BioFactors

Iron in neuronal function and dysfunction

*Gabriela A. Salvador**

Instituto de Investigaciones Bioquímicas de Bahía Blanca, Universidad Nacional del Sur and Consejo Nacional de Investigaciones Científicas y Técnicas, Bahía Blanca, Argentina

Abstract.

Iron (Fe) is an essential element for many metabolic processes, serving as a cofactor for heme and nonheme proteins. Cellular iron deficiency arrests cell growth and leads to cell death; however, like most transition metals, an excess of intracellular iron is toxic. The ability of Fe to accept and donate electrons can lead to the formation of reactive nitrogen and oxygen species, and oxidative damage to tissue components; contributing to disease and, perhaps, aging itself. It has also been suggested that iron-induced oxidative stress can play a key role in the pathogenesis of several neurodegenerative diseases. Iron progressively accumulates in the brain both during normal aging and neurodegenerative processes. However, iron accumulation occurs without the concomitant increase in tissue ferritin, which could increase the risk of oxidative stress. Moreover, high iron concentrations in the brain have been consistently observed in Alzheimer's disease (AD) and Parkinson's disease (PD). In this regard, metalloneurobiology has become extremely important in understanding the role of iron in the onset and progression of neurodegenerative diseases. Neurons have developed several protective mechanisms against oxidative stress, among them the activation of cellular signaling pathways. The final response will depend on the identity, intensity, and persistence of the oxidative insult. The characterization of the mechanisms involved in high iron induced in neuronal dysfunction and death is central to understanding the pathology of a number of neurodegenerative disorders.

Volume 36, Number 2, March/April 2010, Pages 103–110 •
E-mail: salvador@criba.edu.ar

Keywords: iron, neuron, signal transduction, neurodegenerative diseases

1. Introduction

Iron is the most common cofactor within the oxygen-handling biological machinery mainly due to its ability to participate directly as a donor or an acceptor in electron transfer reactions. For this reason, iron is considered an essential trace element for cell function. The distinguishing property that enables iron to participate in oxygen metabolism also explains its potential damaging effects; consequently, cell iron content must be properly handled by the cell [1].

Although total body content of iron is about 4 g in an adult male, erythropoiesis, the most relevant iron-requiring physiological event, utilizes about 0.02 g [2,3]. The amount of iron needed by an adult male can be obtained by absorbing 1–2 mg of iron. In this context, mechanisms that participate in iron conservation and recycling are essential because typical human diets contain just enough iron to replace small losses [1].

The brain particularly needs iron for multiple physiological processes. Iron is a cofactor for tyrosine hydroxylase, tryptophan hydroxylase, xanthine oxidase, and ribonucleoside reductase [4,5]. Fe also participates in myelination, mitochondrial energy generation, and DNA replication/cell cycling [1]. The uptake pathway of brain iron starts in the intestines where dietary Fe^{3+} (ferric iron) is reduced by duodenal cytochrome B to Fe^{2+} (ferrous iron). The divalent metal transporter (DMT) can carry Fe^{2+} across the duodenal epithelium into the blood. Ceruloplasmin or hephaestin regulates iron homeostasis in the blood, by oxidizing Fe^{2+} to Fe^{3+} and promoting its binding to the predominant serum iron carrier, transferrin [1,6–8]. Transferrin-iron circulating in the blood outside of the central nervous system (CNS) cannot directly cross the blood–brain barrier (BBB). The most common pathway for iron transference across the BBB is

Abbreviations: AP-1, activator protein-1; AD, Alzheimer Disease; BER, base excision repair; BBB, blood brain barrier; DMT, divalent metal transporter; DMT1, divalent metal transporter 1; HOs, heme oxygenases; HPxR, hemopexin receptor; IA, iron accumulation; ID, iron deficiency; IRPs, iron regulatory proteins; IRE, iron responsive elements; LIP, labile iron pool; Lf, lactoferrin; Lfr, lactoferrin receptors; MAPK, mitogen-activated protein kinase; NF-κB, nuclear factor kappa B; PD, Parkinson's disease; ROS, reactive oxygen species; Tf, transferring; Tfr, transferrin receptors; βA, β amyloid peptide.

*Address for correspondence: Gabriela A. Salvador, Ph.D., Instituto de Investigaciones Bioquímicas de Bahía Blanca, Universidad Nacional del Sur and Consejo Nacional de Investigaciones Científicas y Técnicas, CC857, B8000FWB Bahía Blanca, Argentina. Tel.: +54-291-4861201; Fax: +54-291-4861200; E-mail: salvador@criba.edu.ar.

Received 31 January 2010; accepted 31 January 2010
DOI: 10.1002/biof.80
Published online 15 March 2010 in Wiley InterScience (www.interscience.wiley.com)

through transferrin receptors on brain endothelial cells, which bind iron circulating in the form of transferrin that then enters the brain by endocytosis. Other transporter systems, such as DMT and the lactoferrin receptor, also participate in delivering iron across the BBB [7,9]. Lactotransferrin receptor (Lfr) has also been localized in some human neurons and its expression increases in the midbrain of patients with Parkinson's disease (PD) [10]. Lfr in neurons serve in the transferrin-independent pathway to transport iron from iron-containing lactoferrin across neuronal membranes (Fig. 1). The transferrin-receptor mediated pathway also participates in iron transport within the CNS (i.e., into various cell types). The amount of iron taken up and stored by the cells exclusively depends on the level of the transferrin receptor expression and its ligand [5,9,11–13]. This phenomenon is controlled at the post-transcriptional level by iron regulatory proteins (IRPs) that interact with iron responsive elements (IREs) in mRNA, thus regulating the expression of ferritin and the transferrin receptors in brain endothelial cells, neurons, glia, and oligodendrocytes [5,9,11–13]. A decreased iron concentration in the extracellular millieu promotes the binding of IRPs to IREs to increase the stability of the transferrin receptor and decrease the expression of ferritin. This mechanism allows an efficient utilization of iron by the cell, with iron not being bound to ferritin first [13].

In the brain, ferritin is the most common iron-storage protein (Fig. 1). Ferritin posses two different subunits, the heavy (H) and light (L) chains. H-ferritin is found in organs with high iron utilization and little iron storage, whereas L-ferritin is found in organs with elevated iron storage [12,14]. Immunohistochemical studies demonstrate that iron, ferritin, and transferrin are present in extensive brain regions. Oligodendrocytes show the highest staining for iron, ferritin, and transferrin, followed by microglia in the cerebral cortex and astrocytes in the basal ganglia [5,11,15]. In neurons, although ferritin presents a variable and inconsistent staining, neuromelanin staining is the predominant iron storage protein detected [16,17]. In this aspect, neuromelanin is found in high concentrations in the substantia nigra and locus ceruleus, and it has been proposed as the main chelator of neuronal iron. Experiments carried out by Zecca and coworkers suggest that neuromelanin acts to reduce potentially toxic iron by chelating the free metal found in the cytosol of neurons [16,17].

Neurons can take up transferrin-bound iron because they contain both transferrin receptors (Tfr) and divalent metal transporter 1 (DMT1) [18,19]. The expression of Tfr is potently upregulated during iron deficiency (ID), this fact explains the role of this receptor on iron uptake [20,21]. Neurons also express ferroportin, which probably functions as a carrier for the excretion of nonrequired iron (Fig. 1). Ferroportin neuronal labeling was detected in perikarya, axons, and dendrites. The labeling intensity within the neurons varies among the different ages. The content of ferroportin was more pronounced in the neuronal axons of the developing brain suggesting that elevated axonal transport and export of iron are related to high metabolic rate in the developing brain [20,21]. Iron accumulation (IA) has been shown to lead to cell damage. Ferritin-bound iron is considered harmless because, in this complex, the metal is not able to react with surrounding molecules. It has been proposed that the iron–ferritin complex in neurons is assembled in the cell body and then transported along axons. This transport could be interrupted in disease processes or normal aging which possibly represent one cause of IA [22].

As a consequence of its active metabolic rate, the brain consumes a high fraction of the total body oxygen that generates large amounts of reactive oxygen species (ROS). Altogether, high oxygen consumption, elevated concentrations of polyunsaturated fatty acids, and the nonregenerative nature of neurons make the brain highly vulnerable to oxidative damage [23–25]. Although brain antioxidant defenses function properly during most of human life, a number of

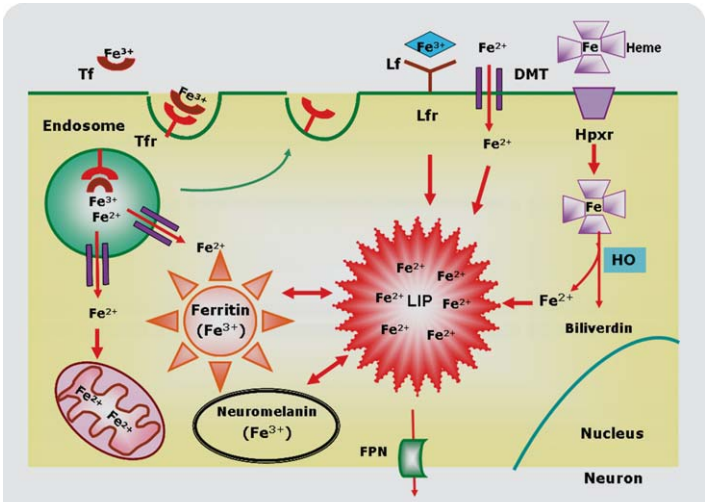

Fig. 1. Iron metabolism pathways in neurons. Iron bound to extracellular transferrin can be transported into neurons through transferrin receptors (Tfr). Intracellularly, iron bound to transferrin/Tfr is located in endosomes. Lactoferrin receptors (Lfr) in neurons provide a transferrin-independent pathway to transport iron from iron containing lactoferrin across the cell membranes. Iron can also reach the neuronal body through its transport by divalent metal transporter 1 (DMT1). Intracellular iron can also be generated after heme degradation by constitutive hemoxygenase-2 (HO-2) present in neurons. Extracellular heme can enter into neurons by way of the hemopexin receptor (HPxR). This iron is likely to be immediately bound to H-ferritin, the predominant isoform in neurons. Iron can also accumulated bound to neuromelanin in dopaminergic neurons. The cellular labile iron pool (LIP) is a pool of chelatable and redox-active iron. Ferroportin (FPN) that transports iron outside the neuron. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

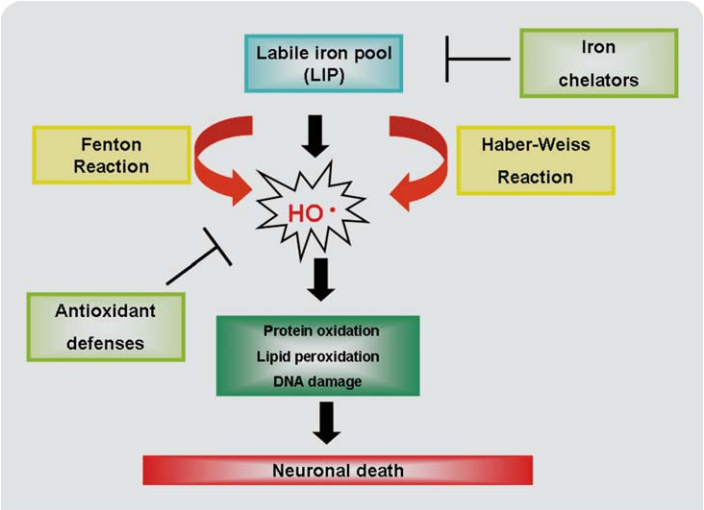

Fig. 2. Chemistry of labile iron pool in the neuron. Free iron (Fe^{2+}) or labile iron pool (LIP) reacts with hydrogen peroxide in the Fenton reaction, leading to the production of the very damaging and reactive hydroxyl radical ($HO^{\bullet}$). Superoxide ($O_2^{\bullet}-$) can also react with ferric iron (Fe^{3+}) in the Haber-Weiss reaction to produce Fe^{2+} thus initiating redox cycling. An imbalance in the hydroxyl radical ($HO^{\bullet}$) production leads to protein and DNA oxidation and lipid peroxidation, causing neuronal death. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

neurodegenerative processes involving IA are associated with the aging process [18].

Free iron interacts with molecular oxygen, generating ROS through Haber-Weiss and Fenton reactions [26,27] (Fig. 2). Uncontrolled ROS production leads to the oxidation of cellular components, a condition termed "oxidative stress." Intracellular redox-sensitive targets of ROS include protein phosphatases, protein kinases, and transcription factors. Oxidative injury induces lipid peroxidation and impairment of glutamate and glucose transport, mitochondrial dysfunction, elevation of intracellular free calcium concentration, and finally, cell dysfunction and death (Fig. 2) [25,28].

Although IA causes neuronal oxidative damage, ID generates neuronal function impairment. The ID during early brain development leads to several deficits, many of which turn out to be irreversible without iron supplementation [29]. Both ID and IA cause neuronal impairment and cell death. This review will focus on the description of the molecular events that operate in neurons under iron overload as well as on iron depletion.

2. Iron overload and neuronal dysfunction

Multiple evidence highlights the involvement of IA in the onset of neurodegenerative processes [30,31]. An age-related increase in the iron content of different areas of the brain was demonstrated in rat, monkey, and humans [32,33]. It has been also documented that iron accretion in redox highly oxidant-sensitive tissues, such as substantia nigra, occurs without the concomitant increase in tissue ferritin [34]. As previously stated, augmented concentrations of non-ferritin-bound iron increase the risk of oxidative stress. Another important mechanism involved in free iron generation is the degradation of heme catalyzed by the heme oxygenases (HO). Whereas *HO-2* expression is constitutive in normal brain, the *HO-1* gene is highly sensitive to induction by a wide range of pro-oxidants and other stressors. After intraperitoneal injection with ferric nitrilo-triacetate in adult rats, *HO-1* expression is induced in both the cortex and hypothalamus [35].

Neurons displays an active system to regulate iron content. Experiments done in hippocampal and neuroblastoma cells demonstrated that during iron overload, cells upregulate the expression of ferritin and ferroportin and decrease the expression of DMT1 [36]. However, the mechanisms underlying DMT1 regulation in the brain remain unclear. It has been shown that the overexpression of human DMT1 and IREs in dopaminergic neurons causes an increased intracellular IA and, concomitantly, oxidative stress leading to cell apoptosis [37]. In human neurons, DMT1 binds to the adaptor protein Ndfip 1 (Nedd4 family-interacting protein 1), and this binding promotes its ubiquitination in response to iron exposure [38]. This interaction results in DMT1 degradation binding to reduced metal uptake. Induction of Ndfip1 expression protects neurons from metal toxicity and removal of Ndfip1 by shRNAi results in increased sensitivity to metals [38]. Additionally, Ndfip1(−/−) knockout mice accumulate Fe within neurons [38].

Mitochondrial oxidative stress is one of the main contributing factors in the etiology of neurodegenerative diseases. Recent studies provide strong evidence about the role of mitochondrial-aconitase as a source of neurotoxic Fenton reactants (H_2O_2 and Fe^{2+}). The treatment of primary midbrain cultures with exogenous oxidative stress via paraquat resulted in a time- and concentration-dependent increase in H_2O_2, $Fe^{2+,}$ and cell death [39]. This paraquat-induced increases in H_2O_2, $Fe^{2+,}$ and cell death were exacerbated in neurons overexpressing m-aconitase. Concomitantly, the removal of Fenton reactants using a mitochondrial permeable iron chelator and catalase ameliorated cell death [39].

Iron, like other positively charged transition metals, also compromises genomic DNA integrity. Transition metals can bind directly to DNA, and they also alter basic properties of repair proteins including, catalytic activities, DNA binding, stability, and compartmentalization. In the brain, the base excision repair (BER) pathway is central for handling oxidative damaged DNA. Assays *in vitro* demonstrated that DNA repair by BER was inhibited by iron [40]. Moreover, in primary neuronal cultures, the repair of oxidatively damaged genomic DNA was markedly delayed. This suggests that under pathological conditions, which involve deregulation of iron, DNA repair is significantly compromised [40]. Thus, iron overload may adversely affect the neuronal DNA repair

capacity and thereby compromise genomic integrity initiating events that can lead to neuronal dysfunction and death [23].

A number of genetic neurodegenerative diseases such as ferrinopathy and Hallervorden-Spatz syndrome have been associated with mutations in the ferritin light chain gene and the pantothenate kinase gene demonstrating a direct correlation between mutations in iron handling proteins and neurodegenerative processes [41,42]. It has been also demonstrated that in both Alzheimer's disease (AD) and PD, iron levels are elevated in cortex (AD) and in substantia nigra (PD) [16,43].

Elevated nonheme brain iron can be detected *in vivo* by magnetic resonance imaging in patients with AD [44]. The abnormal cellular distribution of iron in AD was also confirmed in postmortem tissues [16]. This IA coupled to the presence of β amyloid peptide (βA) in AD causes oxidative stress, including elevated levels of protein carbonyls, 8-hydroxyguanine, and lipid peroxidation [45]. The critical role of iron in βA neurotoxicity was confirmed in a *Drosophila* model of AD. These studies determined that the iron-binding protein ferritin and the H_2O_2 scavenger catalase are the most potent suppressors of the toxicity of βA peptide (1–42). Likewise, treatment with the iron-binding compound clioquinol increased the lifespan of flies expressing βA (1–42). The effect of iron appears to be mediated by oxidative stress as ferritin heavy chain coexpression greatly reduced carbonyl levels in βA (1–42) flies and restored the survival and locomotion function to normal [46]. The link between iron overload and AD was also confirmed by immunocytochemistry studies in neurons and astrocytes of the cerebral cortex and hippocampus, where HO-1 protein is overexpressed and colocalizes to senile plaques and neurofibrillary tangles [47].

Several genetic mutations associated with iron metabolism such as ferritin-H, IRP2, and ceruloplasmin genes have been found in PD [30]. The connection between IA and the pathogenesis of PD can be explained by the concerted action of monoamine oxidase B and superoxide dismutase. These enzymes produce hydrogen peroxide that reacts with unbound iron thus generating hydroxyl radicals. The generation of higher levels of free radicals can cause the misfolding of α-synuclein protein and the release of iron from neuromelanin. Thus, a decrease in the production or activity of peroxidases can be an upstream factor in iron-generated oxidative stress. Moreover, *HO-1* is markedly overexpressed in astrocytes of the substantia nigra and colocalizes with Lewy bodies in affected dopaminergic neurons. Besides its role in oxidative stress generation, IA may also affect the function of IRP-1 and IRP-2 proteins with the final result of decreasing the storage of iron by ferritin and increasing its cellular import [4,48].

3. Neuronal signaling during iron overload

Besides the regulation of the protein machinery involved in iron metabolism, the activation of death or survival signaling pathways is another mechanism developed by neurons against oxidative stress. The final response will depend on the identity, intensity, and persistence of the oxidative insult.

Neurons incubated with Aβ peptide in the presence of iron shows a marked decrease in protein kinase C isoforms, a reduced Akt serine/threonine kinase activity, Bcl 2-associated death promoter (BAD) phosphorylation, and enhanced p38 mitogen-activated protein kinase (MAPK) and caspase-9 and -3 activation [49–52]. In addition, the iron chelator, deferoxamine, was able to block all the proapoptotic signaling triggered by Aβ-Fe. Moreover, the Aβ peptide alone did not activate proapoptototic signaling. This evidence demonstrates that apoptotic cell death can be only triggered by the presence of iron in an *in vitro* AD experimental model [49–52].

Several studies in the last two decades have demonstrated that the transcription factor nuclear factor kappa B (NF-κB) has become a crucial regulator of cell survival, playing important functions in cellular resistance to oxidants and chemotherapeutic agents. Although the knowledge of NF-κB gene targets in neurons is limited, NF-κB can promote cell survival and synaptic plasticity. NF-κB activity and COX-2 gene transcription are affected in brain regions of patients with AD [51–53]. Oxidative stress may impair NF-κB functions given that membrane lipid peroxidation inhibits NF-κB activity, possibly by a direct interaction of 4-hydroxynonenal with NF-κB subunits. Moreover, oxidative stress caused by Zn deficiency impairs NF-κB activation and translocation in human neuroblastoma cells [54]. To date, no direct correlation between NF-κB activation and iron overload-induced oxidative stress has been established. However, NF-κB and some of their upstream kinases, such as MAPK, phosphatidylinositol 3-kinase, or protein kinases A, C are responsible for the *HO-1* gene expression [55].

Another redox-sensitive transcription factor is the activator protein-1 (AP-1). This transcription factor modulates important cellular processes including the decision of cells to proliferate, differentiate, survive, or die by apoptosis. Strong evidence supports the involvement of AP-1 in oxidative stress signaling in neurons. In rat cortical neurons and astrocytes, H_2O_2 activates MAPKs [56]. Upstream of AP-1, the stress-related MAPKs, c-Jun-N-terminal kinase, and p38 are also activated by increases in the intracellular levels of oxidants [57,58]. MAPKs and AP-1 are implicated in normal physiological functions of the brain. c-Jun, a component of AP-1, has been recently attributed a dual role. Cjun could mediate neurodegeneration and cell death, as well as participate in plasticity and repair mechanisms. In this regard, a link between MAPK pathways and HO-1 expression has been recently established in cortical cell neurons. The MEK inhibitor Uo126 decreased HO activity in cortical cell cultures [59]. Hemoglobin exposure causes widespread neuronal injury, and *HO-1* induction that is decreased by MAPK inhibitors [59]. It is hypothesized that reduction in HO activity may contribute to the protective effect of MEK and ERK inhibitors against heme-mediated neuronal injury [60]. Moreover, studies carried out in rat hippocampus after kainate induced neuronal injury postulated that transcription factors acting on putative AP-1, NF-κB binding sites, or gamma-interferon

responsive elements on the DMT1 promoter may also play a role in up regulating the expression of the transporter. This could promote iron influx into the brain areas undergoing neurodegeneration and might be a factor contributing to neuronal damage after the initial excitotoxic injury [61].

Pioneering work at Mattson Laboratory demonstrates the role of neuronal synapses in oxidative stress signaling in the nervous system [62,63]. Synapses are sites where the first manifestations of neurodegenerative processes occur. Activation of glutamate receptors, which are concentrated in postsynaptic regions of distal dendrites throughout the brain, is believed to play a major role in the neuronal death occurring in stroke, epileptic seizures, traumatic brain injury, AD, PD, and Huntington's disease [51,52,64–66]. Synapse vulnerability to iron-induced oxidative stress has been clearly demonstrated by the presence of membrane lipid peroxidation, impairment of membrane ion-motive ATPases, glucose and glutamate transport, and mitochondrial function [62,63,67–69]. In this regard, several signaling pathways like Erk, phosphoinositide 3-kinase, Akt, and glycogen synthase 3-β are activated in isolated synaptic endings exposed to iron-induced oxidative injury [68,69]. Moreover, several key biochemical events that are known to occur in intact neurons undergoing apoptosis (i.e., exposure of phosphatidylserine on the plasma membrane surface; activation of caspase-3; mitochondrial calcium uptake and ROS accumulation) also occur in isolated synaptosomes exposed to iron-induced oxidative injury [70]. Synapse loss occurring in both acute and chronic neurodegenerative disorders might also involve *in situ* synapses apoptotic cascades that might occur before, or independently of, neuronal death. This hypothesis is supplemented by the degenerative morphological changes in synapses preceding amyloid deposition and neuronal degeneration in aging monkeys; synapse degeneration before neuronal cell body damage in AD; and synaptic plasticity in the striatum during the course of PD [71–73]. However, mechanisms whereby apoptotic events triggered by iron-induced oxidative stress in synapses propagate to the cell body remain to be elucidated.

4. Iron chelation in the treatment of iron-induced oxidative stress

Apart from the oxidative stress hypothesis, it has been well-documented that iron also induces the aggregation of inert alpha-synuclein and beta-amyloid peptides [74]. Thus, oxidative stress, protein aggregation, and active redox iron can be considered promising pharmacological targets for the treatment of AD and PD [74]. In this regard, iron chelation is a potential therapeutic strategy in both oxidative stress and protein aggregation processes.

BBB permeable metals chelators can be used as potential therapeutic agents in the treatment of neurodegenerative diseases [75]. New iron chelators have been recently designed for their potential use in the treatment of PD and AD [76]. This new family of compounds comprises desferrioxamine, clioquinol, and aroylhydrazones. *In vitro* and *in vivo* experiments showed that these compounds can significantly reduce neuronal death caused by oxidative stress in PD models. Desferrioxamine (also known as Desferal) is a hexadentate ligand with a higher binding affinity for Fe^{3+} than Fe^{2+} [77]. Administration of desferrioxamine prevented up to 60% of dopaminergic neurons from death in a rat model of PD [77]. In the same animal model, this chelator displayed neuroprotective actions when the concentration of iron in the substantia nigra was increased [78]. In a dopaminergic cell line desferrioxamine also inhibited iron-induced oxidative damage [79]. Despite these promising results, the main disadvantage of a desferroxiamine therapy is that it cannot cross the BBB, due to the size and hydrophilic character of the molecule, when administered orally [80]. Clioquinol has proved to have beneficial effects in several cellular and animal models of neurodegenerative diseases as well as in patients with AD [81]. This compound is a small lipophilic iron chelator that can cross the BBB and can be administrated orally. It has been used in phase II clinical trials for moderate AD cases [77]. Clioquinol treatment reduces the concentration of iron in the substantia nigra of mice [81]. However, clioquinol is not iron selective, and this may produce unwanted effects such as a decrease in S-adenosylmethionine levels, a sign of vitamin B12 deficiency [77]. Aroylhydrazones are novel lypophilic iron chelators that are still in the early stages of investigation in animal studies.

Other important compounds proposed for the treatment of oxidative stress-triggered neurodegenerative disorders are plant polyphenols. In some polyphenols, antioxidant activities can be attributed to strong iron-binding affinity rather than to direct radical scavenging [82–84]. Significantly, both free polyphenols and polyphenol–metal complexes have been reported to have antioxidant properties [85]. Moreover, polyphenols have also been reported to be successful at affecting metal homeostasis *in vivo*. In this connection, it has been shown that polyphenols baicalin and quercetin can reduce liver damage caused by iron overload in mice [86]. A major limitation of these compounds is their limited capacity to be absorbed at the gastrointestinal tract and subsequently transported through the BBB.

5. Iron deficiency and neuronal dysfunction

Iron deficiency (ID) is the most prevalent micronutrient deficiency in the world. To date, it is still not clear whether the effect of dietary ID on the brain is due to the lack of neuronal iron or to other processes occurring in conjunction with ID (e.g., hypoxia due to anemia that affects neurobehavioral outcomes) [29,33,87].

One of the most characteristic effects of ID is the biochemistry of post-translational events. The failure of iron incorporation into protein structures (e.g., cytochromes or iron-sulfur proteins) results in premature protein degradation and loss of function that could have adverse consequences on the nervous system [29,33,87].

Late infancy is characterized by peak hippocampal and cortical regional development, as well as by myelinogenesis, dendritogenesis, and synaptogenesis in the brain. Several studies demonstrated a high vulnerability of the developing hippocampus to early ID. A significant loss of the neuronal metabolic marker cytochrome c oxidase in the CA1 and CA3 hippocampal areas and in the frontal cortex lobes has been observed in rats with ID [88]. Additionally, several studies demonstrated that the metabolic changes observed in the hippocampus were persistent and long-lasting [87,88]. Dendritic extension and remodeling during synaptogenesis are very active metabolic processes dependent on energy and a high rate of local iron regulation [89]. In this aspect, it was also observed that ID causes alterations in the dendritic structure in CA1 pyramidal cells in the hippocampus. This failure in dendritic structure was related to anomalous protein scaffolding for microtubule extension and retraction in brain regions involved in recognition memory processing [90].

The most prevalent age-risk for ID coincides with key periods in the maturation of neurotransmitter systems. Iron is essential for a number of enzymes involved in neurotransmitter synthesis that include tryptophan hydroxylase (serotonin) and tyrosine hydroxylase (norepinephrine and dopamine). Experiments carried out by several laboratories in rodent models determined the colocalization of iron with dopaminergic neurons, and its participation in dopamine and norepinephrine metabolism including functioning of monoamine transporters and receptors [91]. Moreover during ID, extracellular levels of DA and NE are increased mainly due to the effects on the activity and expression of the all the monoamino transporters [92–95]. The degree of alterations in these parameters have been strongly associated with the degree of ID in the examined brain regions [92–95].

Some of the neurotransmitter alterations related to early ID have been related to the neuronal surface protein Thy1. In a rodent model of dietary iron-deficiency, Thy1 was decreased in the developing brains of ID rats [96]. Thy1 deficit could affect the release of neurotransmitters and synaptic efficacy contributing to a variety of aberrant neuron to neuron communications [96].

Conditional knockout mice for DMT1 in hippocampal neurons displayed lower hippocampal iron content; altered developmental expression of genes involved in iron homeostasis, energy metabolism, and dendrite morphogenesis and morphology; reductions in markers for energy metabolism and glutamatergic neurotransmission; and presented altered spatial memory. This conditional knockout mice constitute the first model of iron uptake in the brain and proves the role of ID in hippocampal neuronal development and spatial memory behavior [97]. Epidemiological studies in human infants showed that ID is associated with delayed maturation and lengthened central conduction times in auditory evoked potential studies [98]. Moreover, several studies in rhesus monkey infants indicate that different syndromes of behavioral effects are associated with prenatal and postnatal ID [99–101]. The above evidence indicates that the neuronal dysfunction, and the behavioral consequences of ID, will be a function of the persistence, severity, and duration of this condition.

6. Summary

This review summarized the latest knowledge on iron involvement in neuronal function and dysfunction. Iron, a redox active transition metal, has been proposed as an important contributing factor to the neuropathology of AD and PD and of other neurodegenerative disorders. The field called metallo-neurobiology has greatly expanded in the last 20 years. Although there is increasing experimental evidence that supports the structural biology of metal binding, iron participation in oxidative stress events and in protein aggregation, we are still far from fully understanding the role of iron in the onset and progression of neurodegenerative disorders. The advancement in this understanding will be crucial for the establishment of new therapies aimed at neuronal protection during iron mismanagement conditions.

Acknowledgements

The author thanks Dr. Patricia Oteiza for her mentorship to work in the captivating world of transition metals. G.A.S is a research member of the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) and was a Fulbright Fellow in the Department of Nutrition, University of California, Davis.

References

[1] Kell, D. B. (2009) Iron behaving badly: inappropriate iron chelation as a major contributor to the aetiology of vascular and other progressive inflammatory and degenerative diseases. *BMC. Med. Genomics* **2**, 2.

[2] Andrews, N. C. (1999) The iron transporter DMT1. *Int. J. Biochem. Cell Biol.* **31**, 991–994.

[3] Ganz, T. (2005) Cellular iron: ferroportin is the only way out. *Cell Metab.* **1**, 155–157.

[4] Berg, D., Gerlach, M., Youdim, M. B., Double, K. L., Zecca, L., Riederer, P., and Becker, G. (2001) Brain iron pathways and their relevance to Parkinson's disease. *J. Neurochem.* **79**, 225–236.

[5] Connor, J. R. and Fine, R. E. (1987) Development of transferrin-positive oligodendrocytes in the rat central nervous system. *J. Neurosci. Res.* **17**, 51–59.

[6] Connor, J. R. and Menzies, S. L. (1995) Cellular management of iron in the brain, *J. Neurol. Sci.* **134**(Suppl),33–44.

[7] Ponka, P. (2004) Hereditary causes of disturbed iron homeostasis in the central nervous system. *Ann. N. Y. Acad. Sci.* **1012**, 267–281.

[8] Roskams, A. J. and Connor, J. R. (1994) Iron, transferrin, and ferritin in the rat brain during development and aging. *J. Neurochem.* **63**, 709–716.

[9] Ke, Y. and Ming, Q. Z. (2003) Iron misregulation in the brain: a primary cause of neurodegenerative disorders. *Lancet Neurol.* **2**, 246–253.

[10] Wagner, K. R., Sharp, F. R., Ardizzone, T. D., Lu, A., and Clark, J. F. (2003) Heme and iron metabolism: role in cerebral hemorrhage. *J. Cereb. Blood Flow Metab.* **23**, 629–652.

[11] Connor, J. R., Roskams, A. J., Menzies, S. L., and Williams, M. E. (1993) Transferrin in the central nervous system of the shiverer mouse myelin mutant. *J. Neurosci. Res.* **36**, 501–507.

[12] Connor, J. R., Boeshore, K. L., Benkovic, S. A., and Menzies, S. L. (1994) Isoforms of ferritin have a specific cellular distribution in the brain. *J. Neurosci. Res.* **37**, 461–465.

[13] Pantopoulos, K. (2004) Iron metabolism and the IRE/IRP regulatory system: an update. *Ann. N. Y. Acad. Sci.* **1012**, 1–13.
[14] Levi, S., Yewdall, S. J., Harrison, P. M., Santambrogio, P., Cozzi, A., Rovida, E., Albertini, A., and Arosio, P. (1992) Evidence of H- and L-chains have co-operative roles in the iron-uptake mechanism of human ferritin. *Biochem. J.* **288**(Part 2),591–596.
[15] Lin, H. H., Snyder, B. S., and Connor, J. R. (1990) Transferrin expression in myelinated and non-myelinated peripheral nerves. *Brain Res.* **526**, 217–220.
[16] Zecca, L., Zucca, F. A., Albertini, A., Rizzio, E., and Fariello, R. G. (2006) A proposed dual role of neuromelanin in the pathogenesis of Parkinson's disease. *Neurology* **67**, S8–S11.
[17] Zucca, F. A., Bellei, C., Giannelli, S., Terreni, M. R., Gallorini, M., Rizzio, E., Pezzoli, G., Albertini, A., and Zecca, L. (2006) Neuromelanin and iron in human locus coeruleus and substantia nigra during aging: consequences for neuronal vulnerability. *J. Neural. Transm.* **113**, 757–767.
[18] Moos, T. and Morgan, E. H. (2004) The metabolism of neuronal iron and its pathogenic role in neurological disease: review. *Ann. N. Y. Acad. Sci.* **1012**, 14–26.
[19] Moos, T. and Morgan, E. H. (2004) The significance of the mutated divalent metal transporter (DMT1) on iron transport into the Belgrade rat brain. *J. Neurochem.* **88**, 233–245.
[20] Moos, T., Skjoerringe, T., Gosk, S., and Morgan, E. H. (2006) Brain capillary endothelial cells mediate iron transport into the brain by segregating iron from transferrin without the involvement of divalent metal transporter 1. *J. Neurochem.* **98**, 1946–1958.
[21] Moos, T., Rosengren, N. T., Skjorringe, T., and Morgan, E. H. (2007) Iron trafficking inside the brain. *J. Neurochem.* **103**, 1730–1740.
[22] Rouault, T. A. (2001) Iron on the brain. *Nat. Genet.* **28**, 299–300.
[23] Aracena, P., Aguirre, P., Munoz, P., and Nunez, M. T. (2006) Iron and glutathione at the crossroad of redox metabolism in neurons. *Biol. Res.* **39**, 157–165.
[24] Floyd, R. A. (1991) Oxidative damage to behavior during aging. *Science* **254**, 1597.
[25] Keller, J. N., Mark, R. J., Bruce, A. J., Blanc, E., Rothstein, J. D., Uchida, K., Waeg, G., and Mattson, M. P. (1997) 4-Hydroxynonenal, an aldehydic product of membrane lipid peroxidation, impairs glutamate transport and mitochondrial function in synaptosomes. *Neuroscience* **80**, 685–696.
[26] Halliwell, B. (1992) Reactive oxygen species and the central nervous system. *J. Neurochem.* **59**, 1609–1623.
[27] Halliwell, B. (1996) Free radicals, proteins and DNA: oxidative damage versus redox regulation. *Biochem. Soc. Trans.* **24**, 1023–1027.
[28] Springer, J. E., Azbill, R. D., Mark, R. J., Begley, J. G., Waeg, G., and Mattson, M. P. (1997) 4-Hydroxynonenal, a lipid peroxidation product, rapidly accumulates following traumatic spinal cord injury and inhibits glutamate uptake. *J. Neurochem.* **68**, 2469–2476.
[29] Lozoff, B. and Georgieff, M. K. (2006) Iron deficiency and brain development. *Semin. Pediatr. Neurol.* **13**, 158–165.
[30] Berg, D. and Youdim, M. B. (2006) Role of iron in neurodegenerative disorders. *Top. Magn. Reson. Imag.* **17**, 5–17.
[31] Mattson, M. P. (2004) Metal-catalyzed disruption of membrane protein and lipid signaling in the pathogenesis of neurodegenerative disorders. *Ann. N. Y. Acad. Sci.* **1012**, 37–50.
[32] Hardy, P. A., Gash, D., Yokel, R., Andersen, A., Ai, Y., and Zhang, Z. (2005) Correlation of R2 with total iron concentration in the brains of rhesus monkeys. *J. Magn. Reson. Imag.* **21**, 118–127.
[33] Shoham, S. and Youdim, M. B. (2002) The effects of iron deficiency and iron and zinc supplementation on rat hippocampus ferritin. *J. Neural. Transm.* **109**, 1241–1256.
[34] Bartzokis, G., Tishler, T. A., Shin, I. S., Lu, P. H., and Cummings, J. L. (2004) Brain ferritin iron as a risk factor for age at onset in neurodegenerative diseases. *Ann. N. Y. Acad. Sci.* **1012**, 224–236.
[35] Nakatsuka, I., Maeda, S., Andoh, T., Hayashi, Y., Mizuno, R., Higuchi, H., and Miyawaki, T. (2009) Oxidative changes in the rat brain by intraperitoneal injection of ferric nitrilotriacetate. *Redox. Rep.* **14**, 109–114.
[36] Aguirre, P., Mena, N., Tapia, V., Arredondo, M., and Nunez, M. T. (2005) Iron homeostasis in neuronal cells: a role for IREG1. *BMC Neurosci.* **6**, 3.
[37] Xu, H. M., Jiang, H., Wang, J., Luo, B., and Xie, J. X. (2008) Overexpressed human divalent metal transporter 1 is involved in iron accumulation in MES23.5 cells. *Neurochem. Int.* **52**, 1044–1051.
[38] Howitt, J., Putz, U., Lackovic, J., Doan, A., Dorstyn, L., Cheng, H., Yang, B., T. Chan-Ling, Silke, J., Kumar, S., and Tan, S. S. (2009) Divalent metal transporter 1 (DMT1) regulation by Ndfip1 prevents metal toxicity in human neurons. *Proc. Natl. Acad. Sci. USA* **106**, 15489–15494.
[39] Cantu, D., Schaack, J., and Patel, M. (2009) Oxidative inactivation of mitochondrial aconitase results in iron and H2O2-mediated neurotoxicity in rat primary mesencephalic cultures. *PLoS One.* **4**, e7095.
[40] Li, H., Swiercz, R., and Englander, E. W. (2009) Elevated metals compromise repair of oxidative DNA damage via the base excision repair pathway: implications of pathologic iron overload in the brain on integrity of neuronal DNA. *J. Neurochem.* **110**, 1774–1783.
[41] Crompton, D. E., Chinnery, P. F., Fey, C., Curtis, A. R., Morris, C. M., Kierstan, J., Burt, A., Young, F., Coulthard, A., Curtis, A., Ince, P. G., Bates, D., Jackson, M. J., and Burn, J. (2002) Neuroferritinopathy: a window on the role of iron in neurodegeneration. *Blood Cells Mol. Dis.* **29**, 522–531.
[42] Hayflick, S. J. (2003) Unraveling the Hallervorden-Spatz syndrome: pantothenate kinase-associated neurodegeneration is the name. *Curr. Opin. Pediatr.* **15**, 572–577.
[43] Gotz, M. E., Double, K., Gerlach, M., Youdim, M. B., and Riederer, P. (2004) The relevance of iron in the pathogenesis of Parkinson's disease. *Ann. N. Y. Acad. Sci.* **1012**, 193–208.
[44] Bartzokis, G., Sultzer, D., Cummings, J., Holt, L. E., Hance, D. B., Henderson, V. W., and Mintz, J. (2000) In vivo evaluation of brain iron in Alzheimer disease using magnetic resonance imaging. *Arch. Gen. Psychiatry* **57**, 47–53.
[45] Calabrese, V., Lodi, R., Tonon, C., V. D'Agata, Sapienza, M., Scapagnini, G., Mangiameli, A., Pennisi, G., Stella, A. M., and Butterfield, D. A. (2005) Oxidative stress, mitochondrial dysfunction and cellular stress response in Friedreich's ataxia. *J. Neurol. Sci.* **233**, 145–162.
[46] Rival, T., Page, R. M., Chandraratna, D. S., Sendall, T. J., Ryder, E., Liu, B., Lewis, H., Rosahl, T., Hider, R., Camargo, L. M., Shearman, M. S., Crowther, D. C., and Lomas, D. A. (2009) Fenton chemistry and oxidative stress mediate the toxicity of the beta-amyloid peptide in a *Drosophila* model of Alzheimer's disease. *Eur. J. Neurosci.* **29**, 1335–1347.
[47] Schipper, H. M. (2004) Heme oxygenase expression in human central nervous system disorders. *Free Radic. Biol. Med.* **37**, 1995–2011.
[48] Kaur, D. and Andersen, J. (2004) Does cellular iron dysregulation play a causative role in Parkinson's disease? *Age. Res. Rev.* **3**, 327–343.
[49] Kuperstein, F. and Yavin, E. (2003) Pro-apoptotic signaling in neuronal cells following iron and amyloid beta peptide neurotoxicity. *J. Neurochem.* **86**, 114–125.
[50] Kuperstein, F., Brand, A., and Yavin, E. (2004) Amyloid Ab1-40 preconditions non-apoptotic signals in vivo and protects fetal rat brain from intrauterine ischemic stress. *J. Neurochem.* **91**, 965–974.
[51] Mattson, M. P. (2005) NF-kB in the survival and plasticity of neurons. *Neurochem. Res.* **30**, 883–893.
[52] Mattson, M. P. and Meffert, M. K. (2006) Roles for NF-kB in nerve cell survival, plasticity, and disease. *Cell Death. Differ.* **13**, 852–860.
[53] Lukiw, W. J. and Bazan, N. G. (1998) Strong nuclear factor-kB-DNA binding parallels cyclooxygenase-2 gene transcription in aging and in sporadic Alzheimer's disease superior temporal lobe neocortex. *J. Neurosci. Res.* **53**, 583–592.
[54] Mackenzie, G. G., Keen, C. L., and Oteiza, P. I. (2006) Microtubules are required for NF-kB nuclear translocation in neuroblastoma IMR-32 cells: modulation by zinc. *J. Neurochem.* **99**, 402–415.
[55] Ferrandiz, M. L. and Devesa, I. (2008) Inducers of heme oxygenase-1. *Curr. Pharm. Des.* **14**, 473–486.
[56] Scortegagna, M., Galdzicki, Z., Rapoport, S. I., and Hanbauer, I. (1999) Activator protein-1 DNA binding activation by hydrogen peroxide in neuronal and astrocytic primary cultures of trisomy-16 and diploid mice. *Brain Res. Mol. Brain Res.* **73**, 144–150.
[57] Aimo, L. and Oteiza, P. I. (2006) Zinc deficiency increases the susceptibility of human neuroblastoma cells to lead-induced activator protein-1 activation. *Toxicol. Sci.* **91**, 184–191.
[58] Toone, W. M., Morgan, B. A., and Jones, N. (2001) Redox control of AP-1-like factors in yeast and beyond. *Oncogene* **20**, 2336–2346.
[59] venisti-Zarom, L., Chen-Roetling, J., and Regan, R. F. (2006) Inhibition of the ERK/MAP kinase pathway attenuates heme oxygenase-1 expression and heme-mediated neuronal injury. *Neurosci. Lett.* **398**, 230–234.

[60] Chen-Roetling, J., Li, Z., Chen, M., Awe, O. O., and Regan, R. F. (2009) Heme oxygenase activity and hemoglobin neurotoxicity are attenuated by inhibitors of the MEK/ERK pathway. *Neuropharmacology* **56**, 922–928.
[61] Huang, E., Ong, W. Y., Go, M. L., and Connor, J. R. (2006) Upregulation of iron regulatory proteins and divalent metal transporter-1 isoforms in the rat hippocampus after kainate induced neuronal injury. *Exp. Brain Res.* **170**, 376–386.
[62] Guo, Z. H. and Mattson, M. P. (2000) Neurotrophic factors protect cortical synaptic terminals against amyloid and oxidative stress-induced impairment of glucose transport, glutamate transport and mitochondrial function. *Cereb. Cortex* **10**, 50–57.
[63] Guo, Z. H. and Mattson, M. P. (2000) In vivo 2-deoxyglucose administration preserves glucose and glutamate transport and mitochondrial function in cortical synaptic terminals after exposure to amyloid beta-peptide and iron: evidence for a stress response. *Exp. Neurol.* **166**, 173–179.
[64] Obrenovitch, T. P. and Urenjak, J. (1997) Altered glutamatergic transmission in neurological disorders: from high extracellular glutamate to excessive synaptic efficacy. *Prog. Neurobiol.* **51**, 39–87.
[65] Oppenheim, R. W. (1987) Muscle activity and motor neuron death in the spinal cord of the chick embryo. *Ciba Found. Symp.* **126**, 96–112.
[66] Oppenheim, R. W., Prevette, D., A. D'Costa, Wang, S., Houenou, L. J., and McIntosh, J. M. (2000) Reduction of neuromuscular activity is required for the rescue of motoneurons from naturally occurring cell death by nicotinic-blocking agents. *J. Neurosci.* **20**, 6117–6124.
[67] Hauptmann, S., Keil, U., Scherping, I., Bonert, A., Eckert, A., and Muller, W. E. (2006) Mitochondrial dysfunction in sporadic and genetic Alzheimer's disease. *Exp. Gerontol.* **41**, 668–673.
[68] Uranga, R. M., Mateos, M. V., Giusto, N. M., and Salvador, G. A. (2007) Activation of phosphoinositide-3 kinase/Akt pathway by FeSO4 in rat cerebral cortex synaptic endings. *J. Neurosci. Res.* **85**, 2924–2932.
[69] Uranga, R. M., Giusto, N. M., and Salvador, G. A. (2009) Iron-induced oxidative injury differentially regulates PI3K/Akt/GSK3beta pathway in synaptic endings from adult and aged rats. *Toxicol. Sci.* **111**, 331–344.
[70] Mattson, M. P. (2000) Apoptotic and anti-apoptotic synaptic signaling mechanisms. *Brain Pathol.* **10**, 300–312.
[71] Anglade, P., Mouatt-Prigent, A., Agid, Y., and Hirsch, E. (1996) Synaptic plasticity in the caudate nucleus of patients with Parkinson's disease. *Neurodegeneration* **5**, 121–128.
[72] DeKosky, S. T., Scheff, S. W., and Styren, S. D. (1996) Structural correlates of cognition in dementia: quantification and assessment of synapse change. *Neurodegeneration* **5**, 417–421.
[73] Martin, L. J., Pardo, C. A., Cork, L. C., and Price, D. L. (1994) Synaptic pathology and glial responses to neuronal injury precede the formation of senile plaques and amyloid deposits in the aging cerebral cortex. *Am. J. Pathol.* **145**, 1358–1381.
[74] Barnham, K. J. and Bush, A. I. (2008) Metals in Alzheimer's and Parkinson's diseases. *Curr. Opin. Chem. Biol.* **12**, 222–228.
[75] Richardson, D. R. (2000) The 10th International Conference on Oral Iron Chelators in the treatment of beta-thalassemia and other diseases and biomed meeting. *Expert. Opin. Investig. Drugs* **9**, 1151–1154.
[76] Park, M. J., Lee, S. K., Lim, M. A., Chung, H. S., Cho, S. I., Jang, C. G., and Lee, S. M. (2006) Effect of alpha-tocopherol and deferoxamine on methamphetamine-induced neurotoxicity. *Brain Res.* **1109**, 176–182.
[77] Perez, C. A., Tong, Y., and Guo, M. (2008) Iron chelators as potential therapeutic agents for parkinson's disease. *Curr. Bioact. Compd.* **4**, 150–158.
[78] Jiang, H., Luan, Z., Wang, J., and Xie, J. (2006) Neuroprotective effects of iron chelator Desferal on dopaminergic neurons in the substantia nigra of rats with iron-overload. *Neurochem. Int.* **49**, 605–609.
[79] Sangchot, P., Sharma, S., Chetsawang, B., Porter, J., Govitrapong, P., and Ebadi, M. (2002) Deferoxamine attenuates iron-induced oxidative stress and prevents mitochondrial aggregation and alpha-synuclein translocation in SK-N-SH cells in culture. *Dev. Neurosci.* **24**, 143–153.
[80] Rakba, N., Aouad, F., Henry, C., Caris, C., Morel, I., Baret, P., Pierre, J. L., Brissot, P., Ward, R. J., Lescoat, G., and Crichton, R. R. (1998) Iron mobilisation and cellular protection by a new synthetic chelator O-Trensox. *Biochem. Pharmacol.* **55**, 1797–1806.
[81] Wang, Y., Branicky, R., Stepanyan, Z., Carroll, M., Guimond, M. P., Hihi, A., Hayes, S., McBride, K., and Hekimi, S. (2009) The anti-neurodegeneration drug clioquinol inhibits the aging-associated protein CLK-1. *J. Biol. Chem.* **284**, 314–323.
[82] Andrade, R. G., Jr., Ginani, J. S., Lopes, G. K., Dutra, F., Alonso, A., and Hermes-Lima, M. (2006) Tannic acid inhibits in vitro iron-dependent free radical formation. *Biochimie* **88**, 1287–1296.
[83] Hider, R. C., Liu, Z. D., and Khodr, H. H. (2001) Metal chelation of polyphenols. *Methods Enzymol.* **335**, 190–203.
[84] Lopes, G. K., Schulman, H. M., and Hermes-Lima, M. (1999) Polyphenol tannic acid inhibits hydroxyl radical formation from Fenton reaction by complexing ferrous ions. *Biochim. Biophys. Acta* **1472**, 142–152.
[85] Mandel, S. A., Amit, T., Kalfon, L., Reznichenko, L., Weinreb, O., and Youdim, M. B. (2008) Cell signaling pathways and iron chelation in the neurorestorative activity of green tea polyphenols: special reference to epigallocatechin gallate (EGCG). *J. Alzheimers Dis.* **15**, 211–222.
[86] Zhang, Y., Li, H., Zhao, Y., and Gao, Z. (2006) Dietary supplementation of baicalin and quercetin attenuates iron overload induced mouse liver injury. *Eur. J. Pharmacol.* **535**, 263–269.
[87] Rao, R., Tkac, I., Townsend, E. L., Ennis, K., Gruetter, R., and Georgieff, M. K. (2007) Perinatal iron deficiency predisposes the developing rat hippocampus to greater injury from mild to moderate hypoxia-ischemia. *J. Cereb. Blood Flow Metab.* **27**, 729–740.
[88] de, D. M., Rao, R., Wobken, J. D., Luciana, M., Nelson, C. A., and Georgieff, M. K. (2000) Perinatal iron deficiency decreases cytochrome c oxidase (CytOx) activity in selected regions of neonatal rat brain, *Pediatr. Res.* **48**, 169–176.
[89] Yoo, Y. E., Hong, J. H., Hur, K. C., Oh, E. S., and Chung, J. M. (2004) Iron enhances NGF-induced neurite outgrowth in PC12 cells. *Mol. Cells* **17**, 340–346.
[90] Jorgenson, L. A., Wobken, J. D., and Georgieff, M. K. (2003) Perinatal iron deficiency alters apical dendritic growth in hippocampal CA1 pyramidal neurons. *Dev. Neurosci.* **25**, 412–420.
[91] Bianco, L. E., Wiesinger, J., Earley, C. J., Jones, B. C., and Beard, J. L. (2008) Iron deficiency alters dopamine uptake and response to L-DOPA injection in Sprague-Dawley rats. *J. Neurochem.* **106**, 205–215.
[92] Beard, J., Finch, C. A., and Mackler, B. (1981) Deleterious effects of iron deficiency. *Prog. Clin. Biol. Res.* **77**, 305–310.
[93] Beard, J. (2003) Iron deficiency alters brain development and functioning. *J. Nutr.* **133**, 1468S–1472S.
[94] Beard, J., Erikson, K. M., and Jones, B. C. (2003) Neonatal iron deficiency results in irreversible changes in dopamine function in rats. *J. Nutr.* **133**, 1174–1179.
[95] Beard, J. (2007) Recent evidence from human and animal studies regarding iron status and infant development. *J. Nutr.* **137**, 524S–530S.
[96] Wang, X., Wiesinger, J., Beard, J., Felt, B., Menzies, S., Earley, C., Allen, R., and Connor, J. (2004) Thy1 expression in the brain is affected by iron and is decreased in Restless Legs Syndrome. *J. Neurol. Sci.* **220**, 59–66.
[97] Carlson, E. S., Tkac, I., Magid, R., M. B. O'Connor, Andrews, N. C., Schallert, T., Gunshin, H., Georgieff, M. K., and Petryk, A. (2009) Iron is essential for neuron development and memory function in mouse hippocampus. *J. Nutr.* **139**, 672–679.
[98] Burhans, M. S., Dailey, C., Wiesinger, J., L. E. Murray-Kolb, Jones, B. C., and Beard, J. L. (2006) Iron deficiency affects acoustic startle response and latency, but not prepulse inhibition in young adult rats. *Physiol Behav.* **87**, 917–924.
[99] Golub, M. S., Hogrefe, C. E., Germann, S. L., Capitanio, J. P., and Lozoff, B. (2006) Behavioral consequences of developmental iron deficiency in infant rhesus monkeys. *Neurotoxicol. Teratol.* **28**, 3–17.
[100] Golub, M. S., Hogrefe, C. E., and Germann, S. L. (2007) Iron deprivation during fetal development changes the behavior of juvenile rhesus monkeys. *J. Nutr.* **137**, 979–984.
[101] Golub, M. S., Hogrefe, C. E., Widaman, K. F., and Capitanio, J. P. (2009) Iron deficiency anemia and affective response in rhesus monkey infants. *Dev. Psychobiol.* **51**, 47–59.