhoA/Rho kinase, thereby altering vascular contractility and tone in the vasculature [21]. ROS also inactivate protein tyrosine phosphatases in a dose- and time-dependent manner [22, 23]. ROS activate metalloproteinases, and this stimulation is blocked by *N*-acetyl cysteine [24]. In addition, ROS modulate transcription factors (NF-κB, HIF, CREB, AP-1, ATF2, A-1, CHOP-1, and E2F), modulate the cell cycle, and ion transport (Fig. 1.3). Although the molecular mechanisms underlying ROS-mediated alterations of kinases and transcription factors are not fully understood, it is becoming increasingly evident that the regulation of stress-responsive proteins by ROS may be closely associated with the above alterations. ROS-mediated cellular changes involve (a) the direct effect of ROS on the kinase or transcription factor, which can alter conformation and activity, and (b) the effect of cysteine-rich, redox-sensitive proteins, which are associated with the regulation of stress-responsive proteins. Oxidative stress not only produces conformational changes in redox-responsive proteins but also facilitates the generation of dimers/multimers of these proteins. The redox-responsive proteins include thioredoxin and glutathione *S*-transferase [25]. Emerging evidence suggests that low levels of ROS induce minor changes in levels of Ca^{2+}, enzyme activities, transcription factors, cell cycle, and ion transporters, which support and maintain normal cell function through the tight regulation of diverse intracellular signaling networks. However, moderate and high levels of ROS can inflict damage to all subcellular organelles (e.g., mitochondria, endoplasmic reticulum, etc.), eventually leading to cell death.

The brain processes large amounts of O_2 in relatively small mass and has a high content of substrates available for oxidation in conjunction with low antioxidant activities making polyunsaturated fatty acids found in glycerophospholipids extremely susceptible to oxidative damage. In addition, neurons of certain regions of the brain, such as the hippocampus, may be particularly vulnerable to oxidative stress because of their low endogenous levels of vitamin E and glutathione relative to other brain regions. Such a depressed defense system may be adequate under normal circumstances. However, generation of high levels of ROS following acute neural trauma (stroke, spinal cord trauma, and traumatic brain injury) and neurodegenerative diseases, such as Alzheimer disease (AD), Parkinson disease (PD), and amyotrophic lateral sclerosis (ALS), and low antioxidant defenses can predispose the brain to high oxidative stress leading to neuronal injury and death [3].

1.2.2 Modulation of Genes by ROS

In nonneural cells, ROS regulate many genes, including adhesion molecules and chemotactic factors, antioxidant enzymes, and vasoactive substances [2]. Some of these genes are associated with adaptive responses. This includes the induction of superoxide dismutase (SOD), catalase, and glutathione peroxidase (Gpx) by H_2O_2, supporting the view that newly synthesized protective proteins are needed for adaptive responses [26, 27]. Most redox-sensitive genes have been identified on the basis of their responsiveness to externally applied oxidant stress; only a few have been shown to be

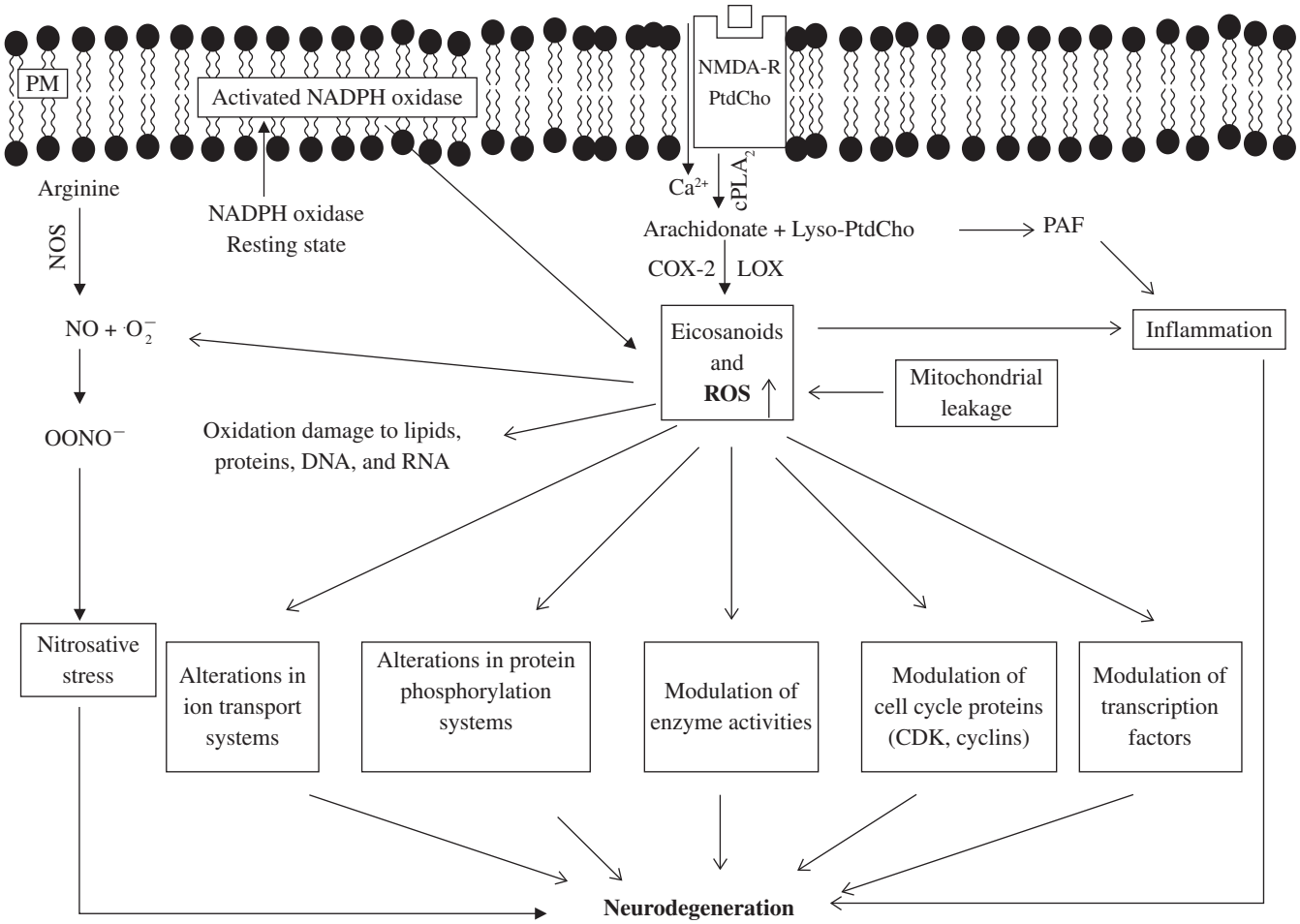

Fig. 1.3 Roles of reactive oxygen species (ROS) in the brain. $cPLA_2$, cytosolic phospholipase A_2; COX-2, cyclooxygenase-2; LOX, lipoxygenase; NOS, nitric oxide synthase; NO, nitric oxide; $OONO^-$, peroxynitrite; PAF, platelet-activated factor. In brain, ROS are generated through mitochondrial dysfunction, ARA oxidation, and NADPH oxidase activation. Low ROS levels modulate ion transport, protein phosphorylation, enzymic activities, cell cycle, and transcription factors, but high ROS levels damage neural membrane lipids, protein, and nucleic acid.

downstream of an endogenous source of ROS, such as the NADPH oxidase. These include TNF-α- and lactosylceramide-mediated induction of intercellular adhesion molecule (ICAM-1) [28] and Ang II, PDGF, and TNF-α stimulation of monocyte chemotactic protein (MCP)-1 [29]. In contrast, stimulation of MCP-1 by IL-1β in vascular smooth muscle cells (VSMCs) is not affected by antioxidants, suggesting that the control of gene expression by ROS is both stimulus- and tissue specific [30]. Neural and nonneural cells possess signaling pathways that can sense oxidative stress and launch adaptive responses that bolster the antioxidant defense networks. Accumulating evidence suggests that modulation of gene expression by ROS occurs at cellular and subcellular levels. Low ROS levels involve modulation of some neuroprotective genes along with redox-sensitive transcription factors (AP-1 and Nrf2). These factors modulate genes for antioxidant response element (ARE), endogenous antioxidants, phase II detoxifying enzymes, and transporters. Nrf2 is a transcription factor that regulates the basal and inducible expression of a wide array of antioxidant genes. After phosphorylation and dissociation from the cytosolic protein Keap1, a scaffolding protein that binds Nrf2 and Cul3 ubiquitin ligase for proteasomal degradation, Nrf2 rapidly translocates to the nucleus, where it activates the ARE in the promoter region of many antioxidant genes [31]. Nrf2 activates transcription primarily through the formation of a dimer with a small musculoaponeurotic fibrosarcoma oncogene family of proteins (Maf) [32, 33]. The binding

of the small Maf-Nrf2 dimers to ARE sequences leads to a coordinated transcriptional activation of a battery of antioxidant enzymes and detoxifying proteins. This regulated adaptive response is called the "phase II detoxification response" [34]. Activation of Nrf2 not only increases the abundance of thioredoxins and glutathione-synthesizing enzymes and glutathione *S*-transferases but also enhances the expression of molecular chaperones, proteasome subunits, and various other cytoprotective proteins [35]. Expression of the Nrf2-dependent proteins is critical to maintaining cellular redox homeostasis through elimination of toxins [2]. Modulation of other genes involves translocation of a specific transcription factor NF-κB, which facilitates expression of proinflammatory enzymes, chemokines, and cytokines. The mechanism by which cytokines (TNF-α) induce neurodegeneration appears to be related not only to the depletion of GSH but also to the redox-dependent generation of ceramide from sphingomyelin, formation of 4-HNE and isoprostane from membrane glycerophospholipids, and generation of hydroxyl- and ketocholesterol from cholesterol [5, 6]. Hydroperoxy fatty acids and H_2O_2 promote the expression of c-Fos and Jun 2 proteins that form heterodimers and activate AP-1 [36]. Activation of nitric oxide synthase (NOS) during oxidative stress generates $NO^{\bullet}$, an important signaling molecular and vasodilator. $NO^{\bullet}$ increases the transcription of Iκ-B, the inhibitory factor that binds NF-κB and facilitates its retention in the cytoplasm [37]. The turnover of I-κB protein is also oxidant sensitive, and antioxidants can block agonist-mediated stimulation of I-κB phosphorylation and degradation [2, 37]. Conversely, H_2O_2 increases translocation of NF-κB to the nucleus, where it facilitates the transcription of responsive genes [38]. In addition, several other mammalian transcription factors are directly modified by ROS or by reducing proteins that modify cysteine residues involved in DNA binding [2]. These transcription factors include AP-1, NF-κB, and hypoxia-inducible factor-1 (HIF-1) [2, 39, 40]. Both c-Fos and c-Jun contain a conserved cysteine in a basic motif that, when oxidized, interferes with the interaction of these proteins with AP-1 consensus sequences. Conversely, if c-Fos/c-Jun heterodimers are complexed with AP-1, they cannot be oxidized [39]. The oxidation state of these important proteins is modulated by redox factor-1 [1], a protein that, in cooperation with thioredoxin, facilitates the cycling of the critical cysteines between reduced and oxidized forms [2, 39]. Thioredoxin also modulates HIF-1-dependent transcription [40] and modifies the DNA binding and transcriptional activity of NF-κB by reducing cysteine 62 [41]. Collectively, these studies indicate the importance of the nuclear redox state in regulating ROS-mediated gene expression [2].

1.2.3 Modulation of Long-Term Potentiation, Cognition, and Memory Formation by ROS

It is well known that hippocampus is involved in synaptic plasticity associated with cognitive function and learning and memory. This region is highly susceptible to oxidative stress [42]. Long-term potentiation (LTP) is defined as a long-lasting increase in synaptic efficacy following high-frequency stimulation of afferent fibers. Treatment of hippocampal slices with H_2O_2 at millimolar concentrations produces oxidative stress [43] and inhibition of LTP, whereas micromolar concentrations of H_2O_2 enhance LTP [44, 45]. The action of H_2O_2 is mediated through the release of calcium ions from internal stores, modulating the activity of specific calcium-dependent protein phosphatases, PLA_2, and phospholipase C (PLC). These enzymes modulate synaptic plasticity. The above observations are supported by studies in aged mice overexpressing extracellular SOD. These mice perform better in a water maze memory task than aged control mice [46]. It is also reported that an increase in SOD activity, which impairs LTP, is caused by a secondary increase in H_2O_2 levels and catalase reverses the effects of SOD. The molecular mechanism associated with H_2O_2-mediated impairment of LTP is not fully understood. However, it is becoming increasingly evident that serine/threonine phosphatases (PP2A) contribute to the impairment of LTP [45, 47]. The ketogenic diet (high-fat and low-carbohydrate with anticonvulsant), which induces ketonemia, not only downregulates PP2A activity and expression of this enzyme but also prevents oxidative stress-mediated impairment of LTP by inhibiting PP2A [48–50]. It is proposed that oxidative stress-mediated impairment of hippocampal LTP is associated with low levels of ROS production, changes in synaptic plasticity, and activation of PP2A, and that ketone bodies prevent this impairment of LTP through the inhibition of PP2A. The regulation of synaptic activity by ROS is not confined to hippocampal synapses. A series of studies have indicated that H_2O_2 modulates dopamine release in dorsal striatum through a ROS sensor on potassium channels that control the excitability of the dopamine-releasing neurons [51]. Emerging evidence suggests that the signal transduction network associated with synaptic plasticity involves many players, including protein kinases, phosphatases, phospholipases, transcription factors, and other Ca^{2+}-dependent enzymes [52], which contribute to the generation of ROS.

1.2.4 Modulation of Cell Death by ROS

As mentioned above, the brain consumes large quantities of oxygen relative to its contribution to total body

mass. This, together with low levels of vitamin E, glutathione, and lipoic acid and low activities of SOD, catalase, and peroxidase, places the brain at the risk for damage mediated by ROS [6]. ROS generation through mitochondrial dysfunction gradually disrupts the intracellular calcium homeostasis, which modulates neuronal excitability and synaptic transmission, making neurons more vulnerable to additional oxidative stress, and leads to neurodegeneration. Among neural membrane components lipids are most susceptible to oxidative modification. Lipid peroxidation produces lipid radicals, which can further attack the subsequent lipid molecules and propagate through a chain reaction. Lipid peroxidation leads to the formation of a number of aldehyde byproducts, including malondialdehyde (MDA), 4-HNE, and acrolein. The most abundant aldehydes are 4-HNE and MDA, while acrolein is the most reactive. In addition, stimulation of PLA_2s, sphingomyelinases, and cytochrome *P*450 hydroxylases produces high levels of glycerophospholipid-, sphingolipid-, and cholesterol-derived lipid mediators, which support inflammatory processes leading to neural cell death in the brain [5, 6]. The highly reactive $OH^{\bullet}$, generated through the Fenton reaction, and $ONOO^{-}$ formed from the reaction between $O_2^{\bullet -}$ and nitric oxide ($NO^{\bullet}$), target protein components of neural membranes. Irreversible protein oxidation includes nitrosylation of cysteine sulfhydryl groups, tyrosine, methionine, and tryptophan by $ONOO^{-}$. Nitration of tyrosine residues may inhibit its phosphorylation or adenylation, important for protein function [53]. Severe oxidative stress can induce disulfide bond-mediated protein cross-linkage or secondary oxidative modifications such as adduct formation between oxidized proteins and lipid peroxides or glycation products, leading to accumulation of damaged proteins and cell death [54]. Some protein modifications, such as phosphorylation, are reversible modifications that can be overcome by specific enzymes (protein phosphatases) that cause a protein to "revert" back to its original protein structure, while other protein modifications, such as protein nitration and HNE-mediated modification (4-HNE-histidine and glutathione-4-HNE Michael adducts), are irreversible. Oxidative modification of proteins may induce alterations in the structure of proteins with subsequent loss of normal physiological cell functions leading to cell death.

Compared with lipids and proteins, neural cell DNA is less susceptible to oxidative modifications because of its double-helix structure and the protective shield from histone and other coating proteins. However, under severe oxidative stress nuclear DNA damage is also oxidized with the generation of 8-hydroxy-2-deoxyguanosine (8-OHdG) [55]. Collective evidence suggests that ROS-mediated alterations in neural membrane components, generation of high levels of glycerophospholipid-, sphingolipid-, and cholesterol-derived lipid mediators, modifications of proteins and DNA, loss of Ca^{2+} homeostasis, and induction of mitochondrial dysfunction may result in neural cell death.

1.3 ROS-MEDIATED SURVIVAL SIGNALING IN NEURAL CELLS

The cellular response to ROS depends not only on their concentration but also on their chemical nature. Low concentrations of ROS do not cause cell death but instead induce an adaptive and survival response to the oxidative stress through modulation of proliferation, synaptic plasticity, gene transcription, and neuronal excitability (Fig. 1.4). Adaptive and survival responses are modulated by cellular Ca^{2+} gradient. In neural cells, maintenance of Ca^{2+} gradients requires reduction in ATP level, which is associated with generation of ROS through respiratory control mechanisms. The selective oxidation of calmodulin (a Ca^{2+} binding protein) and alteration in Ca^{2+}-ATPase (a Ca^{2+}-dependent enzyme associated with efflux of Ca^{2+}) activity during oxidative stress may represent an adaptive response to oxidative stress that functions to downregulate energy metabolism and the associated generation of ROS. During oxidative stress, enhanced sensitivity of Ca^{2+} binding proteins is closely associated not only with modulation of signal transduction processes but also with intracellular energy metabolism, supporting the view that the selective oxidation of critical signal transduction proteins may represent a regulatory mechanism that functions to minimize the generation of ROS through respiratory control. Thus decrease in the rate of ROS formation, in turn, may promote cellular survival under conditions of low oxidative stress, when ROS overwhelm cellular antioxidant defense systems, by minimizing the nonselective oxidation of a range of lipid, proteins, and nucleic acids [56, 57]. In addition, ROS may function as signaling molecules that fine-tune neural cell metabolism through the selective oxidation of Ca^{2+} binding proteins in order to minimize widespread oxidative damage and protein aggregation. Formation of low ROS levels also minimizes protein oxidation, which promotes intracellular repair mechanisms that function to eliminate damaged and partially unfolded proteins. Since the rates of protein repair or degradation compete with the rate of protein aggregation, the modulation of intracellular Ca^{2+} concentrations and energy metabolism through the selective oxidation of critical signal transduction proteins (Ca^{2+} binding proteins) maintains cellular function by minimizing protein aggregation [58]. Furthermore, ROS, specifically H_2O_2, are also essential for

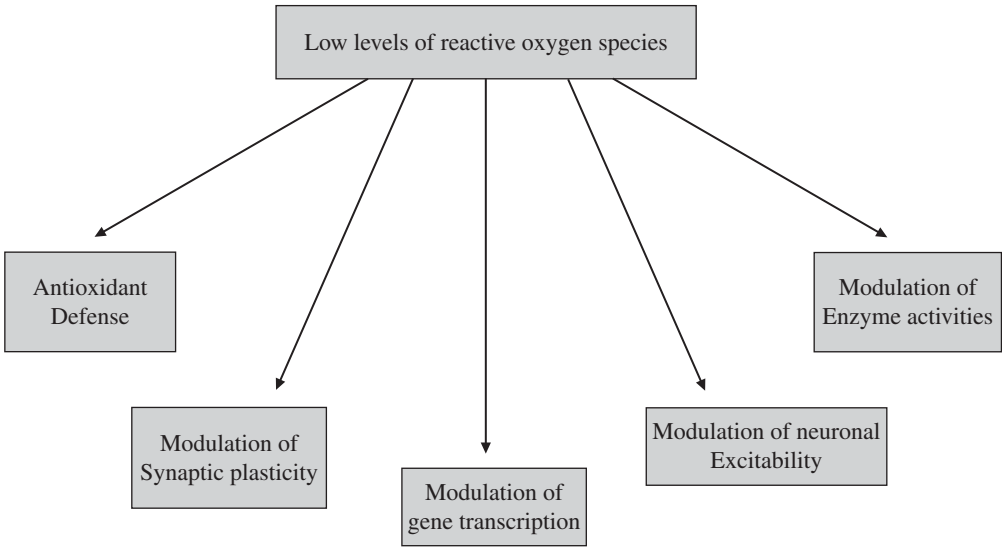

Fig. 1.4 Modulation of neural cell survival by low levels of ROS generated during normal metabolic conditions.

growth factor-mediated signal transduction, mitochondrial function, and maintenance of normal thiol redox-balance. These processes are closely associated with neural cell survival.

ROS activate both acidic and basic sphingomyelinases (SMases). The activation of SMases results in generation of ceramide. Low levels of this lipid mediator stimulate and modulate signaling pathways involved in the regulation of cell viability, differentiation, growth, and survival [59]. However, excessively high levels of ceramide can trigger apoptosis through the release of cytochrome *c*. Ceramides generated in response to membrane-associated oxidative stress have been implicated in the dysfunction and death of cells in neurotraumatic and neurodegenerative diseases [5].

1.4 ROS-MEDIATED INJURY IN NEURAL CELLS

High ROS levels induce neurodegeneration through apoptotic and necrotic cell death. The reaction between high ROS levels and proteins leads to a chemical cross-linking of membrane proteins and phospholipids resulting in alterations in membrane-bound enzymes and reduction in membrane unsaturation [60]. Alterations in activities of membrane-bound enzymes and depletion of unsaturation in membrane lipids are associated not only with decrease in activities of membrane-bound enzymes but also with decreased membrane fluidity and altered activities of ion channels and receptors [6, 61]. Oxidative damage to cellular proteins along with the loss of calcium homeostasis contributes to protein aggregation and deposition, a process that occurs in neurodegenerative diseases [58]. In addition, high levels of 4-HNE generated during severe oxidative stress contribute to neurodegeneration by forming adducts with sulfhydryl groups (thiols) on proteins involved in neurotransmission [4, 62]. Moreover, in the presence of metal ions, such as Fe^{2+} and Cu^{2+}, H_2O_2 can be further transformed into hydroxyl radical ($^{\bullet}OH$) through the Fenton reaction. Hydroxyl radicals can attack polyunsaturated fatty acids in membrane phospholipids, forming the peroxyl radical ($^{\bullet}ROO$), and then propagate the chain reaction of lipid peroxidation. Furthermore, high levels of ROS modulate the expression of genes responsible for modulating activities of cytokines and chemokines [3, 6]. Neurons are most susceptible to ROS-mediated oxidative injury. ROS also contribute to brain damage by activating a number of cellular pathways resulting in the expression of stress-sensitive genes and proteins associated with oxidative injury [63]. ROS-mediated injury to astrocytes induces apoptosis-like cell death through a caspase-3-independent mechanism [63]. In reactive microglia, activation of NADPH oxidase orchestrates the generation of superoxide, which is converted into O_2 and H_2O_2 by SOD.

Under severe oxidative stress, high levels of $NO^{\bullet}$ are formed through enzymic oxidation of L-arginine to citrulline. $O_2^{\bullet-}$ reacts with $NO^{\bullet}$ to form peroxynitrite ($ONOO^-$), a strong oxidant that can initiate lipid peroxidation and formation of nitrotyrosine in proteins (Fig. 1.5). This metabolite not only inhibits enzymes of the mitochondrial respiratory chain and inactivates glyceraldehyde-3-phosphate dehydrogenase, but also inhibits membrane Na^+/K^+-ATPase and inactivates sodium channels in the membrane [64]. In addition, *S*-nitrosylation or covalent reaction of NO with specific

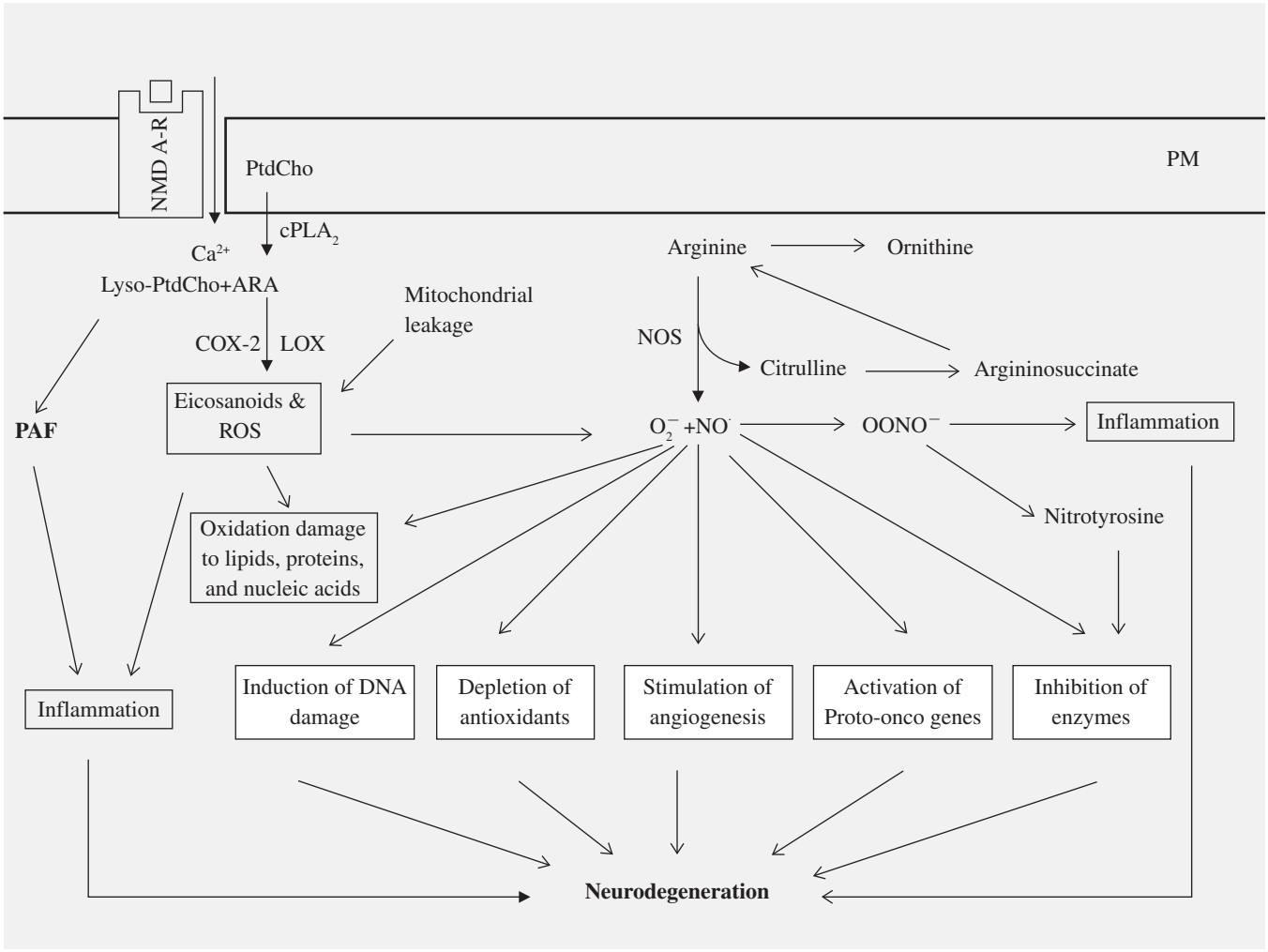

Fig. 1.5 Generation of nitric oxide ($NO^{\bullet}$) and its roles in signal transduction processes. $cPLA_2$, Cytosolic phospholipase A_2 COX-2, cyclooxygenase-2; LOX, lipoxygenase; NOS, nitric oxide synthase; $OONO^{-}$, peroxynitrite and PAF, platelet-activating factor.

protein thiol groups represents one mechanism contributing to $NO^{\bullet}$-mediated protein misfolding and neurotoxicity [65, 66]. Although nitrosative stress has long been considered as a major mediator of neurodegeneration, the molecular mechanism of how $NO^{\bullet}$ can contribute to neurodegeneration is not fully established. It has been suggested recently that nitration and nitrosylation of proteins contribute to the neurodegenerative process by inducing protein aggregation [66–68]. Under severe oxidative and nitrosative stress, the activation of NAD^{+}-consuming enzyme poly(ADP-ribose) polymerase-1 (PARP-1) is another likely mechanism for $NO^{\bullet}$-mediated energy failure and neurotoxicity. Although under mild oxidative stress the activation of PARP-1 is a repair process for neuronal protection, under high oxidative stress it causes neuronal energy compromise leading to neurodegeneration [6, 69]. As stated above, oxidative stress activates both acidic and basic SMases and promotes the generation of ceramide [59]. This lipid mediator at high levels triggers apoptosis. Ceramides generated in response to membrane-associated oxidative stress are implicated in cell death in neurotraumatic and neurodegenerative diseases [5]. Although the molecular mechanism of ceramide-mediated apoptosis is not fully understood, ceramide has been reported to modulate the opening of the mitochondrial permeability transition pores (PTPs), which disrupts the transmembrane potential, thus causing the release of cytochrome *c* and the generation of hydrogen peroxide. These molecules induce the release of APAF-1 and caspase-3 activation, leading to apoptotic cell death. In addition, ceramide also induces changes in the expression of the Bcl-2 family of proteins by activating specific transcription factors such as NF-κB and c-Jun [70]. Similarly, neural

membrane cholesterol under oxidative stress is transformed into hydroxy- and ketocholesterols through the action of cytochrome *P*450 hydroxylases. Generation of hydroxy- and ketocholesterols in neural cells also facilitates neurodegeneration through the loss of mitochondrial transmembrane potential ($\Delta\Psi_m$), the release of cytochrome *c*, and activation of caspase-3 [5].

At high levels, ROS-mediated hydroxylation of nucleic acid bases generates 8-OHdG from deoxyguanosine and alters DNA. DNA alterations are associated with aging, glutamate toxicity, and Alzheimer disease (AD) [3]. Upon DNA repair, 8-OHdG is excreted in the urine. Urinary 8-OHdG not only is a biomarker of generalized cellular oxidative stress but also is a risk factor for neurodegenerative diseases, various types of brain and visceral cancers, atherosclerosis, cardiovascular diseases, hypertension, and ischemia-reperfusion injury. Under low oxidative stress moderate DNA damage triggers cell-cycle arrest and initiates DNA-repair processes that ensure DNA integrity. However, if the intensity of ROS-mediated oxidative stress is high, then DNA repair does not occur and the neural cell dies by either apoptosis or necrosis [71]. 4-HNE forms Michael adducts with deoxyguanosine, yielding four diastereomeric 1,N(2)-dG adducts (6*R*, 8*S*, 11*R*), (6*S*, 8*R*, 11*S*), (6*R*, 8*S*, 11*S*), and (6*S*, 8*R*, 11*R*) with 8-hydroxyl and 6-(1-hydroxyhexyl) in the *trans* configuration [72]. These adducts may interfere with DNA replication and transcription, thereby contributing to the etiology of diseases associated with oxidative stress [73].

Generation of $ONOO^-$ also triggers DNA damage-including DNA strand breakage and base modification. $ONOO^-$ activates the nuclear enzyme PARP, resulting in energy depletion and apoptosis/necrosis of cells. $ONOO^-$-modified DNA may also lead to the generation of autoantibodies in various autoimmune disorders such as systemic lupus erythematosus (SLE). In chronic inflammatory diseases, $ONOO^-$ formed by phagocytic cells may cause damage to DNA, generating neoepitopes leading to the production of autoantibodies [74]. Emerging evidence suggests that severe oxidative stress can cause mutations and epigenetic perturbation by damaging DNA and proteins that modify chromatin.

1.5 CONCLUSION

Oxidative stress refers to cytotoxic consequences caused by oxygen free radicals generated in a cell by processes that utilize molecular oxygen. The major sources of ROS include the mitochondrial respiratory chain, xanthine/xanthine oxidase, myeloperoxidase, cytochrome *P*450, COX, LOX, and NADPH oxidase. The presence of redox-active metals, such as Fe^{2+} and Cu^{2+}, also contributes to ROS generation. ROS-mediated activation of transcription factors (AP-1, NF-κB, HIF-1) results in their translocation to the nucleus, leading to the transcription of genes involved in cell growth regulatory pathways. Although a decade ago the traditional view was that oxidative stress causes cellular damage, studies from the past several years indicate that low levels of ROS are needed for signal transduction processes associated with synaptic plasticity, memory formation, and gene expression associated with cell survival. The emerging view is that ROS can either enhance neural cell survival or promote cell death, depending on the magnitude and duration of the oxidative stress, genetic background, and redox states of the cells [75]. Generation of ROS not only serves as a stimulus for triggering stress-response induced signal-transduction pathways but also can modulate neural cell death/survival through direct oxidative modifications of neural membrane components and generation of lipid mediators. Under normal conditions, the balance between generation and elimination of ROS ensures the proper maintenance of neural cell metabolism and other functions. The final decision of whether the neural cell survives or dies is the result of the overall outcome of the integration of signals from redox-sensitive factors, levels of glycerophospholipid-, sphingolipid-, and cholesterol-derived lipid mediators, and other regulatory mechanisms [75]. Accumulation of oxidative damage products (lipid mediators) and failure of cells to neutralize ROS-mediated stress may result in excessive cell death as occurs not only in neurotraumatic and neurodegenerative diseases but also in normal aging.

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