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MOLECULAR MECHANISMS OF LEAD NEUROTOXICITY

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Abstract

Lead (Pb^{2+}) is a non-essential metal with numerous industrial applications that have led to ts ubiquity in the environment. Thus, not only occupational-exposed individuals' health is compromised, but also that of the general population and in particular children. Notably, although the central nervous system is particularly susceptible to Pb^{2+}, other systems are affected as well. The present study focuses on molecular mechanisms that underlie the effects that arise from the presence of Pb^{2+} *in situ* in the brain, and the possible toxic effects that follows. As the brain barriers represent the first target of systemic Pb^{2+}, mechanisms of Pb^{2+} entry into the brain are discussed, followed by a detailed discussion on neurotoxic mechanisms, with special emphasis on theories of ion mimicry, mitochondrial dysfunction, redox imbalance, and neuroinflammation. Most importantly, the confluence and crosstalk between these events is combined into a cogent mechanism of toxicity, by intertwining recent and old evidences from humans, *in vitro* cell culture and experimental animals. Finally, pharmacological interventions, including chelators, antioxidants substances, anti-inflammatory drugs, or their combination are reviewed as integrated approaches to ameliorate Pb^{2+} harmful effects in both developing or adult organisms.

Keywords

Metals; Lead; Neurotoxicity; Ion mimicry; Mitochondrial dysfunction; Redox imbalance; Neuroinflammation; Chelators; Antioxidants; Anti-inflammatory drugs

1. INTRODUCTION

Lead is the second most hazardous substance according to the Agency for Toxic Substances and Disease Registry (ATSDR)'s Substance Priority List, a classification that consisted in prioritization of substances based on a combination of their frequency, toxicity, and potential for human exposure at National Priorities List (NPL) sites. Metallic lead (Pb^0) is a bluish-

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gray heavy metal, resistant to corrosion because, when it is exposed to air or water, thin films of lead compounds (oxides and carbonates) are formed and protect this metal from further attacks; additionally, it can combine other metals to form various alloys. This non-essential element (atomic weight 207.2) is primarily found in various mineral forms in the earth's crust in the divalent form (Pb^{2+}), although Pb^{4+} is present in organolead. It has been widely used for centuries because it is readily shaped, molded, and resistant to corrosion. It is a ubiquitously distributed environmental toxicant that is naturally present in the earth cortex, but anthropogenic activities are responsible for its widespread in all compartments of the environment. The phasing out of leaded gasoline for vehicles between 1973 and 1995 and the removal of the metal from paint by 1978 have resulted in a substantial lowering of mean blood lead levels (BLL). However, because Pb^{2+} is a persistent metal, it is still present in all compartments of the environment, water, soil, and dust, making human exposure to occurs via food, water, air, and soil (Gupta, 2016; Patrick, 2006a). After exposure, Pb^{2+} is absorbed into the bloodstream, binds to circulating erythrocytes with a half-life of approximately 30 days to be distributed throughout the body, transiently accumulating in the brain, liver, and kidney to be eventually stored in the bone for 20–30 years. Nutritional status is a risk factor for Pb^{2+} intoxication and its effects. Thus, metals like iron (Fe^{2+}), zinc (Zn^{2+}), and calcium (Ca^{2+}) deficiency increase gastrointestinal Pb^{2+} absorption, and affect the susceptibility to Pb^{2+} neurotoxicity as will be described later in this chapter. Also, physiological conditions such as pregnancy, menopause, lactation, and aging favor the release of the metal from storage compartments. In the laboratory, several animal models (mostly rodents) are used to simulate Pb^{2+} exposure on the human body, with the metal mainly administrated via gavage, intraperitoneal injection or drinking water. Following absorption, Pb^{2+} interferes with many organs such as the liver, kidney, and central nervous system (CNS), the most vulnerable system, particularly during development. Once in the brain, Pb^{2+} effects can be classified as either morphological or pharmacological (Silbergeld, 1992). Morphological effects are related to structural and pathological changes in the brain cells, including neuronal differentiation, myelination, and synaptogenesis. The pharmacological perspective, on the other side, approaches Pb^{2+} neurotoxicity from the ion-mimicry mechanism, with special emphasis on Pb^{2+} competition with Ca^{2+} and to a lesser extent Zn^{2+} and Fe^{2+} for their insertion sites and essential functions. This characteristic is somehow responsible of brain Pb^{2+} incorporation, synaptic neurotransmission disruption, and impacts on intracellular Ca^{2+} concentration with consequent mitochondrial dysfunction and redox imbalance. These effects, conjugate with new evidence that ascribe a neuroinflammatory potential to Pb^{2+}, creating a highly disrupted microenvironment in the brain that leads to the neurotoxicant manifestations at both, the neurobehavioral and neurobiological/biochemical level, all pathognomonic of Pb^{2+} intoxication. Therefore, the present review will focus solely on Pb effects on the nervous system, mostly in cellular models or experimental animals and clinical evidence, with special emphasis on changes associated with the *in-situ* presence of the metal in the brain. Nevertheless, developmental effects will be also cited in the context of existing effects and potentially enduring consequences of Pb^{2+} neurotoxicity.

2. CELLULAR AND MOLECULAR MECHANISMS INVOLVED ON LEAD NEUROTOXICITY

2.1. THE ROLE OF THE BRAIN BARRIERS: *LEADING* Pb^{2+} INTO THE BRAIN

The brain, an organ with high complexity and functions that are vital for an individual's life is protected by barriers. They are the *blood-brain barrier* (BBB) a physical, metabolic and transport-specialized interface shaped by the endothelial cells joined by tight junctions that form the walls of the capillaries accompanied by the astroglia, pericytes, perivascular macrophages, and a basal lamina; the *blood-cerebrospinal fluid barrier* (BCB) an interface formed by the epithelial cells of the choroid plexus facing the cerebrospinal fluid; and the *arachnoid barrier* formed by avascular arachnoid epithelium. Among the functions, the BBB provides a stable microenvironment for physiological neural function, ensuring brain homeostasis and thereby protecting a tissue with limited capability to regenerate from chemical insult and damage of endogenous and exogenous toxins. In occasions, although with high restriction, the BBB allows several paths of permeation for incorporation or efflux of substances to and out of the CNS, namely: a) passive diffusion for lipophilic and non-polar molecules; b) efflux transporters including adenosine triphosphate (ATP)-binding cassettes transporters (ABC); c) solute carriers for the incorporation of essential nutrients; d) transcytosis of macromolecules, and e) monocyte-lineage-cell movement in pathological conditions (Abbott et al., 2010). A large number of toxicants including metals, pesticides, mycotoxins, drugs of abuse, and therapeutic drugs are known to disrupt the BBB and/or BCB morphology or function.

In the case of metals, several elements have specialized processes to enters the CNS because they are necessary for optimal CNS function, while other metals that are non-essential can also cross or modify the barriers, including Pb^{2+}. Although severe Pb^{2+} intoxication in newborn rats and in infants may cause micro vessel damage characterized by brain edema, there is little evidence that blood Pb^{2+} levels lower than 80 μg/dl were able to damage or disturb transport mechanisms (Bradbury and Deane, 1993). Once inside the brain, Pb^{2+} accumulates in the choroid plexus, which is not directly affected by the metal toxicity but rather mediated by the inhibition of production and secretion of transthyretin, a protein involved with the transport of thyroid hormones to the developing brain. This selective neurotoxicity may be the consequence of the incomplete tightness of the interepithelial junctions of the BCB or the high expression of numerous organic anion and cation transporters in this interface. In this respect, *in vitro* studies demonstrated that in contrast to later Pb^{2+} exposure, the presence of the metal prior to the formation of intercellular tight junctions inhibits the expression of claudin-1, an essential protein for interepithelial junctions (Lewis and Zheng, 2007). A more recent study established that Pb^{2+}-induced a reduction in Cx43 gap junction hemichannel levels by promoting extracellular signal-regulated kinase (ERK) phosphorylation, constituting a novel pathway of cellular uptake through the BCB barrier (Song et al., 2016).

In terms of the BBB, several processes have been described (Martinez-Finley et al., 2012). On one side, Pb^{2+} may enter the CNS by passive diffusion as free Pb^{2+} or by the exchange of $PbCO_3$ with an anion, or passively in the form of an inorganic complex, such as $PbOH^+$

with a high dependence on pH. This was suggested after experiments of ^{203}Pb uptake into the brain, with the calcium-ATP-dependent pump playing a pivotal role of Pb^{2+} transport back into the capillary lumen (Deane and Bradbury, 1990). Other studies performed in cerebellar granule cells from newborn rats indicate that 1,4-dihydropyridine insensitive calcium channels and *N*-methyl-D-aspartate receptors (NMDAr) are permeable to Pb^{2+} (Mazzolini et al., 2001). It is also possible that lead traverses the BBB by the Divalent Metal (Ion) Transporter 1 (DMT1), a 12-transmembrane domain protein present in endothelial cells of the BBB in both glia and neurons as an uptake mechanism for Fe^{2+} and other essential divalent metals (Luo et al., 2012). Furthermore, Fe^{2+} deficiency enhances Pb^{2+} transport and DMT1 overexpression promotes an increase in Pb^{2+} transport (Wang et al., 2011). Related to Ca^{2+}, pioneer experiments performed in bovine adrenal medullary cells, they demonstrate that Pb^{2+} does not compete for binding sites in Ca^{2+}-dependent channels, but rather permeates through them (Simons and Pocock, 1987; Tomsig and Suszkiw, 1991). Thus, Pb^{2+} incorporation into the brain seems to be mediated by mechanisms independent of voltage-gated-calcium channels (VGCCs) and of protein transport, although the activation of cation channels by the depletion of intracellular Ca^{2+} stores may also play a role in the Pb^{2+} transport through cellular endothelium (Kerper and Hinkle, 1997). More recently, the transient receptor potential (TRPC) canonical channels that mediate Ca^{2+} influx into the brain and BBB integrity also play a role in Pb^{2+} incorporation into the brain, provided that these channels can be activated by a variety of signals, including intracellular Ca^{2+} store depletion (Weber and Muller, 2017). Moreover, Pb^{2+} is a relatively potent stimulator of the TRPC5 channel and augments Ca^{2+} entry to the cells (Bouron et al., 2015; Sukumar and Beech, 2010), although TRPC role in Pb^{2+} incorporation into the brain has not been completely elucidated. It should be noted that modifications in intracellular *vs.* extracellular Ca^{2+} balance have detrimental effects in the BBB tight junctions assembly and functionality (Abbott et al., 2010). Most importantly, Pb^{2+} activates the Ca^{2+} dependent protein kinase C (PKC) in both BBB and BCB cells (Markovac and Goldstein, 1988; Zhao et al., 1998), an event that determines a loss of epithelial barrier function, an increase in trans endothelial permeability, and the inhibition of astroglia-induced endothelial differentiation (Laterra et al., 1992). Additionally, it has also been reported that Pb^{2+} induces morphological damage to the BBB accumulating in the brain endothelial cells, opening of the tight junctions and promoting an enhanced pinocytotic activity as evidenced by an extensive cerebral hemorrhage and extravascular distribution of albumin (Gupta and Gupta, 2019; Zheng et al., 2003). Moreover, Pb^{2+} exposure activated intracellular non-receptor protein tyrosine kinase Src, leading to a reduction of occludin level, a tight junction protein involved in Pb^{2+}-induced BBB leakage (Song et al., 2014). Besides, Pb^{2+} may indirectly affect the BBB through its accumulation in the astrocytes (Tiffany-Castiglioni and Qian, 2001), a type of neural cell with a pivotal role in the formation and maintenance of BBB integrity (Bressler and Goldstein, 1991). Although Pb^{2+} accumulates in both astrocytes and neurons, there is a preferential deposit of the metal in the immature astroglia, providing the basis for the Pb^{2+} sink hypothesis in which astrocytes act as Pb^{2+} depot in encephalopathy (Lindahl et al., 1999; Tiffany-Castiglioni et al., 1989), resulting in the accumulation of unfolded protein in the endoplasmic reticulum (ER) and consequent inhibition in the cell-cycle progression and the transcription of certain proteins (Zhang et al., 2008). In this line, Pb^{2+} alters the astroglial-endothelial interactions, leading to cerebral microvascular dysfunction due to

alterations in the expression of human fetal astrocyte genes (Hossain et al., 2000). Finally, oligodendrocytes, the myelin-forming cells in the CNS, are pointed out as the most vulnerable brain cell type for Pb^{2+} toxicity (Tiffany-Castiglioni, 1993), particularly at the early stages of maturation, affecting both oligodendrocytes and astrocytes progenitor cells with a direct impact in the nerves' myelin cover (Deng et al., 2001).

In summary, these findings indicate that Pb^{2+}-induced damage to mature and immature brain cellular components may contribute to the neurotoxicant consequences of adult occupational Pb^{2+} exposure and to the higher vulnerability of children to environmental Pb^{2+} exposure. Thus, additional research is needed to increase the understanding of how Pb^{2+} and other neurotoxicants appropriate of the physiological mechanisms aimed to maintain homeostatic control of the CNS microenvironment and the direct or indirect damage to the morphology or functionality of the brain barriers and cellular types (Zheng et al., 2003).

2.2. THE IONIC MIMICRY MECHANISM: THE ROLE OF DIVALENT ESSENTIAL METALS

The ability of Pb^{2+} to interact with O_2 and thiols, both of critical relevance as part of protein metal binding sites, allows its substitution for diverse essential divalent cations such as Ca^{2+} and Zn^{2+}. It is important to emphasize that the living organisms are provided with many mechanisms of control to maintain physiological intracellular levels of essential metals, whereas the nonessential metals lack of homeostatic regulation. Although there is a consensus now that not a single mechanism can be ascribed to Pb^{2+} neurotoxicity, its ability to substitute or compete with essential divalent cations, preferentially Ca^{2+} but also Fe^{2+} and Zn^{2+}, and thus altering the whole cellular microenvironment, was for some time the prevalent mechanism of Pb^{2+} toxic action. Pb^{2+} has an ionic radius 20% larger, broader coordination chemistry, greater electronegativity than Ca^{2+} and forms complex preferentially with thiols groups and complex ions rather than with O_2 ligands. Though all these characteristics lead to a lower affinity of Pb for to Ca^{2+} binding sites, provided the extensive distribution and physiological importance of Ca^{2+} effects, including synaptic activity and its actions as an intracellular second messenger, the Pb^{2+}/Ca^{2+} mimicry mechanism has the most profound impact in living organisms (Garza et al., 2006; Giorgi et al., 2018).

2.2.1. Calcium—The ability of Pb^{2+} to interchange with Ca^{2+} is central to its detrimental effects. Unlike Ca^{2+}, which intracellular levels are highly regulated, Pb^{2+} is an unregulated non-essential heavy metal The 10,000-fold difference between extracellular and cytosolic Ca^{2+} levels respond to the impermeability of the plasma membrane to Ca^{2+} and transport mechanisms that remove Ca^{2+} from the cytoplasm (Florea et al., 2013). Pb^{2+} binds to the same/near sites than Ca^{2+} and enters the cell through Ca^{2+} channels to mimic, substitute, or compete with Ca^{2+} by a) direct interference with Ca^{2+} transport systems or storage sites; b) indirect alteration of Ca^{2+} homeostasis in cells functionality, including the elevation in intracellular cytosolic free Ca^{2+}; or c) competition for Ca^{2+} binding sites and Ca^{2+} effector mechanisms (Pounds, 1984; Simons, 1993). Thus, in the first place, Pb^{2+} gains entrance to the cell in part by permeating Ca^{2+} channels, contributing to the early stages of toxic metal accumulation being the membrane and its proteins the first components of the cell to be exposed to the metal. Once inside the cell, Pb^{2+} further competes with Ca^{2+} to activate or

inhibit Ca^{2+}-regulated processes via interactions with Ca^{2+} binding-proteins (CABPs) such as the ones mentioned below:

2.2.1.1. VGCCs: presynaptic neuronal depolarization leads to the influx of Ca^{2+} via the VGCCs, being Pb^{2+} a potent blocker of all VGCCs types probably through the binding to an external site, as there is no change in the voltage dependence upon Pb^{2+} binding. The inhibition of presynaptic VGCCs prevents the required rise in intracellular Ca^{2+} levels ($[Ca^{2+}]i$), and eventually permits the passage of Pb^{2+} which is incorporated into the Ca^{2+} transport systems in the nervous system, all events impacting on synaptic activity with net detrimental effects on neurotransmitter release in a tissue-dependent-fashion (Atchison, 2003; Audesirk and Audesirk, 1993; Piatt and Biisselberg, 1994; Sadiq et al., 2012). Besides, Pb^{2+} can induce catecholamine release from its storage vesicles in a Ca^{2+}-independent manner, a mechanism that involves exocytosis stimulation probably due to direct stimulation of calcineurin by Pb^{2+} (Westerink and Vijverberg, 2002). Notably, several *in vivo* evidence demonstrate alterations on catecholamine functionality (Cory-Slechta et al., 2008; Verstraeten et al., 2008), revealing that Pb^{2+} effects on these systems might be the result of either an overall metal-mediated cellular damage or a direct interaction with these enzymes and neurotransmitters.

2.2.1.2. CABPs: Pb^{2+} competes with Ca^{2+} for its binding sites on multiple proteins involved in vesicular mobilization and docking at the presynaptic cleft. This is known to occur by one of the following three mechanisms: 1) occupation of Ca^{2+} sites and structural inhibition of the protein's activity; 2) inappropriate activation of the protein with detrimental consequences on downstream events, or 3) allosteric modulation of the protein activity with a binding site different than the one for Ca^{2+} (Gorkhali et al., 2016). These Ca^{2+}-binding structures are characterized by two main types in terms of their conformational structure: the "EF-hand" motifs, which are characteristic of calmodulin conformation, and the C2 domains, exhibited by protein kinase C (PKC), both having a higher affinity to Pb^{2+} compared to Ca^{2+}.

2.2.1.2.1 Calmodulin: small changes in $[Ca^{2+}]i$ modify calmodulin subcellular distribution, its interaction with other proteins, and changes its conformational states (Simons, 1986). Several reports underscore the importance of the cross-reactions between Pb^{2+} and Ca^{2+} with calmodulin-dependent systems. In the first place, there are evidence that Pb^{2+} modifies the expression of calmodulin-related genes (Li et al., 2015). Secondly, pioneer reports indicate that Pb^{2+} activates calmodulin-dependent phosphodiesterase, calmodulin-inhibitor-sensitive-potassium channels, and calmodulin-independent PKC (Gill et al., 2003; Goldstein, 1993; Habermann et al., 1983). In addition, low Pb^{2+} concentrations activate calmodulin enzymatic activity by mimicking Ca^{2+} in the EF-hand binding sites, which leads to an increased calmodulin-mediated syntaxin I that opens VGCCs by phosphorylation. This results in an elevated number of readily releasable synaptic vesicles whereby binding Pb^{2+} to synaptotagmin I, a synaptic-vesicle-associated protein, whose interaction with Pb^{2+} induces inhibition of vesicular membrane fusion and therefore a decreased neurotransmitter release. In contrast, at high Pb^{2+} levels, calmodulin activity is inhibited, preventing the interaction between syntaxin I and synaptotagmin I and suppressing neurotransmitter release (Florea et

al., 2013). Besides, calmodulin inhibition could be the result of Pb^{2+} binding to another region of the protein with resulting allosteric inhibition, a mechanism independent of simply ionic displacement (Kasten-Jolly and Lawrence, 2018). In fact, as a result of binding studies (Kirberger et al., 2013; Kirberger and Yang, 2008) it is demonstrated that Pb^{2+} alters the conformation of calmodulin in the Ca^{2+}-bound state, particularly at the molecular recognition site, resulting in Pb^{2+} opportunistic binding to secondary sites of diverse geometric structure and to which Ca^{2+} does not bind. The authors concluded that: "this allosteric mechanism suggests that the promiscuous nature of lead allows for multiple molecular targets and by extension offers a comprehensive explanation for the resulting systemic pathology of lead toxicity". Therefore, the presence of Pb^{2+} in the neurons interferes with a myriad of cellular events resultant of inadequate calmodulin functionality, including phosphorylation and dephosphorylation processes, signaling events, and the interaction with other proteins, all which convert this protein as a recently-described bridging and adaptor molecule (Villalobo et al., 2018), and likely playing a prominent role in Pb^{2+} neurotoxicity.

2.2.1.2.2. PKC: Resembling calmodulin, Pb^{2+} has been reported to interact with PKC isozymes, possibly through different binding sites and at even lower concentrations than those required to activate calmodulin. These enzymes are activated by signals such as increases in the concentration of diacylglycerol (DAG) or in $[Ca^{2+}]i$. Upon activation, the enzyme is translocated from the cytosol to the membrane and undergo phosphorylation to initiate the activation of transcription factors through signaling cascades (Kasten-Jolly and Lawrence, 2018). In this respect, studies in PC12 cells demonstrate that Pb^{2+} induces *c-jun* and *egr-1* mRNA, in addition to PKC-dependent *c-fos* mRNA, as well as the corresponding encoded protein products (Kim et al., 2000). Moreover, PKC activation by picomolar Pb^{2+} concentrations (Markovac and Goldstein, 1988) triggers a variety of events such as cellular phosphorylation, stimulated basal secretion of the neurotransmitter, spontaneous transmitter release within the nerve terminals (Bressler and Goldstein, 1991; Goldstein, 1993), all of which inhibit Ca^{2+} entry into cells, and thus inducing an elevation in $[Ca^{2+}]i$ (Simons, 1993). It was later reported that Pb^{2+} in the micromolar range inhibited the enzyme through a mechanism not related to the competition with Ca^{2+} (Marchetti, 2003). Overall, Pb^{2+} modulation of cellular PKC activity remains a controversial subject, although it seems that at low Pb^{2+} levels, both PLC and PKC activities are enhanced, whereas higher Pb^{2+} concentrations inhibit these enzymes (Toscano and Guilarte, 2005). In this respect, a recent review indicates that Pb^{2+} shifts Ca^{2+} from the two Ca^{2+} binding loops within the C2 domain of all isoforms of the conventional PKC molecule, with different activation/inactivation outcomes according to the assay system (Kasten-Jolly and Lawrence, 2018).

2.2.1.2.3. AMPA glutamate receptor 2 (GluR2) subunit: Ionotropic glutamate receptors include NMDA and a-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptors. The overactivation of glutamate receptors increases cytosolic $[Ca^{2+}]i$ leading to cell death. Different from NMDAr which are Ca^{2+} permeable, AMPAr containing the GluR2 subunit are impermeable to Ca^{2+}. On the basis of the premise that long-term exposure of rat cortical neurons to Pb^{2+} acetate decreases GluR2 expression (Ishida et al., 2013), the same authors demonstrated the protective effects of the non-selective AMPA receptor blocker 6-

cyano-7-nitroquinoxaline-2,3-dione (CNQX) against Pb^{2+}-induced activation of ERK 1/2, mitogen-activated protein kinase 38 (MAPK p38), PKC, and neuronal cell death, suggesting that Ca^{2+}-permeable AMPAr resulting from GluR2 decrease may be involved in Pb^{2+}-induced neurotoxicity (Ishida et al., 2017).

Overall, this evidence indicates that Pb^{2+} not only affects cell signaling by replacing Ca^{2+} in protein binding sites, but also alters Ca^{2+} cellular concentration by modulating the activity of cationic channels.

2.2.2. Zinc—Besides Ca^{2+}, Pb^{2+} interaction at Zn^{2+} binding sites is also important. In the CNS, this element is second only to Fe^{2+} in trace metal abundance and it is considered redox inert. Although mostly protein-bound, it is an essential component of numerous proteins involved in biological defense mechanisms against oxidative stress. There are small pools of the metal in presynaptic vesicles of glutamatergic neurons as well as in intracellular non-vesicular pools.

2.2.2.1. Zn^{2+} finger proteins: are transcription factors that contain a Cys2-His2 Zn^{2+} binding domain, a major structural motif required for sequence-specific DNA-binding. Thus, provided the Pb^{2+} affinity for thiol sulfur atoms, the Zn^{2+}-binding sites based on thiol groups are those with a higher affinity for Pb^{2+} (Garza et al., 2006). Most importantly, Pb^{2+} competes with the Zn^{2+} binding site of several Zn^{2+} finger proteins, probably as a result of structural alteration in the Zn^{2+} coordination site (Ordemann and Austin, 2016; Zawia et al., 2000). In this regard, a report indicates that Pb^{2+} inhibits the DNA-binding mechanism of 1) the transcription factor IIIA (TFIIIA), which is related to the internal control region of the gene (Zawia et al., 2000); 2) the specificity protein 1 (Sp1), which is highly important in development (Basha et al., 2003); and, 3) of the early growth response transcription factor 1 (Egr-1), that is implicated in plasticity, learning and memory processes (Reddy and Zawia, 2000).

2.2.2.2. NMDAr channel: *is* another putative target of Pb^{2+} neurotoxicity in the Zn^{2+} allosteric binding site (Guilarte et al., 1995; Toscano and Guilarte, 2005; Marchetti, 2014), although the exact interaction with Zn^{2+} binding site is still under debate (Gavazzo et al., 2008). This glutamatergic receptor subtype is implicated in the regulation of neuronal development and cognitive functions, including long term-potentiation (LTP), a form of synaptic plasticity NMDAr-dependent that is believed to form the cellular basis for the cognitive process, including learning and memory. Pioneer studies by Alkondon et al. (1990) concluded: “the elucidation of the actions of Pb^{2+} on the NMDAr ion channel complex provides important insights into the clinical and toxic effects of this cation” (Alkondon et al., 1990). The extracellular portion of the NMDAr subunits consists of an N-terminal domain that binds Zn^{2+} allosterically, and a contiguous ligand-binding domain that binds glutamate and glycine, a step required for NMDAr activation. This is accompanied by the removal of the Mg^{2+} blockade through the depolarization of the neuron’s cellular membrane and the opening of the associated ion channel permeable to Ca^{2+}, Na^{+} and K^{+} (Baranowska-Bosiacka et al., 2012). Evidence indicates that Pb^{2+} is a potent, non-competitive antagonist of the NMDAr, with a high affinity to the NR2A subunit of the receptor by binding at the Zn^{2+} regulatory site of the NMDAr (Nihei and Guilarte, 1999) in a voltage-independent

fashion (Neal and Worley, 2011). Further studies in rats chronically exposed to Pb^{2+} performed by these authors demonstrated that impairments of spatial learning and hippocampal LTP are associated with changes in gene and protein expression of NMDAr subunits, conforming the mechanistic basis for the well-known deficits in cognitive function resultant of Pb^{2+} exposure (Nihei et al., 2000; Nihei and Guilarte, 2001). Importantly, the involvement of the glutamatergic system, and in particular NMDA participation in Pb^{2+}-induced learning impairments was already reported in a study that examined the mechanistic relationships between Pb-induced alterations in glutamate neurotransmission and behavioral toxicity (Cory-Slechta et al., 1997). Other studies performed *in vitro* in cultured hippocampal neurons reported that Pb^{2+} exposure during synaptogenesis decreases synaptophysin and synaptobrevin proteins, resulting in impaired vesicular release at both the glutamatergic and GABAergic synapses. The authors conclude that these presynaptic deficits resulting from Pb^{2+} exposure are mediated by disruption of NMDAr-associated cAMP response element-binding (CREB)-dependent transcription of activity-regulated genes such as brain-derived neurotrophic factor (BDNF) that alters the function of its related binding site, the tyrosine receptor kinase B (TrkB) and downstream signaling, being BDNF a trans-synaptic molecule released from both dendrites and axons whose levels are decreased in response to Pb^{2+} exposure (Neal et al., 2010; Neal and Guilarte, 2010; Stansfield et al., 2012). As a regulator of Ca^{2+}-signaling and homeostasis, BDNF perturbation led to alterations in Ca^{2+}-dependent pathways, which in turn can also be interfered by Pb^{2+}. In further studies, the same authors showed that Pb^{2+} exposure markedly inhibits presynaptic vesicular release in hippocampal Schaffer collateral-CA1 synapses in young adult rats. This effect was associated with a reduction in vesicle number and a decreased number of presynaptic terminals with multiple mitochondria, while no change in presynaptic Ca^{2+} influx was reported, effects that contribute to the deficits in synaptic plasticity and intellectual development observed in children (Zhang et al., 2015). More recently, Ding et al. (2018) reported that Pb^{2+} decreased both excitatory and inhibitory presynaptic transmission due to a disarrange in the distribution and density of presynaptic vesicles, a process dependent on the phosphorylation level of synapsin 1 via cyclin-dependent kinase 5 (CDK5) (Ding et al., 2018).

In addition to the blockage of the NMDAr, Pb^{2+} inhibits the passage of Na^{+}, Ca^{2+}, and other ions through the postsynaptic membrane, thus reducing excitatory postsynaptic potentials (EPSPs) and the likelihood for generating an action potential (Florea et al., 2013). Moreover, and besides these effects, Pb^{2+} alters hippocampal NMDAr subunits mRNA expression in rats, modifying the receptor levels or subtypes and altering the development of defined neuronal connections that require its activation (Sanders et al., 2009). However, evidence also exists that the interaction between Pb^{2+} and Zn^{2+} occurs via independent allosteric binding sites (Lasley and Gilbert, 1999) that are associated to an elevation in NMDAr density as a result of prenatal Pb^{2+} exposure in rats (Lasley et al., 2001). Furthermore, developmental Pb^{2+} exposure reduces the ability of the NMDA antagonist MK-801 to suppress LTP in the rat dentate gyrus (Gilbert and Lasley, 2007) and produces MK-801 sub-sensitivity at the behavioral level associated with postnatal Pb^{2+} exposure (Cory-Slechta, 1995). Finally, and based on the premise that Pb^{2+} affinity for NMDAr may not completely explain the mechanisms of Pb^{2+}-induced neurotoxicity, further studies aimed to examine

metabotropic glutamate receptors (mGluR) in rat hippocampal neurons. In this regard, both *in vitro* and *in vivo* studies revealed that expression of mGluR5 mRNA and protein was decreased, revealing that mGluR5 might be involved in Pb^{2+}-induced neurotoxicity by disturbing mGluR5-induced long-term depression (LTD) and decreasing NMDAr-dependent LTP (Xu et al., 2009; Xu et al., 2009).

2.2.2.3. δ-aminolaevulinic acid dehydratase (δ-ALA-d): is an enzyme involved in heme synthesis formed by eight identical subunits with eight Zn^{2+} ions as cofactors. It is also a target of Pb^{2+} due to mimicry with Zn^{2+} whereby Pb^{2+} binds to the sulfhydryl moiety at the active site of the enzyme, resulting in the characteristic anemia observed in organisms exposed to Pb^{2+} (Bergdahl et al., 1997). In addition, δ-ALA-d inhibition results in δ-ALA accumulation, an unstable compound that can be rapidly converted into free radicals (Ahamed and Siddiqui, 2007) and in part responsible for the redox imbalance associated to Pb^{2+} exposure, a crucial aspect that will be discussed below.

2.2.3. Iron—Iron is the most abundant trace element in the brain and is considered a redox-active metal that undergoes redox-cycling, including the crucial Fenton reaction. It is an essential cofactor for normal brain development and function as well as for a wide variety of important cellular processes, such as O_2 transport, respiration, the tricarboxylic acid (TCA) cycle, lipid metabolism, heme biosynthesis, gene regulation, and DNA and RNA synthesis. It is incorporated in the heme group of hemoglobin, myoglobin, and cytochromes, or is associated with nonheme moieties or Fe-S motifs (Cairo et al., 2006). As a divalent cation, Fe^{2+} is another important metal with the potential to be a target for Pb^{2+} displacement, particularly in the DMT-1, which as previously discussed may be involved in the transport and cellular uptake of Pb^{2+} (Kirberger et al., 2013). Intriguingly, few studies have examined the effect of Pb^{2+} exposure on cellular Fe^{2+} homeostasis to provide a hint on possible interactive mechanisms. In this regard, a recent report indicates that Pb^{2+} exposure increases Fe^{2+} content in rat brain, which was associated with the decreased expression of ferroportin 1 (FP1), a pivotal Fe^{2+} efflux protein (Zhu et al., 2013). Further studies from the same group showed that Pb^{2+} exposure induced an increase in cellular Fe^{2+} levels which was accompanied by a decrease in FP1 expression, while FP1 overexpression could attenuate Fe^{2+} accumulation in Pb^{2+}-exposed PC12 cells (Zhou et al., 2014).

2.3. THE MITOCHONDRIAL DYSFUNCTION MECHANISM

Mitochondria, as the major source of cellular energy metabolism, have been studied extensively in xenobiotic-induced neurotoxicity. In particular, metals have been reported to produce some of the toxic effects by targeting mitochondria (Meyer et al., 2013). Although a non-redox-active metal, Pb^{2+} promotes an imbalance in the mitochondrial homeostasis, in part by decreasing glutathione (GSH) as the result of the inhibition of thiols groups, reducing the antioxidant enzymes activities, substituting Ca^{2+}, Fe^{2+}, or Zn^{2+} or altering the integrity, permeability and functionality of membranes, favoring lipid peroxidation (Caito and Aschner, 2015). All these events will be discussed in detail in the next section. In comparison to other organelles and on the basis of the high content of unsaturated lipids, the mitochondrial membranes represent a structure with a particular susceptibility to Pb^{2+}. In the same line, mitochondrial enzymes that contain essential sulfhydryl groups are also a

target for Pb^{2+} species. Thus, direct damage to mitochondrial components may disrupt homeostatic mitochondrial functions leading to cell death (Blajszczak and Bonini, 2017). In this regard, pre- and neonatal Pb^{2+} exposure in rats affects the energy status of cultured primary cerebellar granule neurons from rats through a decrease in ATP concentrations by the inhibition of Na^+/K^+ ATPase, and the increase in intracellular and mitochondrial reactive oxygen species (ROS) concentration (Baranowska-Bosiacka et al., 2011).

2.3.1. Intramitochondrial ion mimicry—Different from other organelles, the mitochondrion has its own genome, a circular double chain DNA molecule (mtDNA), 22 transference RNAs, and 2 ribosomal RNAs that lack repair mechanisms, although playing a key role in mitochondrial functionality. This unique characteristic is emphasized by Meyer et al, 2013, who consider this organelle as a preferential target of environmental toxicants, with a particular focus on mtDNA (Meyer et al., 2013). In this regard, recent data from participants in the PROGRESS cohort revealed that Pb^{2+} levels at different time frames during pregnancy were associated with increased mtDNA content in cord blood (Sanchez-Guerra et al., 2019). Notably, mitochondrial dysfunction can affect key regulators of the intracellular signaling such as ROS and Ca^{2+} that all can be transmitted to the nuclei (mitochondrial to nuclei signaling or retrograde signaling), resulting in changes in the genic expression.

From the ultrastructural view, the mitochondrion is semi-autonomous with a double membrane system, the internal membrane (IMM), and external membrane (EMM) that delimit an intermembrane space (IMS), whereas the IMM brands the matrix, a viscous microenvironment with high enzymatic content. Mitochondrial membranes facilitate the accumulation of lipophilic substances, while the matrix's negative charge and slightly alkaline pH as a result of the proton pumping associated with oxidative phosphorylation permits the incorporation of cationic metals, including Pb^{2+} (Castellino and Aloj, 1969). In addition, as mentioned in the previous section, Ca^{2+}-dependent transporters represent another influx/efflux mechanism resultant of molecular mimicry (Lidsky and Schneider, 2003; Meyer et al., 2013; Rocha and Trujillo, 2019; Silbergeld, 1977). In the same line, it is important to highlight that essential metal ions such as manganese (Mn^{2+}), Fe^{2+}, copper (Cu^{2+}), and Zn^{2+} play pivotal roles in this organelle and their dysregulation can impact on mitochondrial compartments and functions (Nam et al., 2018), which opens up the possibility of Pb^{2+} competition with these ions, thus affecting mitochondrial homeostasis.

In terms of function, the mitochondria are the energetic center of the cell as a consequence of the electron flux through the mitochondrial respiratory complexes I-IV with resultant oxidative phosphorylation. The complete nutrients oxidation by the tricarboxylic acid cycle produces reduced coenzymes such as reduced nicotinamide adenine dinucleotide (NADH), flavin adenine dinucleotide 2 (FADH2) that act as electron donors. The electron flux through the electron transition chain (ETC) produces an electrochemical gradient that is used by the complex V to generate ATP. Mitochondrial dysfunction is characterized by the inhibition of mitochondrial O_2 consumption, altered membrane potential, and reduction in ATP levels by an imbalance in the energy intake and expenditure. Any defect in the ATP-generating capacity causes decreased cellular energy metabolism, cellular dysfunction, and cell death (Fariss et al., 2005). There are some evidence that support the effect of Pb^{2+} on oxidative

mitochondrial processes. First, it was reported that neonatal exposure to low levels of Pb^{2+} produces changes in phosphorylation activity in rat brain mitochondria (Bull et al., 1979), while *in vitro* studies in brain mitochondria of young rats (Dumas et al., 1985) and in synaptosomes (Rafałowska et al., 1996) showed that Pb^{2+} ions alter mitochondrial respiration and Adenosine diphosphate (ADP) phosphorylation. In addition, Pb^{2+} can also act on cytochrome c and ATP synthase to cause dysfunction of mitochondrial ETC and generation of free radicals (Dressier et al., 1999). Furthermore, it was demonstrated that relatively high Pb^{2+} concentrations inhibited both the Pb^{2+}-induced respiration and the respiratory response to ADP in cerebral and cerebellar mitochondria from both, immature and adult rats. The authors concluded that matrix NAD-linked dehydrogenase, were more sensitive to inhibition by Pb^{2+} than were the inner-membrane enzymes (Holtzman et al., 1978).

While necrosis and apoptosis are the major forms of regulated cell death, the role of mitochondria in more recently described necroptosis, pyroptosis, ferroptosis, and other cell death processes is still poorly defined. In relation to apoptosis, mitochondrial dysfunction initiated by the opening of the mitochondrial permeability transition pore (MPTP) leads to membrane permeabilization, the release of cytochrome c, followed by activation of caspases and cleavage of downstream effector proteins, resulting in cell death. This is a suicide event triggered by extrinsic, receptor-mediated, or intrinsic, mitochondria-mediated signaling pathways. *In vivo* evidence indicate that Pb^{2+} exposure affects the mitochondrial permeability, thereby stimulating the release of mitochondrial proteins, facilitating apoptosis in several brain regions, increasing the Bax/Bcl-2 ratio by down-regulation of Bcl-2 and up-regulation of Bax expression, being their relationship a key indicator of apoptosis susceptibility by the intrinsic pathway (Sharifi et al., 2002) (Pulido and Parrish, 2003). Further studies reported that Pb^{2+} induced developmental apoptosis in several brain regions, consistent with dying neurons expressing activated caspase-3 early in the degenerative process (Dribben et al., 2011), is the hippocampus the region most vulnerable where caspase 3, Bcl-2, and BDNF increase in developmental Pb^{2+} exposed rats (Chao et al., 2007). Moreover, *in vitro* experiments in PC12 cells demonstrated that p53 activation by DNA damage leads to an imbalance in Bax/Bcl-2 and mitochondrial dysfunction that was followed by Pb^{2+}-induced apoptosis (Xu et al., 2006). Likewise, an increase in the production of $[Ca^{2+}]_i$, ROS, and associated oxidative stress in Pb^{2+}-treated PC12 cells accompanied by an upregulation in the expression in both mRNA and protein caspase-3, caspase-9, Bax, p53, and cytochrome-c and downregulation in Bcl-2 (Kumar et al., 2015). In addition, ROS triggers the apoptotic pathway that can be activated by an overload in mitochondrial $[Ca^{2+}]$i, being the ER-mitochondrial Ca^{2+} interchange a highly regulated process in the cell in homeostatic conditions (Marchi et al., 2018; Orrenius et al., 2003; Rizzuto et al., 2003). Thus, apoptosis can be caused by loss of control of Ca^{2+} homeostasis or by changes in Ca^{2+} distribution within intracellular compartments (Garza-Lombó et al., 2018). In addition, Ca^{2+} homeostasis is regulated by mitochondrial functions that control the buffering capacity of ER-Ca^{2+} channels. Ryanodine receptors (RyRs), the major intracellular Ca^{2+} release channels located in the ER, are crucial for brain function because they mediate Ca^{2+} release. Interestingly, Pb^{2+} exposure affects RyRs leading to an increase in $[Ca^{2+}]_i$ in cultured cells (Fan et al., 2013; Jia et al., 2018). Thereby, increased $[Ca^{2+}]$i alter cellular

energy balance because high cytoplasmic Ca^{2+} concentrations increase mitochondrial Ca^{2+} uptake by the Ca^{2+} uniporter. In these conditions, the MMP is dissipated, and ATP synthesis is reduced. Additionally, Ca^{2+} may also impair ATP synthesis by causing oxidative injury to the IMM. Furthermore, a sustained rise in cytoplasmic Ca^{2+} increases ATP consumption by forcing the Ca^{2+}-ATPases to work to eliminate the excess Ca^{2+}. Importantly, Pb^{2+}-induced elevation of $[Ca^{2+}]_i$ resulting from the ER Ca^{2+} release in resting cells may be involved in the neurotoxicity (including learning and memory impairment) on account of $[Ca^{2+}]$i which play a major role in the induction and maintenance of LTP (Fan et al., 2013). It should be noted that Ca^{2+} uptake is driven by a difference in mitochondrial potential product of the ETC force that facilitates positively charged ions to enter the matrix through macromolecular complexes Ca^{2+} antiporters. In turn, Ca^{2+} is rapidly extruded into the cytoplasm by different pumps, channels, and auxiliary proteins, in order to restore the basal state. Thus, any alteration of the coordination of these events will disrupt mitochondrial Ca^{2+} homeostasis (Giorgi et al., 2018). It is the case of Pb^{2+}, a metal that as mentioned affects many Ca^{2+}-dependent events product of ion-mimicry and of its accumulation in the same subcellular compartments than Ca^{2+} (Bressler and Goldstein, 1991; Dressier et al., 1999; Lidsky and Schneider, 2003; Rocha and Trujillo, 2019). In this respect, Chavez et al. (1987) proposed that Pb^{2+} stimulates mitochondrial Ca^{2+} release by promoting a reduction in the NAD(P)H/NAD(P) ratio and a collapse in the transmembrane potential, due to its ability to pass through the Ca^{2+} uniporter and its affinity to the thiol groups located in the matrix side of the IMM (Chávez et al., 1987). These results confirm previous reports in terms of interference with Ca^{2+} entrance to the mitochondria (Jurkowitz et al., 1974; Parr and Harris, 1976; Silbergeld and Adler, 1978), and complement others (Kapoor and Van Rossum, 1984), suggesting that suggested that Pb^{2+} accumulated in the mitochondria displaces Ca^{2+} from its intramitochondrial binding sites. Thereby, the inhibition of Ca^{2+}uptake into brain mitochondria by Pb^{2+} would be expected to increase $[Ca^{2+}]$i in the cytosol, affecting normal neuronal function (Goldstein, 1977).

On the other hand, and as mentioned before, the ETC is also the main source of ROS due to an incomplete O_2 reduction in the Complex I and Complex III, affecting proteins, membranes, and promoting caspase activation and the mitochondrial apoptotic pathway. ROS can also facilitate the opening of the MPTP leading to the IMM permeability (Moro, 2020), a mechanism reported in human neuroblastoma SH-SY5Y cells treated with Pb^{2+} (Ye et al., 2016).

In summary, in addition to its role in energy metabolism, mitochondria regulate cellular redox status, control Ca^{2+} homeostasis, initiate the intrinsic pathway of apoptosis through activation of MPTP, carry-out anabolic biochemical processes including synthesis of heme and Fe^{2+}-sulfur clusters, regulate the levels of essential intracellular intermediates that activate cellular signal transduction pathways including retrograde signaling, and contribute to additional energy production by other pathways including Krebs cycle and fatty acid oxidation (Meyer et al., 2013). Important homeostatic processes include replication of the mitochondrial genome, mitochondrial biogenesis such fusion and fission processes, and degradation pathways including mitophagy (Murata et al., 2020). The inclusion of the study of mitochondrial targets beyond the ETC, such as mitochondrial metabolism, oxidative

stress, mtDNA genetics, and signaling response will broaden the spectrum of environmental agents considered mitotoxicants.

2.3.2. Mitochondrial ROS generation—ROS are chemically reactive chemical species containing O_2. At low levels, these species may participate in cell signaling processes, whereas at higher levels they may damage cellular macromolecules, including proteins, lipids, DNA, and RNA. Most ROS are generated in the mitochondria (mtROS), with a reduced proportion is mediated by the activity of enzymes such as nicotinamide adenine dinucleotide phosphate (NADPH)-dependent oxidases (NOx), enzymes that generate $O_2^{\bullet-}$ or H_2O_2, xanthine oxidase (that contains a Fe^{2+}-sulfur cluster), the heme proteins cyclooxygenases, cytochrome p450 enzymes, lipoxygenases, and myeloperoxidases, as well as the protein folding machinery in the endoplasmic reticulum (Turrens, 2003). ROS generation leads to $OH^{\bullet}$ formation through Fenton/Haber-Weiss reactions, in where H_2O_2 oxidizes either Fe^{2+} or Cu^{2+} respectively, that are reduced back by $O_2^{\bullet-}$ initiating free radical chain reactions. Once formed, $O_2^{\bullet-}$ undergoes rapid dismutation into H_2O_2 through the action of superoxide dismutase (SODs), including the Cu/Zn-dependent cytosolic SOD1, extracellular SOD3, and MnSOD (SOD2) which is exclusively localized in the mitochondrial matrix. H_2O_2 is removed by peroxisomal enzyme catalase (CAT) or by the selenoprotein glutathione peroxidase (GPx) enzymes, the latter one of the most abundant intrinsic antioxidants that help in preventing lipid peroxidation and the resulting cell death by ferroptosis, a process assisted by glutathione reductase (GR) to recycle oxidized glutathione (GSSG). Additionally, other H_2O_2-removing systems do exist, including peroxidases that use the dithiol-containing protein thioredoxin as a substrate (peroxiredoxins); whereas thioredoxin reductase, which converts oxidized thioredoxin back to the reduced form is also a selenoprotein. The above-mentioned are the main components of the enzymatic antioxidant system that counteracts ROS deleterious effects. The tripeptide GSH constitutes the main non-enzymatic antioxidant, particularly in the mitochondria. Most GSH is used by GPx and peroxiredoxins to catalyze H_2O_2 reduction. Additional natural antioxidants are ascorbic acid, vitamin E, α-tocopherol, cysteine, Zn^{2+}, Se^{2+}, among others (Halliwell, 2001). The interference with nitric oxide (NO) production might represent another mechanism accounting for Pb neurotoxicity. Reactive nitrogen species (RNS) include the diffusible second messenger NO, considered a free radical because it has an unpaired electron, the nitrogen dioxide radical (NO_2), and the peroxynitrite ($OONO^-$), which protonates at relevant pH to form peroxynitrous acid (ONOOH), both strong oxidizing agents (Aschner, 1996). The synthesis of NO is a process initiated by the presynaptic release of glutamate, which binds to its receptors and allows Ca^{2+} to enter into the cell. The binding of Ca^{2+}/calmodulin to neuronal NO synthase (nNOS) facilitates the oxidation of L-arginine to conclude with the synthesis of L-citrulline and NO. Several shreds of evidences demonstrate that the mimicry between Pb^{2+} and Ca^{2+} can alter the activity and expression of Ca^{2+}-dependent nNOS and endothelial NOS synthase (eNOS) in different brain regions, preventing the accessibility of Ca^{2+} to NOS, thus leading to a decreased activity of nNOS/eNOS and resulting in a reduced NO production in different brain regions (Garcia-Arenas et al., 1999; Nava-Ruiz et al., 2012). In agreement, nNOS expression and activity were reported to be reduced by Pb^{2+} in the developing rat brain, alterations attributed to changes in $[Ca^{2+}]i$ homeostasis (Chetty et al., 2001). Additionally, provided the

importance of the NO signaling in the induction of LTP in the hippocampus, the impairment on the NO transduction system might affect synaptic development and plasticity of brain regions involved in higher cognitive functions that are associated with clinical Pb^{2+}-exposure manifestations (García-Arenas et al., 2004; Selvín-Testa et al., 1997). In addition, Pb^{2+} competes with Ca^{2+}, Zn^{2+} is also an important structural element of NOS enzymes, in particular at the NMDAr level, thus interfering with nNOS activation and consequent NO production (Garza-Lombó et al., 2018).

Mitochondrial ROS production depends on the electron flux in the ETC. When reduced, such as by age, pathological conditions, or the presence of xenobiotics, namely, the possibility that electrons move from O_2 to form ROS it's enhanced. Another factor influencing mtROS generation is Ca^{2+} signaling that involves all the associated processes described above (Meyer et al., 2018). Moreover, the inhibition of the ETC by xenobiotics or by NADH augmentation due to the low ATP demand and consequent low respiration rate will increase the $NADH/NAD^+$ ratio and lead to the $O_2^{\bullet-}$ formation. In contrast, in most of the situations in which the mitochondria have a normal functionality and the $NADH/NAD^+$ relationship is relatively low, only small $O_2^{\bullet-}$ amounts are produced (Murphy, 2009). Additionally, both the Complex I and the enzyme alpha ketoglutarate dehydrogenase will produce more ROS when the $NADH/NAD^+$ relation is high; then increased ROS generation will compromise the ability of the Complex I to oxidize NADH, originating a vicious circle that may lead to greater mitochondrial dysfunction (Stefanatos and Sanz, 2011).

Additionally, the membrane polyunsaturated fatty acids are very susceptible to the lipidic peroxidation, playing a critical role in the redox state of the cell by the production of toxic aldehydes such as malonaldehyde (MDA) and 4-hydroxynonenal (4-HNE), a process triggered by ROS and catalyzed by both Fe^{2+} and Cu^{2+}. These aldehydes are highly reactive and able to form adducts with nucleic acids, proteins, amino acids, and polypeptides, leading to enzymatic inactivation, mitochondrial dysfunction, and cell death (Halliwell, 1992). Thus, essential metal ions (Mn^{2+}, Cu^{2+}, Fe^{2+}, and Zn^{2+}) and ROS are capable to alter mitochondrial lipid environments and membrane integrity. In addition, either the overload or depletion of essential metal ions (or their substitutes) interferes with mitochondrial functions, such as the TCA cycle, oxidative phosphorylation, and GSH metabolism (Nam et al., 2018).

In summary, both enzymatic and non-enzymatic antioxidants are involved in the neural protection from oxidative harm resultant of mitochondrial dysfunction. Thus, the orchestrated formation of excessive free radicals, combined with a decrease in cellular enzymatic defenses accompanied by GSH and natural antioxidant depletion might lead to increased sensitivity of neural cells to Pb toxicity. Evidence of these effects will be provided in detail in the next section.

2.4. THE PRO-OXIDANT/ANTI-OXIDANT MECHANISM

Oxidative stress is the redox state resulting from an imbalance between the generation and detoxification of ROS and RNS. Under carefully controlled situations, ROS function as important physiological regulators of intracellular signaling pathways. However, an overloaded amount of ROS promotes a redox imbalance that damage the cells by oxidizing

cellular biomolecules, including nucleic acids, proteins, and lipids, all of which lead to and facilitate apoptosis (Finkel, 2011)

The brain is particularly susceptibility to oxidative damage. In the first place, because of elevated O_2 consumption, the product of high ATP demand of neurons results in efficient mitochondrial functionality. Secondly, the brain presents low levels of antioxidant defenses when compared to other cells, as well as an abundance of highly polyunsaturated fatty acids. In addition, although mostly stored in ferritin, Fe^{2+} is present in the brain, which along with Cu^{2+} in Fenton and Haber-Weiss reactions, respectively are capable to promote H_2O_2 formation, lipid peroxidation, and autoxidation of neurotransmitters, in particular catecholamines considered highly auto-oxidized molecules. Moreover, the brain generates H_2O_2 *in situ* via dopamine metabolism by monoamine oxidases (MAOs), flavoprotein enzymes located in the OMM. In the same line, cytochromes P450 (CYPs), enzymes present in the brain can 'leak away' ROS from the catalytic intermediates in the P450 cycle. Finally, brain microglia can produce $O_2^{\bullet-}$ and H_2O_2 upon activation, although neurons and oligodendrocytes seem to be more susceptible to oxidative damage than astrocytes and microglia (Halliwell, 1992; Halliwell, 2001; Wang and Michaelis, 2010).

Even low-environmentally-relevant Pb^{2+} levels are reported to induce a redox imbalance in the cell by the generation of free radicals resulting in oxidative damage to critical biomolecules, lipids, proteins, and DNA (Ahamed and Siddiqui, 2007; Ercal et al., 2005). Evidence on this regard come from *in vitro* experiments or studies conducted in animals, most of them at moderate to high Pb^{2+} doses. In addition, several clinical and epidemiological studies provide associations among workers with high Pb^{2+} exposure and oxidative stress, while there are fewer reports of environmental Pb^{2+} exposure in children and adults that assessed this association (Almeida Lopes et al., 2016). This raises the need to develop oxidative stress biomarkers of early Pb^{2+}-adverse effects and potential therapeutically interventions in Pb^{2+}-related negative outcomes. Thus, in the first place, the putative mechanisms of Pb^{2+} on the pro- and antioxidant balance will be described in detail with a summary of the most relevant results of this topic in experimental and clinical settings, followed by a brief description of state-of-the-art studies aimed to ameliorate these detrimental effects.

2.4.1. Mechanisms for free radical generation

2.4.1.1. Interactions in the heme biosynthetic pathway: pro-oxidative effect of δ-ALA: The δ-ALA undergoes enolization and auto-oxidation at pH 7.0–8.0. The conversion of the δ-ALA keto form into the δ-ALA enol form is shown to be necessary for auto-oxidation reactions, being the latter autoxidized to generate $O_2^{\bullet-}$ and reduced ferricytochrome c. In parallel, the reaction between δ-ALA/oxyhemoglobin (oxyHb)-coupled oxidation results in metaHb, δ-ALA radical, and H_2O_2 generation. The $O_2^{\bullet-}$ and H_2O_2 formed in these reactions can generate $HO^{\bullet}$ radicals. In addition to these mechanisms, some authors have related Pb^{2+} neurotoxicity to the ability of δ-ALA to inhibit either the K^+-stimulated release of γ-aminobutyric acid (GABA) from preloaded rat brain synaptosomes or the binding of GABA to synaptic membranes (Bechara, 1996; Gurer-Orhan et al., 2004).

2.4.1.2. Membrane lipid peroxidation: direct Pb^{2+} effect: Lipids play a pivotal role in neuronal function as they constitute the plasma membrane, the barrier between intracellular and extracellular spaces. They are prone to the attack of free radicals and undergo lipid peroxidation, leading to a decrease in membrane fluidity and leakiness. This facilitates the entrance of substances usually unable to cross the barrier, except through specific channels (e.g., K^+, Na^+, Ca^{2+}, etc.). As the neuronal membrane lipids are rich in polyunsaturated fatty acids (PUFA), the side chains are specifically more vulnerable to ROS/RNS, and thus to oxidative stress processes.

Lead is known to have toxic effects on membrane structure and functions, altering membrane fluidity and/or promoting alterations in membrane permeability, which were associated to Pb neurotoxicity (Verstraeten et al., 2008). On cell membrane, the presence of double bonds in the fatty acid weakens the C–H bonds on the carbon atom adjacent to the double bonds and makes H removal easier. Therefore, fatty acids containing zero to two double bonds are more resistant to oxidative stress than is the PUFA with more than two double bonds. Lipid peroxidation results in the formation of a wide variety of oxidation products, the most common of which are aldehydes. Among the most abundantly produced aldehydes are MDA, propanal, hexanal, and 4-HNE. Another mechanism for Pb^{2+}-induced membrane oxidative damage is the effect on changes in the membrane composition. Because fatty acid chain length and unsaturation are associated with membrane susceptibility to peroxidation, Pb^{2+}-induced arachidonic acid elongation might be responsible for the enhanced lipid peroxidation in the membrane. By causing lateral phase separation and/or by increasing lipid peroxidation rates, Pb^{2+} could affect membrane related processes such as the activity of membrane enzymes, endo- and exocytosis, the transport of solutes across the bilayer, and signal transduction processes. In addition, Pb^{2+} can also stimulate Fe^{2+}-initiated membrane lipid oxidation by changing membrane physical properties (Adonaylo and Oteiza, 1999; Ahamed and Siddiqui, 2007). Taken together, these data suggest that altered lipid composition of membranes due to Pb^{2+} exposure may result in altered membrane integrity, permeability, and function, increasing the susceptibility to lipid peroxidation.

2.4.1.3. Effects on antioxidant defense systems of cells: GSH is a tripeptide containing cysteine that has a reactive thiol group with reductive potency against ROS that represents more than 90% of the non-tissue-related thiol pool of the human body. Eventually, GSH can be involved as a cofactor or a coenzyme in the enzymatic detoxification reactions of ROS. In addition, GSH is an important substrate acting in the metabolism of specific drugs and toxins via conjugation in the liver. Pb^{2+} binds to the ^{-}SH group, which decreases GSH levels and its antioxidant activity. An enzymatic component of the antioxidant defense system, GR, reduces GSSG back to GSH and thereby supports the antioxidant defense system indirectly. GR possesses a disulfide at its active site that constitutes a target for Pb^{2+}, resulting in the inhibition of the enzyme, leading to a decreased GSH/GSSG ratio that will render cells more susceptible to oxidative damage (Carocci et al., 2016; Ercal et al., 1996).

The metalloproteins GPx, CAT, and SOD accomplish their antioxidant functions by enzymatically detoxifying the peroxides (^{-}OOH), H_2O_2, and $O_2^{\bullet -}$, respectively. CAT decomposes H_2O_2 into H_2O and O_2 at higher steady- state H_2O_2 concentration, while GPx requires GSH to decompose H_2O_2 or other peroxides with the simultaneous oxidation of

GSH into GSSG under lower steady-state levels of H_2O_2. Since these antioxidant enzymes depend on various essential trace elements and prosthetic groups for proper molecular structure and enzymatic activity, they are potential targets for Pb^{2+} toxicity. SOD dismutates $O_2^{\bullet-}$ into H_2O_2 and requires $Cu^{2+}$and Zn^{2+} for its activity. Thus, three types of SODs exist in mammalian cells that use an essential metal as a cofactor. Cu/Zn-dependent SOD1 and SOD3 are localized in the cytosol (SOD1), the extracellular space (SOD3) and to a lesser extent, in the IMM space of the mitochondria (SOD1), while MnSOD (SOD2) is solely localized in the mitochondrial matrix. The transition metal (Cu^{2+} or Mn^{2+}) in the SOD active site is required for the breakdown of $O_2^{\bullet-}$ by catalyzing both the one-electron oxidation-reduction of separate $O_2^{\bullet-}$ to give the overall disproportionation reaction that produces O_2 and H_2O_2. Cu^{2+} ions have a functional role in the reaction by undergoing alternate oxidation and reduction, where Zn^{2+} ions confer higher thermal stability to the proteins instead of having a role in the catalytic cycle (Garza-Lombó et al., 2018).

2.4.2. Clinical and experimental studies—Provided the numerous reports that relate Pb^{2+} detrimental effects on redox balance and the possible link to its neurotoxic effects, what follows is a short description of some pivotal studies stressing relevant conclusions on these effects, with a distinction in between clinical evidence and studies performed *in vitro* and in laboratory animals focused on blood and/or brain biomarkers status.

2.4.2.1. Clinical evidence: An excellent review regarding epidemiological data has been published recently (Almeida Lopes et al., 2016). Among the studies that met their criteria, the authors divided the population in environmentally-exposed to Pb^{2+}, mostly composed of children and the general population, or workers occupationally-exposed to the metal. They concluded that Pb^{2+} exposure induced oxidative stress in both populations, with the most frequent biomarkers that showed association with blood Pb^{2+} levels (BLL) were the antioxidant enzymes, as well as GSH and MDA.

2.4.2.1.1. Environmental exposures: many reports compared BLL with biomarkers of effects, in particular δ-ALA-d, SOD and CAT activities, MDA and GSH levels as a biochemical profile of redox toxicity. Ahamed et al., in 2005 and 2006 reported a positive association among BLL, MDA levels, and CAT activity and a negative correlation with GSH levels and δ-ALA-d activity in children (Ahamed et al., 2005) and adolescents living in an urban setting exposed to Pb^{2+} from environmental sources (Ahamed et al., 2006). The authors propose δ-ALA-d not only a biochemical index of Pb^{2+} exposure but also an early biomarker of oxidative stress. However, other authors reported no significant variations in δ-ALA-d, CAT, and SOD activities at low BLL in a pediatric population (Martínez et al., 2013). Additionally, Pb^{2+}-exposed adults living in urban centers showed a negative correlation with antioxidant enzymes SOD, CAT, GPx activity, and GSH levels (Jangid et al., 2016). On the other hand, Pb^{2+}-contaminated opium is a frequent cause of Pb^{2+} toxicity in opium addicts from certain countries. In this subpopulation, high levels of lipid peroxidation and protein carbonylation, and a decrease in total antioxidant capacity and in SOD activity were reported (Shojaeepour et al., 2018).

2.4.2.1.2. Occupational exposures: *A* high increase in δ-ALA distribution and accumulation was indicated as a triggering event in Pb^{2+}-induced oxidative stress responses in occupationally-exposed workers (Costa et al., 1997). In accordance with these results, Gurer-Orhan et al, in 2004 evaluated workers of a battery plant exposed to Pb^{2+}, a study that reported decreased blood GSH/GSSG ratios and δ-ALA-d activity accompanied by increased MDA levels and CAT activity, all indicative of oxidative stress (Gurer-Orhan et al., 2004). Two independent studies also performed in battery manufacturing workers that reported an increase in lipid peroxidation levels, while a reduction in SOD and CAT activity was evident (Ghanwat et al., 2016; Patil et al., 2006). In another study, the gene expression levels of both SOD1 and GPx1 and SOD and GPx enzymatic activity were increased, while the expression and activity of CAT were unchanged in red blood cells from Pb^{2+}-exposed workers (Kasperczyk et al., 2012). More recently, Kshirsagar et al. (2020) reported significantly increased serum lipid peroxide levels in painters occupationally exposed to Pb^{2+}, likely due to exaggerated ROS generation caused by SOD and CAT inhibition (Kshirsagar et al., 2020).

Overall, these studies suggest that high BLL seem to be associated to increased lipid peroxidation and reduced δ-ALA-d, while the activity of antioxidant enzymes is less consistent across the studies with divergent results, reductions in the presence of high BLL and increases at lower BLL, probably as a compensatory mechanism.

2.4.2.2. Experimental animals and in vitro studies

2.4.2.2.1. Adult animals: Pioneer experiments performed by Sandhir et al. (1994) demonstrated that chronic Pb^{2+} exposure accentuated lipid peroxidation in all regions, preferentially in the hippocampus, the area with higher Pb^{2+} accumulation. The antioxidant enzymes SOD, CAT, GR, and GPx activity was diminished, with a similar trend in GSH, results that suggest that Pb^{2+} may exert its neurotoxic effects via peroxidative damage to the membranes (Sandhir et al., 1994). Ercal et al. (1996) demonstrated that chronic adult Pb^{2+} exposure depletes GSH levels, increases GSSG, and promotes MDA production in both liver and brain samples taken from C57BL/6 mice (Ercal et al., 1996). Under the same experimental conditions, Gurer et al. (1998) reported similar results in blood, as well as increases in CAT and glucose 6-phosphate dehydrogenase (G6PD) activity, accompanied by a reduction in δ-ALA-d activity. Resembling clinical studies, the authors suggested that δ-ALA accumulation and autooxidation might contribute to Pb^{2+}-induced oxidative stress (Gurer et al., 1998). In further studies, the same authors reported enhanced MDA content in Pb^{2+}-treated Chinese hamster ovary (CHO) cells and brain tissue of Fisher 344 rats, accompanied by alterations in their antioxidant defense systems, including decreased GSH levels and increased CAT and G6PD activity (Gurer et al., 1999). Several other studies performed in chronically-Pb^{2+}-exposed rats also demonstrated a reduction in δ-ALA-d and an increase in TBARS as compared to controls (Adonaylo and Oteiza, 1999). Liver GSSG and MDA levels were higher in young chronically-exposed Pb^{2+} rats than those in the adult group, although both brain and blood MDA levels were higher in the older animals exposed to identical treatment (Aykin-Burns et al., 2003). In agreement, adult rats chronically exposed to high Pb^{2+} levels had high blood SOD and CAT activity and increased MDA content in blood and brain (Soltaninejad et al., 2003). Moreover, Pb^{2+} exposure caused an

increase in brain TBARs and CAT activity, while brain SOD activity levels were unaffected (Antonio-García and Massó-Gonzalez, 2008a). Interestingly, GPx was increased, while GR was reduced in several brain regions of rats chronically-exposed to Pb^{2+} (Bokara et al., 2009). On the other hand, there is evidence to support that Pb^{2+} exposure causes genotoxicity, probably as a result of the production of free radicals, but also by a metal inhibition on DNA repair (García-Lestón et al., 2010). In this respect, an increase in DNA damage in lymphocytes of young and adult female rats chronically exposed to Pb^{2+} has been reported (Nascimento and Martinez, 2016).

2.4.2.2.2. Developing animals: *Pb^{2+}* concentration in blood and brain regions was associated with enhanced lipid peroxidation in rats continuously exposed to the metal from gestation to adolescence (Villeda-Hernández et al., 2001). However, when SOD, GPx, and GR activity was assessed in several brain regions obtained from pre and postnatally Pb^{2+}-exposed pups, only a decrease in SOD in the hypothalamus was evident (Moreira et al., 2001). Another report indicates that plasma and brain δ-ALA concentrations increased significantly immediately after Pb^{2+}-exposure ended at weaning, along with a decrease in activities of SOD, GPx, and GR enzymes in several brain regions, effects that were not evident in adult rats (Wang et al., 2006). A similar pattern of effects was later published (Bokara et al., 2008) showing an increase in lipid peroxidation and antioxidant enzymes (SOD and CAT) activities in young rats' brains developmentally exposed to Pb^{2+}, an effect that did not persist into adulthood. The consequences of lactational Pb^{2+} exposure on the onset of oxidative damage late in life was assessed by measuring the levels of 8-hydroxyl-2'-deoxyguanosine (oxo^8dG), one of the major products of DNA oxidation, and the associated activity of the DNA repair enzyme 8-oxoguanine DNA glycosylase (Ogg1) in the brain cerebral tissue of aging rats exposed to Pb^{2+} during postnatal brain development and/or during old age. The results demonstrated that oxo^8dG remained elevated in the frontal cortex of these animals only if Pb^{2+} exposure started at early postnatal age. However, no changes in GSH, Cu/ZnSOD, or MnSOD levels were affected in either scheme of Pb^{2+} exposure (Bolin et al., 2006). In addition, both gestational and lactational Pb^{2+} exposure elevated ROS and MDA levels followed by an increase in the Bax/Bcl-2 ratio, promoting apoptosis, while GSH and SOD were significantly decreased, in the pups' hippocampus at early postnatal age (Lu et al., 2013). It was also demonstrated that pre-and post-natal low-level Pb^{2+}-exposure in rats increases the GSSG/GSH ratio by decreasing the GSH levels and SOD, CAT, and GPx activity, with inconsistent changes in mRNA and protein expression, an effect that the authors attribute to impairments in the catalytic function of the enzyme protein or a reduction in the availability of cofactors (Baranowska-Bosiacka et al., 2012). Further evidence confirms that developmental Pb^{2+} exposure induced a redox imbalance evidenced by a significant increase in MDA levels and decrease in antioxidant enzymes (GPx, SOD, and CAT activity) in the pups' hippocampus, the brain region associated with LTP, and learning and memory impairments frequently reported after early Pb^{2+} exposure (Soleimani et al., 2016).

Overall, as concluded from a meta-analysis of the available literature regarding the effects of Pb^{2+} on oxidative stress parameters in rodents, the metal significantly increased the levels of oxidants such as MDA, GSSG, ROS, and H_2O_2, and reduces the levels of antioxidative

substances, such as CAT, GPx, GR, GSH, SOD, and GST (Fan et al., 2020). As mentioned before, the reported decreases in the antioxidant enzymes may be the result of the metal affinity for sulfhydryl groups or Pb^{2+} competition for metal cofactors such as Cu^{2+} and Zn^{2+} in the binding sites of these enzymes.

Finally, and regarding genotoxicity and its impact on redox imbalance, according to the International Agency for Research on Cancer, Pb^{2+} has been identified as a probable human IIB carcinogen, while its inorganic form Pb^{2+} is included in class IIA ("Inorganic and organic lead compounds.," 2006). Experimental evidence suggests a facilitating role for Pb^{2+} in carcinogenesis, allowing or enhancing carcinogenic events involved in DNA damage, repair, and regulation of tumor suppressor and promoter genes (Silbergeld, 2003). From the mechanistic point of view, it has been reported that the genetic damage induced by the metal could be due to indirect mechanisms provided that can substitute Ca^{2+} and/or Zn^{2+} in enzymes involved in DNA processing and repair leading to an inhibition of DNA repair and an enhancement in the genotoxicity when combined with other DNA damaging agents. Besides, oxidative stress produced by the increase in free radical levels induced by Pb^{2+} exposure may also contribute to the indirect genotoxicity of this metal (García-Lestón et al., 2010). However, its role in human carcinogenesis is still a topic of debate with limited epidemiological evidence (Ahn et al., 2020; Fu and Boffetta, 1995; Ferrante et al., 2017).

2.5. THE INFLAMMATORY COMPONENT

The neurotoxic effects of Pb^{2+} have been long recognized, although only recently new insights have linked the neuroimmune system to its toxicity (Chibowska et al., 2016; Liu, et al., 2015; Metryka et al., 2018). Neuroinflammation is a complex biological response that involves microglial and astroglial activation and many signaling proteins, receptors, and cell types including the production and release of inflammatory cytokines, augmented ROS generation, diminished antioxidant activity, resulting in neuronal injury or neuronal loss in the CNS. In the first place and regarding cytokines importance for BBB permeability, it is reported that the coadministration of inflammatory cytokines and Pb^{2+} induces the production of matrix metalloproteinase (MMP)-9, a Zn^{2+}-dependent enzyme that degrades the extracellular matrix and basement membrane in astrocytes, resulting in increased permeability of the BBB (Lahat et al., 2002). There are reports that Pb^{2+} exposure caused a microgliosis and astrogliosis after continuous or intermittent Pb^{2+} exposure, with Iba-1, and glial fibrillary acidic protein (GFAP) indicators of gliosis (Shvachiy et al., 2018). Similarly, an increase in the hippocampal number of immunoreactive microglial cells (Iba1+) and (Iba1+/TLR4+) has also been reported. Moreover, the microgliosis and astrogliosis in the hippocampus of young mice were probably mediated by the innate immune reaction of toll-like receptors 4 (TLR4), the signal transfer by the adapter myeloid differentiation primary response gene 88 (MyD88) to the intracellular pathway with resultant nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kβ) activation and inflammatory responses. In the same study, the authors reported enhanced expression levels of interleukin-1 beta (IL-1β), tumor necrosis factor-alpha (TNF-α), p38MAPK, and ERK1/2 in the hippocampus, indicating potential implication of inflammatory response and MAPK signaling activation in Pb^{2+}-induced microgliosis and astrogliosis (Liu et al., 2015). In another study, Pb^{2+} exposure induced the activation of NF-kβ, activator protein-1 (AP-1), c-jun N-terminal

kinase (JNK), MAPK, and caspases in the rats' brain, which may contribute to its neurotoxic effects by apoptotic mechanisms (Ramesh et al., 2001). In addition, astroglial activation, microglia-induced neuroinflammation, and synaptic degeneration, accompanied by the increased level of glial markers, GFAP and S-100b, as well as the production of proinflammatory cytokines, including IL-1β, TNFα, and IL-6, was reported in immature rats' brain areas as a result of prolonged Pb^{2+} exposure (Struzyńska et al., 2007). Moreover, brain gene expression for IL-6 and the transforming growth factor-β1 (TGF-β1) was induced as the result of developmental Pb^{2+} exposure in mice (Kasten-Jolly et al., 2011). Recent data corroborate these findings, reporting that chronic pre-and neonatal Pb^{2+}exposure induces neuroinflammation in the forebrain cortex, hippocampus, and cerebellum of rat pups evidenced by a significant increase in several cytokines and prostanoids [IL-1, IL-6, TGF, prostaglandin E2 (PGE2) and T-box transcription factor 2 (TXB2)]. The protein and mRNA expression of enzymes involved in the inflammatory process (cyclooxygenases) COX-1 and COX-2 increased in all brain structures, as did NF-kβ expression (Chibowska et al., 2020). Additionally, Kumawat et al. (2014) reported up-regulation of ERK and protein kinase B (Akt) pathways, along with activation of NF-kβ. Moreover, TNF-α, IL-6, and monocyte chemoattractant protein-1 (MCP-1), as well as COX-2 pro-inflammatory enzyme levels were increased in response to Pb^{2+} exposure. It was proposed that pro-inflammatory cytokines secreted by the microglia in response to Pb^{2+} can induce apoptotic pathways culminating in the activation of the caspase-3 enzyme. Notably, both NF-kβ and AP-1 are redox-sensitive transcription factors that regulate the induction of antioxidant-specific genes by the "antioxidant responsive element" in the promoter region of the CAT or SOD genes (Kumawat et al., 2014). It has been shown that Pb^{2+}causes the induction of oxidative stress and depletion of cellular GSH content, a situation that triggers the activation of NF-kβ and AP-1 binding (Korashy and El-Kadi, 2008). Finally, microglial and astroglial activation is associated with elevated levels of NADPH oxidase (NOx), the main non-mitochondrial source of $O_2^{\bullet -}$ and ROS. Importantly, NOx is ubiquitously expressed in microglia and may be activated by IL-1β, TNF-α, IFN-γ, and other pro-inflammatory cytokines (Kasten-Jolly et al., 2011). Interestingly, NOx is activated by Pb^{2+}, promoting ROS accumulation in the brain (Verstraeten et al., 2008). In addition, Pb^{2+} stimulates the synthesis and secretion of the proinflammatory cytokine IL-8 in a mechanism dependent on Nrf2, a transcriptional factor responsible for the induction of xenobiotic-metabolizing enzymes (Metryka et al., 2018) and a redox-sensitive counterbalancing component. Notably, the microglia is more sensitive to Pb^{2+}-induced oxidative stress, evidenced as increased production of ROS, down-regulation of GSH, and enhanced Nrf2 protein expression, while no obvious changes in astrocytes were observed (Peng et al., 2019). It is worth to mention that the Nrf2 pathway plays a negative regulatory role in response to oxidative stress, an event that has an important role in primary microglial toxicity caused by Pb^{2+} exposure and may be associated to the pathological basis of Pb-induced neurological dysfunction. On the other hand, Liu et al, 2012 (Liu et al., 2012) raised the hypothesis that activation of microglia participates in the Pb^{2+}-induced hippocampal LTP impairment and neuronal injury. They supported their idea on the bases that microglia activation can impair learning and memory processes via released IL-1. Thus, Pb^{2+} can cause microglia activation, which can up-regulate the levels of IL-1b, TNF-α, and inducible NOS synthase (iNOS), proinflammatory factors that either alone or in combination may cause hippocampal neuronal injury and LTP

impairment leading to learning and memory deficits. Remarkably, iNOS is a Ca^{2+}-independent enzyme with a similar structure to nNOS and eNOS, which is found primarily in immune cells or glial cells (astrocytes and microglia) and is activated in response to pathogen recognition and cytokine release (Nava-Ruiz et al., 2012). Overall, this evidence reveals the tight relationship between pro-oxidant and inflammatory events that may lead to brain injury and eventually cell death, particularly when homeostatic processes are overwhelmed and the Pb^{2+} induction of redox imbalance lead to neuroinflammation.

Finally, it is noteworthy that more recent evidences point-out neuroinflammation characterized by astroglial and microglial activation as a key factor in the onset and progression of sporadic Alzheimer's disease (AD) (Huat et al., 2019). In particular, acute Pb^{2+} exposure is associated with the formation of amyloid plaques as the result of a significant increase of beta-amyloid (Aß) in brain tissue (Gu et al., 2011). Specifically, Pb^{2+} produced IL-1-associated amyloid precursor protein (APP) over-expression, as well as β-secretase (BACE) and presenilin (PS) generation, leading to Aß accumulation. Moreover, based on previous reports indicating that ROS contribute to astrocyte degeneration, these authors ascribed the induction of IL-1 in the developing brain to a metal-catalyzed oxidative stress mechanism (Ashok et al., 2015). Interestingly, there are other theories that associate AD occurrence to "fetal programming" as the result of Pb^{2+}-exposure and the potential role of neuroepigenetics in neurodegenerative diseases. In this regard, it has been recently demonstrated in experimental animals that Pb^{2+} neurotoxicity can be mediated through epigenetic mechanisms, including brain DNA methylation, chromatin remodeling by histone post-translational modifications (both acetylation and methylation), and non-coding RNAs, in particular microRNAs (Khalid and Abdollahi, 2019; Xu et al., 2020). Interestingly, an association between BLLs and aberrant DNA methylation in δ-ALA-d and p16 gene leading to epigenetic silencing was recently reported in an environmentally Pb^{2+}-exposed pediatric population. Given these observations, the authors proposed these epigenetic changes as early biomarkers of susceptibility (Yohannes et al., 2020), while other authors considered them as essential tools in addressing risk assessment and proper decision making in the management of Pb^{2+} exposure (Mani et al., 2019). Furthermore, sex-dependent epigenetic mechanisms were pointed-out as pivotal in the combined developmental neurotoxicity of Pb^{2+} and prenatal stress in rats (Sobolewski et al., 2018). For a complete revision of the existing literature on the role of Pb^{2+} in the "fetal origin of adult disease" hypothesis, in particular related to AD pathogenesis, the reader can refer to (Basha and Rajarami, 2010; Bihaqi, 2019; Wang, et al., 2020).

3. AMELIORATION OF PB-NEUROTOXIC EFFECTS

It is clear that preventive measures are preferred over amelioration because once Pb^{2+} enters the body and reaches its target organs it is almost impossible to remove it completely or to reverse the harmful effects. However, in an event of a Pb^{2+} intoxication, various chelators and antioxidants are approved to be used either alone or in combination. Details on these compounds' benefits and pitfalls are provided below.

3.1. AMELIORATION BY ANTIOXIDANTS

Antioxidants are effective in alleviating and treating the oxidative stress-induced toxicity of Pb^{2+}. By interacting with generated ROS, antioxidants prevent radical chain reactions because they inhibit or delay oxidation of a substrate by counteracting free radical-induced damage to macromolecules. The major limitation in the use of antioxidants is related to their poor solubility in aqueous solvents, low absorption, poor bioavailability, and rapid metabolism. To improve their use, nanoparticles, liposomes, micelles, and phospholipid complex have been formulated for better delivery. The most important source of antioxidants is provided by nutritional factors, which can modify Pb^{2+} absorption, deposition, and toxicity. Next, we will discuss nutrients known to interact with Pb^{2+} as reviewed by (Ahamed and Siddiqui, 2007; Carocci et al., 2016; Flora et al., 2012; Gurer and Ercal, 2000; Hsu and Guo, 2002):

3.1.1. Metals: As reviewed before, Pb^{2+} disrupt Ca^{2+} homeostasis and interact with its cellular and molecular targets; thereby, a diet with Ca^{2+} supplementation reduces Pb^{2+} absorption and toxicity. In this line, there are pieces of evidence that Pb^{2+} administration during pregnancy and lactation induced significant reductions in antioxidant enzymes, and increased the MDA levels in the cerebellum and hippocampus, effects that were partially reversed by Ca^{2+} supplementation (Gottipolu and Davuljigari, 2014). In a similar way, the competition between Zn^{2+} and Pb^{2+} might decrease the absorption of Pb^{2+}, thus Zn^{2+} supplementation prevents Pb^{2+}-induced δ-ALA-d inhibition and Pb^{2+} substitution at various Zn^{2+}-mediated processes and molecular targets, including Cu/ZnSOD. Moreover, combined Zn^{2+} and Ca^{2+} supplementation reversed the Pb^{2+}-induced decreases on antioxidant enzymes and an increase in lipid peroxidation and free radical formation, all involved in Pb^{2+} neurotoxicity in mice (Prasanthi et al., 2010). In the case of Fe^{2+}, its deficiency increases the susceptibility to Pb^{2+} toxicity and in turn, Pb^{2+} inhibits δ-ALA-d enzyme and impairs Fe^{2+} utilization in heme biosynthesis. Finally, selenium influences Pb^{2+} absorption, being also the cofactor for the antioxidant enzyme GPx, enhancing GSH availability which ultimately leads to ROS neutralization. In this regard, N-acetylcysteine (NAC) supplementation can improve the Pb^{2+}-induced oxidative stress in blood and tissue (including the brain), the burden of Pb^{2+} on the body, and molecular alterations in DNA (Sharma et al., 2014).

3.1.2. Vitamins and carotenoids: ascorbic acid (vitamin C) is a low molecular mass antioxidant that binds to and removes Pb^{2+}, increases Pb^{2+} elimination, and reduces Pb^{2+}-induced lipid peroxidation by quenching ROS together with metal chelation properties. In a study performed in battery manufacturing workers, vitamin C supplementation during one month reduced lipid peroxidation and nitrite formation and enhanced the erythrocytes SOD and CAT activity, although these effects were evident in the absence of a reduction in blood Pb^{2+} levels (Ghanwat et al., 2016). Vitamin E (α-tocopherol) on the other side, is the generic term used to describe several naturally-occurring compounds that possess the biological activity of α-tocopherol, although is more effective in combination with other antioxidant agents than alone. Vitamin B6 (pyridoxine) has antioxidant effects by promoting GSH generation while its deficiency inhibits GSH biosynthesis by limiting cysteine availability. Finally, β-carotenes prevent lipid peroxidation and increase antioxidant enzymatic activity.

In support of these shreds of evidence, experimental studies revealed that alterations in antioxidant enzyme activities and the increase in oxidant production and lipid peroxidation levels in the brain in chronically- Pb^{2+}-intoxicated rats during gestation and lactation were prevented by simultaneous treatment with vitamins A, C, E, B6 and Zn^{2+} (Antonio-García and Massó-Gonzalez, 2008b). Moreover, combined vitamin E and garlic oil administration act as detoxifying agents and antioxidants by scavenging free radicals, associated to the independent action of garlic oil on the removal of Pb^{2+} salt as PbS (Sajitha et al., 2010).

3.1.3. Flavonoids and polyphenols: Flavonoids are naturally polyphenolic compounds present in several foods and beverages that exhibit both antioxidants and chelating properties due to its molecular structure. Alpha-lipoic acid employs a two steps antioxidant activity: first by attacking ROS and preventing the lipid peroxides formation, and second, because it can replenish and regenerate other antioxidants like vitamin C and E. It is frequently used in combination with chelating agents like 2,3-dimercaptosuccinic acid (DMSA). In this regard, a beneficial role of lipoic acid was demonstrated when administered with DMSA/Mi-ADMSA (mono isoamyl meso-2,3-dimercaptosuccinic acid) treatment, due to the effective reversal of altered parameters indicative of oxidative stress compared to monotherapy in acute-Pb^{2+}-intoxicated rats (Pande and Flora, 2002). In the same line, the beneficial effects of lipoic acid on oxidative stress parameters in rats are not related to its ability to remove Pb^{2+} from target cells as a chelator but rather associated with its thiol antioxidant capacity (Gurer et al., 1999). Quercetin, on the other side, suppressed Pb^{2+}-induced toxicity by reducing oxidative stress, downregulating Hsp-70 and Bak and upregulating Bcl-2 in the rat brain, revealing the involvement of intrinsic pathways in Pb^{2+}-mediated neuronal cells death (Chander et al., 2014). Moreover, quercetin administration decreased Pb content in blood and brain, markedly increased NO production and PKA activity in the brains of Pb-treated mice, and suppressed Pb^{2+}-induced oxidative stress accompanied by an increase in protein kinase B (Akt), calcium/calmodulin kinase II (CaMKII), eNOS, nNOS, and CREB phosphorylation in mice brains, all parameters known to be inhibited by Pb^{2+} (Liu et al., 2013). Morin is another bioflavonoid that has shown antioxidant and also anti-inflammatory properties. Pb^{2+} exposure enhanced the Bax/Bcl-2 ratio, facilitated cytochrome c release from the mitochondria resulting in neuronal cell death, all events reversed with morin (Thangarajan et al., 2018). In addition, luteolin is a glycosylated flavonoid compound that is demonstrated to attenuate neuronal damage induced by Pb^{2+} through the inhibition of the oxidative damage, neuroinflammation, and the cortical cell death in adult rats chronically exposed to Pb^{2+} (Baty et al., 2020). Moreover, treatment with melatonin can improve motor deficits and oxidative stress protecting the cerebellum against developmental Pb^{2+}-exposure (Bazrgar et al., 2015). In addition, *in vivo* pharmacological activation of TrkB receptors by small molecules such as 7,8-dihydroxyflavone can reverse long-term effects of chronic Pb^{2+} exposure on presynaptic terminals, pointing to BDNF-TrkB receptor activation as a promising therapeutic intervention in Pb^{2+}-intoxicated children (Zhang et al., 2018). Tannic acid is another naturally occurring plant polyphenol with antioxidant, anticancer, and anti-inflammatory effects with chelator properties for metals, including Pb^{2+}. In this respect, its administration restored antioxidant status and histopathological alterations in the rat's brain induced by chronic Pb^{2+} treatment during adulthood (Ashafaq et al., 2016). Levo-carnitine (L-carnitine)

is a water-soluble antioxidant located on the mitochondrial membrane that facilitates the transport of long-chain fatty acids used for the production of energy through the mitochondrial membrane. Its treatment counteracted the effect of Pb^{2+} in rats, reducing the production of ROS and scavenging free radicals by maintaining and protecting the level of the antioxidant enzymes SOD, CAT, and GPx (El-Sherbini et al., 2017). Ferulic acid, a phenolic acid present in aliments abrogated Pb^{2+}-induced ROS generation, lipid peroxidation, and protein carbonyls in the rat's cerebellum and hippocampus, while perturbations in the GSH levels and activity of enzymatic antioxidants were also markedly restored (Kumar and Muralidhara, 2014). Ferulic acid treatment significantly, although not completely, protected the cells and could be blocked by Zn^{2+} protoporphyrin (Zn-PP), and Nrf2 shRNA (Yu et al., 2016). Catechins, on the other side, are polyphenolic compounds contained in green tea, and strong scavengers against $O_2^{\bullet -}$, H_2O_2, $OH^{\bullet}$, and NO that resulted in PC12 cells treated by Pb^{2+} decreased intracellular Ca^{2+} level and ROS formation as well as improved MMP (Chen et al., 2003). Thymoquinone, which is extracted from *Nigella sativa* seeds (commonly known as Black seed), possesses therapeutic effects including, antibacterial, anti-inflammatory, and antioxidant properties. Several evidence demonstrate that this compound greatly improves Pb-induced neurotoxicity in early life and provides neuroprotective and antioxidant characteristics in several mouse brain regions (Butt et al., 2018) and improvement of SOD, CAT, and GPx activity and MDA levels in the maternal and fetal brains (Saleh et al., 2019).

In summary, essential elements mainly alter Pb^{2+} biokinetics both at absorptive and enzymatic sites, whereas vitamins, flavonoids, and polyphenols largely act as antioxidants in preventing toxicity via rebalancing the impaired oxidant/antioxidant balance disrupted by Pb^{2+} exposure. However, caution should be used as an overdose of some nutrients that may overcome their beneficial effects and produce adverse health effects (Ahamed and Siddiqui, 2007).

3.2. AMELIORATION BY CHELATORS

Numerous evidences indicate that metal toxicity to humans can be treated with human-relevant chelating agents. An ideal chelating agent possesses characteristics like great affinity for the toxic metal, high solubility in water, ability to cross cell membranes, the possibility to oral administration, and low metabolism. A chelating agent forming a stable complex with a toxic metal may shield the metal ion from biological targets, thereby reducing the toxicity, or it may expose the metal to the biological environment, prevent it from being scavenged by biological protective mechanisms and thereby increase the toxicity of the metal (Carocci et al., 2016). In addition to their heavy metal chelating properties, most of these agents have a dithiol group that may act as an O_2 radical scavenger and thus inhibit lipid peroxidation (Gurer and Ercal, 2000). Chelating agents are divided into prophylactic chelators, used as a preventive treatment, and therapeutic chelators, used as a treatment for Pb^{2+} intoxication. The recommended chelator treatment for both acute and chronic Pb^{2+} intoxication includes: DMSA (succimer), 2,3-dimercaptopropane-1-sulfonate (DMPS), British Anti-Lewisite (BAL) or 2,3-dimercaprol, calcium disodium ethylenediaminetetraacetate ($CaNa_2EDTA$) and D-penicillamine (DPA) (Bjørklund et al., 2017). Penicillamine was the first chelating agent used in the treatment of Pb^{2+}poisoning;

BAL on the other side, is rarely used due to the narrow therapeutic window and severe side effects. DMSA is the most efficient and safe chelating agent for Pb^{2+} exposure while DMPS is less effective than $CaNa_2EDTA$ or DMSA. In turn, $CaNa_2EDTA$ has side effects because contributes to a greater loss in essential minerals and redistributes Pb^{2+} to the brain (Kim et al., 2015). The following chelators are under experimentation: ascorbic acid, vitamin B, racemic DMSA, and Mi-ADMS (Blanusa et al., 2012). Besides, some ionophores have been shown to transport Pb^{2+} across the cell membrane, providing a novel method for reducing the body burden of the metal. Additionally, macrocyclic decadentate or octadentate ligands are suited for the complexation of large metal ions like Pb^{2+}, whereas picolinate ligand is designed to accommodate the Pb^{2+} ion pair leading to high stability and selectivity (Thuppil and Kaushik, 2012). Finally, herbal compounds are under study, with garlic as a medicinal plant attributed to remarkable therapeutic properties. Its active agent is allicin, which prevents oxidative stress by chelating lead ions and scavenging free radicals (Parsi et al., 2020).

In the first place, the increased ROS, nNOS, and $[Ca^{2+}]i$ along with altered behavioral abnormalities that were supported by changes in neurotransmitter levels as well as a fall in mitochondrial membrane potential, release of cytochrome c, and altered Bcl2/Bax ratio reported in adult rats as a consequence of Pb^{2+} exposure, were all reverted towards normal levels following combination therapy over monotherapy with $CaNa_2EDTA$ or MiADMSA (Flora et al., 2007). In addition, a group of studies performed in developing rats reported an appreciable recovery of Pb^{2+}-induced oxidative stress in several synaptic processes in the brain after ascorbate treatment, suggesting its potential as a therapeutic strategy against early-life Pb^{2+} exposure (Ahmad et al., 2018; Ahmad et al., 2018; Ahmad et al., 2020). In line with these evidences, concomitant administration of ascorbic acid and Pb^{2+} reduced Bax protein and increased Bcl-2 in developing rat hippocampus and decreased Pb^{2+} level in the blood of dams compared with Pb^{2+}-treated only dams, revealing protective effects of ascorbic acid on Pb^{2+}-mediated apoptosis (Han et al., 2007). Further evidence revealed a significant increase in plasma MDA and total oxidant status in Pb-intoxicated rats compared to the control group, while ascorbic acid increased the total antioxidant capacity in these animals and ameliorated the Pb-induced impairment of synaptic plasticity in the hippocampus via its antioxidant activity (Karamian et al., 2015). Furthermore, a group of evidence demonstrated that ascorbic acid treatment during pregnancy can prevent Pb^{2+}-induced impairments in the cerebellar development in rats via the protection of inhibitory neurons and synapses (Nam et al., 2019a, 2019b). On the other hand, Parsi et al. (2020) concluded that garlic is as effective as DPA in significantly reducing blood Pb^{2+} levels, an amelioration effect that showed fewer side effects and more clinical improvement than DPA (Parsi et al., 2020). Interestingly, *Spirulina maxima* are more effective than garlic in reducing oxidative stress and lipid peroxidation parameters as well as caspase-3 gene expression in adult rat brain tissue (cerebrum and cerebellum) (Galal et al., 2019).

Although most therapeutic approaches seek to increase Pb^{2+} excretion by chelation, it should be noted that available chelators can induce adverse health effects and its efficacy in reversing or preventing toxic effects is questionable. Thereby, the search for new substances and therapeutically-safe regimens, including combined chelator administration, is still necessary.

3.3 AMELIORATION BY COMBINATORIAL CHELATING/ANTIOXIDANT THERAPY

When antioxidants are combined with chelating agents, they show a synergism that improved chelating ability. The combination of DMSA with NAC, vitamin E or alpha-lipoic acid was found to have a synergistic effect in preventing oxidative damage. Zinc, at moderate doses, was effective at increasing the chelating ability of $CaNa_2EDTA$. From these assertions, it is evident that antioxidants can be used in standard doses in conjunction with chelating agents with beneficial effects on Pb^{2+} toxicity (Patrick, 2006b). Multiple studies have demonstrated the effectiveness of coadministration of antioxidant nutrients or essential metal with a chelating agent like $CaNa_2EDTA$ or DMSA or the antioxidant alone. Ercal et al, in 1996 demonstrated that pharmacologic interventions with DMSA, which encompass both chelating as well as thiol-mediated antioxidant functions, reduced both, Pb^{2+} levels in blood and brain as well as indices of oxidative stress (Ercal et al., 1996). Treatment with either NAC (a thiol antioxidant) or succimer (a chelating agent) reversed lead-induced alterations in MDA and GSH content, but only succimer appeared to partially restore δ-ALA-d activity (Gurer et al., 1998). S-adenosyl-L-methionine, the GSH precursor, reduced Pb^{2+} concentrations in the brain and had beneficial effects in preventing alterations in some biochemical variables, including brain MDA in animals acutely-administered with Pb^{2+} (Flora and Seth, 1999). The combined administration of NAC and DMSA or MiADMS restored the parameters indicative of Pb^{2+}-induced oxidative stress in rats and decreased the Zn^{2+} levels that were elevated in response to Pb^{2+}-exposure. The authors concluded that the ideal treatment for Pb^{2+} intoxication seems to be a combination of a chelator and an antioxidant (Tandon et al., 2002). Nevertheless, in a study performed in Pb^{2+}-poisoned opioid addicts, NAC treatment recovered their antioxidant capacity, although the advantages of NAC in the improvement of DPA efficacy were not evident (Shojaeepour et al., 2018).

With respect to natural herbal compounds, curcumin is the main active constituent of turmeric rhizome *(Curcuma longa)* with strong anti-inflammatory and antioxidant properties as a radical scavenger and metal chelator against Pb^{2+} intoxication, reducing the metal levels in rat brain. Although its low aqueous-solubility and rapid metabolism result in poor systemic bioavailability, restricting its oral use, nanocurcumin (curcumin encapsulated in chitosan nanoparticles) has good bioavailability, chelating property, and longer retention time than curcumin (Pal et al., 2015). In corroboration, a recent study performed in adult rats chronically exposed to Pb^{2+}, curcumin attenuates Pb-induced neurotoxicity via inhibition of oxidative stress associated with its chelating activity (Abubakar et al., 2019).

3.4. AMELIORATION BY OTHER COMPOUNDS

Gangliosides are sialic acid-containing glycosphingolipids that are part of cell membranes and abundantly expressed in the CNS. They have referred to as being neuroprotective against Pb^{2+} induced injuries in the rats' hippocampal neurons, evidenced as a reduction of cell viability and abnormal autophagic processes (Meng et al., 2016). Olive leaf extract, on the other side, is protected against Pb^{2+}-induced brain damage through apoptosis, oxidative stress, and inflammation inhibition (Seddik et al., 2011). The naturally-occurring cationic polysaccharide, chitosan prevents lipid oxidation in biological systems by scavenging free radical and inhibiting ROS generation. After Pb^{2+} exposure, GSH and total thiols decreased, while protein oxidation, GSSG, MDA, and ROS increased in blood and tissues compared

with the control group. Its administration removed Pb^{2+} from blood and tissues and reversed partially or totally the alterations in the biochemical variables (Wang et al., 2016). In other studies, tert-butylhydroquinone, an Nrf2 and phase II detoxification enzyme inducer protects against oxidative stress and cell death through the Nrf2/HO-1 (heme oxygenase-1) pathway resultant of Pb^{2+} toxicity *in vivo* and *in vitro* (Ye et al., 2016). In this aspect, it was indicated that hemin administration in SH-SY5Y cells attenuates Pb^{2+}-induced cell death and oxidative stress, providing protection against neurotoxicity by HO-1/carbon monoxide activation (Ye et al., 2018).

4. CONCLUSION

This collective evidence indicates that Pb^{2+} is a compelling neurotoxicant with not a single well-established molecular mechanism that completely explain its neurotoxicity. Instead, from the vast literature of *in vitro*, *in vivo,* and clinical studies available one may conclude that a plethora of effects arise in response to exposure to this metal. Undeniably, Pb^{2+}-associated neurotoxicity is multifactorial in nature and encompasses divalent cation mimicry and oxidative stress as major causes of neuronal cell death. Overall, all the proposed mechanisms discussed above congregate, interrelate, entangle, and lead to neurotoxic manifestations when the compensatory responses are overcome (see Figure 1). As reviewed, Pb^{2+} exposure results in ROS accumulation and a decrease in antioxidants. This metal is able to bind to GSH sulfhydryl groups and restrict its ability as a ROS scavenger. In addition, increased lipid peroxidation and decreased mitochondrial antioxidant enzymatic activities are serious consequences of Pb^{2+} toxicity. Thus, the pro-oxidant microenvironment contributes to higher BBB permeability, inducing oxidative damage to cellular molecules, the activation of inflammatory mediators, and damage of tight junctions. Additionally, ROS and RNS overproduction can be secondary to intracellular hypercalcemia, as Ca^{2+} stimulates enzymes that generate ROS and/or RNS by activating dehydrogenases in the citric acid cycle, a process that accelerates hydrogen output, which in turn increases the electrons flux along with the ETC, an event that increases ROS formation. In turn, Ca^{2+} activates proteases that convert xanthine dehydrogenase into xanthine oxidase, another important source of ROS. Finally, neurons and endothelial cells constitutively express nNOS and eNOS, respectively, both enzymes activated by Ca^{2+}, a process that further promotes a high reactivity between NO and ROS. Thereby, Pb^{2+} can perturb Ca^{2+}-dependent functions by direct interference with Ca^{2+} transport, storage, or Ca^{2+} binding sites, or by indirectly altering cell functions required for Ca^{2+} homeostasis. In any case, the Pb^{2}+/Ca^{2+} but also Zn^{2+}, Fe^{2+}, and eventually Cu^{2+} interactions lead to important functional impairments in organelles, cells, tissues, and organ systems intoxicated with Pb^{2+}. Eventually, a presumed sequence of events may start with the rise in $[Ca^{2+}]_i$ that first triggers intrinsic generation of mitochondrial dysfunction and oxidative damage that altogether promotes apoptotic neuronal death.

Certainly, early-life environmental exposures make the brain vulnerable to an adverse priming event. Similarly, late-life vulnerabilities suggest that environmental toxicants may play an important role in the dysfunctions commonly observed in aging and diseases of aging. Accordingly, a better understanding of an organism response to these adverse molecular events may converge to propose corrective approaches for the prevention of Pb-

mediated neurotoxicity. The evidence of ameliorative effects of antioxidants, anti-inflammatory compounds, or chelators alone or in combination may emerge as a new perspective for therapeutic interventions against Pb-induced neurotoxicity.

Thus, in spite of the presumed allostatic processes that may take place as a result of synaptic plasticity or eventual ameliorative approaches, it should be recognized that Pb^{2+} alterations may cause enduring changes in the brain that affect the individual performance at the behavioral, emotional, intellectual, and social level. This is particularly true when exposure occurs during development, making both scenarios not mutually excluding. As Dr. Silbergeld concludes her excellent article: "early-life Pb^{2+} detrimental effects may reverse, persist, be progressive or remain unmasked until a new event challenges the brain allowing them to reemerge". Thus, the research community is encouraged to move on and pursue additional studies to better understand the toxic mechanisms associated with Pb exposure during all life-stages, acknowledging that additional information may also provide insight into therapeutic modalities for Pb poisoning.

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"TWO DISTINCT FORMS OF LEAD NEUROTOXICITY"

As Dr. Silbergeld proposed almost 30 years ago, *"The complexity of lead neurotoxicity in exposed humans and the interactions of age with dose suggest there may be several mechanisms of toxicity, and at least two distinct forms of lead neurotoxicity, involving specific mechanisms and resulting in specific expressions of altered neuronal function. Both expressions of neurotoxicity may occur at the same time within an organism at a given exposure, but the long-term consequences are likely to differ. Basically, I propose that lead exerts neurotoxic effects in the following distinct ways: first, as a neurodevelopmental toxicant, interfering with the hard wiring and differentiation of the CNS; second, as a neuropharmacological toxicant, interfering with ionic mechanisms of neurotransmission"* (Silbergeld, 1992).

This chapter hones on the second proposal, which is to elucidate the molecular mechanisms behind lead (Pb^{2+}) effects in the brain, including those on transmitter release and signal transduction, resulting from the presence of Pb^{2+} *in situ* in the synapse. To this end, a special emphasis was directed to theories of ion mimicry, mitochondrial dysfunction, redox imbalance, and neuroinflammation. Most importantly, the crosstalk among all these factors is proposed as the confluent mechanism of toxicity. These alterations were thought to be potentially reversible if the metal was removed from the synapsis microenvironment as the result of the cessation of the exposure or as a consequence of amelioration interventions, including chelators, antioxidants substances, anti-inflammatory drugs, or their combination as integrated approaches to mitigate Pb^{2+} detrimental effects.

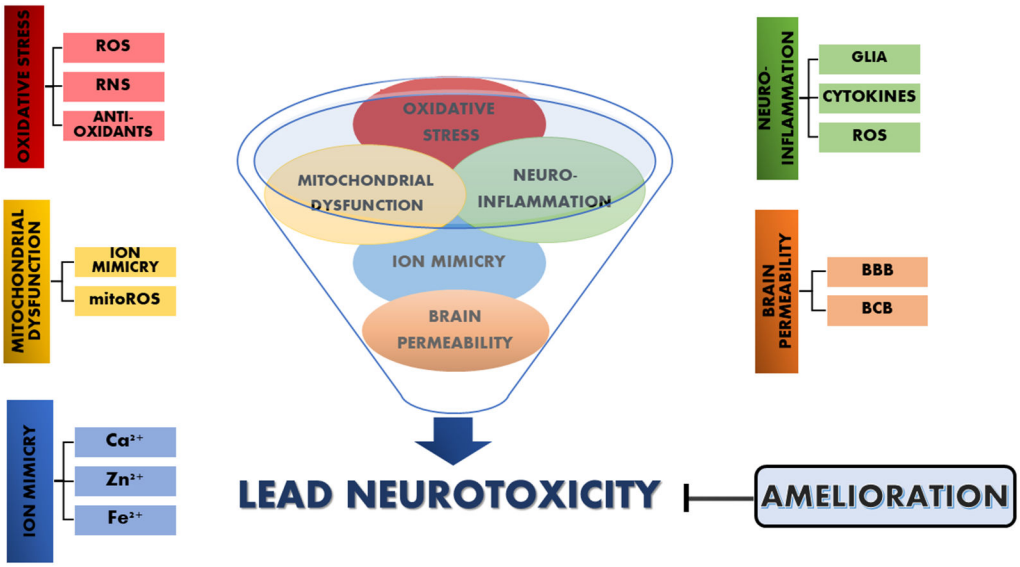

Figure 1: Mechanisms implicated in lead neurotoxicity

The funnel represents the confluent nature of the discussed mechanisms, that alone or in combination may trigger Pb^{2+} neurotoxicity. See the text for details on amelioration strategies and on key players participating in each mechanism (depicted on both sides of the figure). ROS, reactive oxygen species; RNS, reactive nitrogen species; mitoROS, mitochondrial-generated ROS; Ca^{2+}, calcium; Zn^{2+}, zinc; Fe^{2+}, iron; BBB, blood-brain barrier; BCB, blood-cerebrospinal fluid barrier.