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ARTICLE *in* BIOLOGY OF METALS · FEBRUARY 2012

Impact Factor: 2.5 · DOI: 10.1007/s10534-012-9523-0 · Source: PubMed

Biometals (2012) 25:761–776
DOI 10.1007/s10534-012-9523-0

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Received: 20 December 2011 / Accepted: 13 January 2012 / Published online: 9 February 2012

Abstract Iron is an essential element for life on earth, participating in a plethora of cellular processes where one-electron transfer reactions are required. Its essentiality, coupled to its scarcity in aqueous oxidative environments, has compelled living organisms to develop mechanisms that ensure an adequate iron supply, at times with disregard to long-term deleterious effects derived from iron accumulation. However, iron is an intrinsic producer of reactive oxygen species, and increased levels of iron promote neurotoxicity because of hydroxyl radical formation, which results in glutathione consumption, protein aggregation, lipid peroxidation and nucleic acid modification. Neurons from brain areas sensitive to degeneration accumulate iron with age and thus are subjected to an ever increasing oxidative stress with the accompanying cellular damage. The ability of these neurons to survive depends on the adaptive mechanisms developed to cope with the increasing oxidative load. Here, we describe the chemical and thermodynamic peculiarities of iron chemistry in living matter, review the components of iron homeostasis in neurons and elaborate on the mechanisms by which iron homeostasis is lost in Parkinson's disease, Alzheimer's disease and other diseases in which iron accumulation has been demonstrated.

Keywords Iron homeostasis · Mitochondria dysfunction · GSH · Fe–S clusters · Neurodegeneration

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Iron chemistry and toxicity

Iron (atomic number: 26; atomic weight: 55.85) is the 26th element in the periodic table, forming part of the first horizontal triad of transition elements, together with cobalt and nickel. Iron has a maximal oxidation state of 6+, but only the 2+ and 3+ states are common in biological environments. The neutral iron atom has four unpaired electrons in the 3d orbital and two paired electrons in the 4s orbital (Fig. 1). Removal of the 4s electrons gives rise to the 2+ state, while additional removal of one 3d electron gives rise to the 3+ state (Sienko and Plane 1976).

There is a notable difference in solubility between the Fe^{2+} and the Fe^{3+} salts, with the Fe^{3+} salts practically insoluble (Ksp for $Fe(OH)_3$: 2.8×10^{-39}; maximal solubility at pH 7.0: 10^{-17} M), whereas Fe^{2+} salts reach solubilities of 10^{-1} M (Spiro and Salman 1974). Thus, Fe^{2+} is the prevalent species in reductive biological fluids as the intracellular milieu.

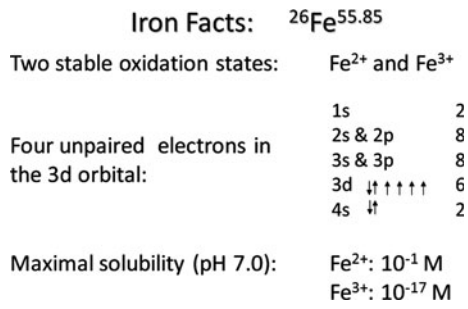


Fig. 1 Iron facts**.** Unpaired electrons in the 3d orbital give rise to the paramagnetic properties of iron and its capacity to exchange 1 electron in oxidation–reduction reactions

Nevertheless, because of oxidation by O_2, iron is continuously cycling between the 2+ and 3+ states.

Iron's capacity to exchange one electron in biological reactions is extraordinary. In the cellular environment, both Fe^{2+} and Fe^{3+} establish coordination complexes with a great diversity of ligands. Iron complexes display a variety of reduction potentials, ranging from very positive to negative values. This property is determined by a basic concept in coordination chemistry, which institutes that the ligand modifies the electron cloud surrounding the metal, thus modifying its reduction potential. This property allows for fine tuning between iron reduction potential and the electron transfer process that iron catalyzes. It is estimated that the predominant reduction potential for iron in the intracellular milieu of the cell is about 0 V (Wood 1988). Given that as the reduction potential nears zero iron more readily exchanges between 2+ and 3+ forms, iron is an extremely flexible element for electron exchange reactions and is widely used in nature.

Iron is an intrinsic reactive oxygen species (ROS) producer. When one or more of its six ligand binding sites is not tightly bound, iron engages in one-electron exchange reactions with the potential of producing free radicals (Graf et al. 1984). Indeed, iron toxicity is the product of this electron-exchange capacity combined with a reductive intracellular environment and the presence of oxygen. Fe^{2+} can react with O_2 to give rise to a superoxide radical which, catalyzed by superoxide dismutase, quickly dismutates to O_2 and H_2O_2. Fe^{2+} also reacts with H_2O_2 to generate the highly reactive hydroxyl free radical (reactions 1–3):

$$2Fe^{2+} + 2O_2 \rightarrow 2Fe^{3+} + 2O_2^{\cdot -} \quad E0 : -0.26\,\mathrm{V}; \quad \Delta G = 25.1\,\mathrm{KJ/mol} \tag{1}$$

$$2O_2^{\cdot -} + 2H^+ \rightarrow H_2O_2 + O_2 \quad E0 : 0.89\,\mathrm{V}; \quad \Delta G = -85.9\,\mathrm{KJ/mol} \tag{2}$$

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + {}^{\cdot}OH \quad E0 : 0.28\,\mathrm{V}; \quad \Delta G = -27.0\,\mathrm{KJ/mol} \tag{3}$$

This set of reactions is known as the Haber–Weiss reactions while reaction 3, the reaction of Fe^{2+} with hydrogen peroxide to produce a hydroxyl radical, is known as the Fenton reaction. Reactions 2 and 3 have strong negative ΔG values and thus drive reaction 1 to the production of a superoxide anion ($O_2^{\bullet -}$). The coupling of reactions 1–3 gives reaction 4:

$$3Fe^{2+} + O_2 + 2H^+ \rightarrow 3Fe^{3+} + OH^- + {}^{\cdot}OH \quad E0 : 0.91\,\mathrm{V}; \quad \Delta G = -87.6\,\mathrm{KJ/mol} \tag{4}$$

The intracellular medium provides plenty of reductant power in the form of ascorbate and reduced glutathione (GSH) to regenerate the ferrous state:

$$2Fe^{3+} + 2GSH \rightarrow 2Fe^{2+} + GSSG + 2H^+ \quad \Delta G = -61.7\,\mathrm{KJ/mol} \tag{5}$$

The balance of reactions 1–5 results in reaction 6:

$$2GSH + O_2 \xrightarrow{Fe^{2+}/Fe^{3+}} GSSG + OH^- + OH^{\cdot} \quad \Delta G = -113.7\,\mathrm{KJ/mol} \tag{6}$$

In summary, in a reductive and O_2-containing environment such as the intracellular milieu, iron promotes the production of a hydroxyl radical at the expense of O_2 and GSH consumption. The in vivo relevance of reaction 6 is accentuated by the consideration that under normal conditions the cytoplasm has millimolar concentrations of GSH and sub-micromolar concentrations of reactive iron (Epsztejn et al. 1997; Kruszewski 2003). It is expected that with age and under iron overload conditions the cell concentration of redox-active iron raises (Zecca et al. 2004; Glickstein et al. 2006) and thus the generation of hydroxyl radicals. In dopaminergic cells, another source of free radicals derives from the non-enzymatic oxidation of dopamine mediated by redox-active iron, resulting in the production of semiquinones and H_2O_2 (Zoccarato et al. 2005).Thus, redox-active iron, both through the Fenton reaction and via dopamine oxidation, is a dangerous pro-oxidant agent.

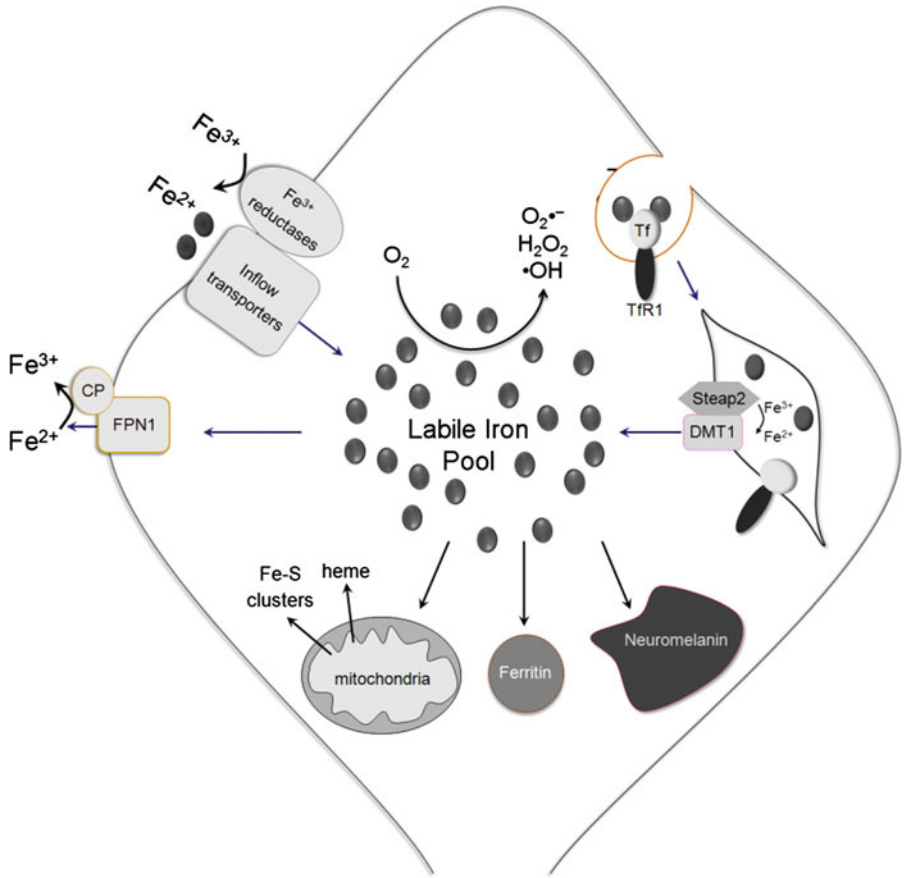


Fig. 2 Molecular components of neuronal iron homeostasis. The scheme includes Tf and its receptor (TfR1); inflow (DMT1, TRPC6, L-type voltage-dependent calcium channels (L-VDCC) and Zip14) and efflux (ferroportin 1, FPN1) membrane transporters; the iron storage protein ferritin; the ferroxidase ceruloplasmin (CP) and the ferrireductase Dcytb that reduces Fe^{3+} to Fe^{2+} previously to inflow transporters. Iron is taken up either through the endocytosis of Fe-containing Tf or transported directly through the plasma membrane. Once in the cytoplasm, iron forms part of the labile iron pool (LIP) from where it distributes to mitochondria, ferritin and neuromelanin, or it is exo-transported by FPN1. In the cellular environment, iron in the LIP is a net ROS producer. Putative Fe^{3+} reductases include Dcytb, its homologous cytochrome b561, SDR2 and Steap2 (see text)

Neuronal iron homeostasis

Iron concentration in cerebrospinal fluid (CSF) ranges between 0.2 and 1.1 μM whereas transferrin (Tf) concentration lies around 0.24 μM (Symons and Gutteridge 1998; Moos and Morgan 1998). Although CSF and the interstitial fluid that bath the neurons are in different compartments, it is likely that the composition of CSF reflects that of the interstitial fluid (Bradbury 1997). Considering that one Tf molecule binds two iron atoms, CSF iron often exceeds the binding capacity of Tf. Hence, non-Tf bound iron (NTBI) uptake is expected to occur in neurons that express divalent metal transporter 1 (DMT1) or other iron uptake transporters. The components of neuronal iron homeostasis are depicted in Fig. 2. NTBI uptake occurs directly at the plasma membrane, mediated by one or more of the transporters shown in Fig. 2. Tf-bound iron uptake initiates with the binding of Tf to the transferrin receptor (TfR), followed by internalization into the endosomal system. Release of iron is mediated by endosomal acidification, Fe^{3+} reduction possibly by Steap2 (Ohgami et al. 2006) and transport into the cytoplasm by endosomal DMT1. Once in the cytoplasm, Fe^{2+} becomes part of the labile or reactive iron pool where it distributes to mitochondria and ferritin or engages in electron exchange reactions (Kakhlon and Cabantchik 2002; Kruszewski 2003).

All the components described in Fig. 2 have been detected in the brain (Moos et al. 2007; Rouault et al. 2009; Haeger et al. 2010). In neurons of the substantia nigra (SN) and in the noradrenergic neurons of the locus coeruleus, iron also distributes to neuromelanin granules (Zecca et al. 2003).

The iron transporters and auxiliary proteins

DMT1 is a Fe^{2+}/H^{+} co-transporter that transports iron into cells (Gunshin et al. 1997). In the brain, DMT1 is expressed in hippocampal pyramidal and granule cells, cerebella granule cells, pyramidal cells of the piriform cortex, SN and the ventral portion of the anterior olfactory nucleus, striatum, cerebellum, hippocampus and thalamus, as well as in vascular cells throughout the brain and ependymal cells in the third ventricle (Gunshin et al. 1997; Williams et al. 2000; Burdo et al. 2001). The pervasive presence of DMT1 in neurons suggests that DMT1 is needed for normal neuronal function (Hidalgo and Núñez 2007; Wright and Baccarelli 2007; Pelizzoni et al. 2011). In support of this notion are our observations that mice trained in the Morris water maze presented increased expression of DMT1 in the hippocampus when compared with untrained mice, and that treatment of hippocampal neurons for a short time (5 min) with NMDA resulted in increased expression of DMT1 for at least 2 days (Haeger et al. 2010).

The mammalian DMT1 gene undergoes alternative splicing generating 4 isoforms, all active in Fe^{2+} transport. The 1A and 1B mRNA DMT1 variants originate from alternative splicing at the 5′ end (exons 1A and 1B), while variants, that contain or do not contain an iron responsive element (IRE), (+)IRE and (−)IRE, respectively, motif in the mRNA, originate from splicing at the 3′ end (exons 16/16A and 17) (Hubert and Hentze 2002; Ludwiczek et al. 2007). The 1B isoforms are expressed in brain and other tissues (Haeger et al. 2010) whereas the 1A isoforms are almost exclusively expressed in intestine (Hubert and Hentze 2002). It is generally accepted that the expression of the (+)IRE isoforms is regulated by the IRE/iron regulatory protein (IRP) system, which post-transcriptionally regulates the expression of iron homeostasis proteins such as TfR1, DMT1, FPN1 and ferritin, in response to the concentration of reactive iron in the cytoplasm (Garrick and Garrick 2009). Knowledge on the transcriptional regulation of DMT1 expression is emerging. The inflammatory cytokine NFκB and nuclear factor Y regulate DMT1(1B) expression in embryonic carcinoma cells (Paradkar and Roth 2006). Hypoxia up-regulates expression of the DMT1(1A) isoform, presumably through activation of HIF-1 (Lis et al. 2005; Wang et al. 2010). HIF-1 also promotes the expression of the 1B isoform by binding to a putative hypoxia-response element sequence in DMT1 exon1B gene (Qian et al. 2011).

Other putative neuronal iron inward transporters are Transient receptor potential cation channel, subfamily C, member 6 (TRPC6), L-type voltage-dependent calcium channels (L-VDCC) and Zrt- and Irt-like protein 13 (Zip14). TRPC6 belongs to a family of store- and receptor-operated calcium channels (Krizaj 2005). TRPC6 was found into the proximal dendrites of Tyrosine hydroxylase (TH)-positive dopaminergic neurons in the SN (Giampa et al. 2007). Although the iron transport capacity of TRPC6 was reported (Mwanjewe and Grover 2004), its role as a putative iron transporter in neurons awaits further evaluation.

Evidence indicates that L-VDCC is partially responsible for iron overload in cardiomyocytes (Oudit et al. 2006). The evidence that L-VDCC channels are a conduit for Fe^{2+} influx into neuronal cells arises from the observation that treatment with the selective L-VDCC channel inhibitor nimodipine prior to an iron challenge provides significant protection against death in cultured neurons. This observation led to the hypothesis that L-VDCCs double as Fe^{2+} channels that can participate in iron overload in neurodegenerative diseases (Gaasch et al. 2007; Lockman et al. 2012).

Zip14 has two-fourth exons, giving rise to ZIP14A and ZIP14B splicing variants (Girijashanker et al. 2008). In mice ZIP14A expression is highest in the duodenum, liver and kidney, whereas ZIP14B expression is high in brain, and testis (Lichten and Cousins 2009). Recent reports have provided compelling evidence demonstrating that Zip14 is a Fe^{2+} transporter. Overexpression of Zip14 in human embryonic kidney 293 (HEK293) cells increases Fe^{2+} uptake that is inhibited both by Zn^{2+} and ZIP14 siRNA (Liuzzi et al. 2006). In mouse models, inflammatory stimuli like lipopolysaccharide (LPS) and turpentine induce upregulation of Zip14 through a mechanism involving IL-6 and induction of iNOS (Liuzzi et al. 2005). The upregulation of ZIP14 by inflammatory stimuli raises

the possibility that this transporter may act as a novel interface between inflammation and tissue iron accumulation.

FPN1 is the only member of the SLC40 family of transporters and the first reported protein that mediates the exit of iron from cells (McKie et al. 2000). FPN1 has a unique role mediating iron exit from enterocytes and macrophages into circulating blood. In enterocytes, FPN1 is responsible for iron efflux during the process of intestinal iron absorption, while in Kupffer cells FPN1 mediates iron export for reutilization by the bone marrow (Devalia et al. 2002). Neither the transport mechanism nor the Fe species transported by FPN1 are known, although the latter is probably Fe^{2+}, the prevalent form of available intracellular iron.

FPN1 is abundantly expressed in the mouse brain, being present in neurons, microglia, astrocytes and oligodendrocytes (Song et al. 2010). Spatiotemporal expression of FPN1 in neurons is variable (Moos and Rosengren Nielsen 2006). In young mice brain (postnatal day 7 to 21) high immunoreactivity is found in the neurons of the striatum and the hippocampus, both in cell bodies and in projection fibers. FPN1 is mildly expressed in the SN pars compacta and the superior colliculus and weakly expressed in the SN pars reticulata (Boserup et al. 2011). In the adult brain, FPN1 immunoreactivity is lower in the projections of the striatum, but no differences have been found in neuronal cell bodies (Moos and Rosengren Nielsen 2006).

Knowledge on the regulation of FPN1 expression is incipient. Most probably, FPN1 expression is regulated at both transcriptional and translational levels (McKie et al. 2000; Knutson et al. 2003). FPN1 has an IRE motif in the 5′-UTR of its mRNA that bestows on FPN1 a ferritin-like response to variations in cell iron, increasing its expression under elevated iron conditions (McKie et al. 2000; Yang et al. 2002; Aguirre et al. 2005).

The ferrireductases

Ferrous iron is the preferred redox state for transport while Fe^{3+} is the predominant redox state in food, so prior to its transport into the cell, Fe^{3+} must be reduced to Fe^{2+}. The reduction of iron is carried out by ferrireductases present either in the plasma membrane or in the endosome/lysosome system. Four membrane ferrireductases involved in iron transport processes have been described: Duodenal cytochrome B (Dcytb), its homologous cytochrome b561, Stromal cell-derived receptor 2 (SDR2) and 6-transmembrane epithelial antigen of the prostate 3 (Steap3) (Vargas et al. 2003; Ohgami et al. 2006). Dcytb, initially described as the ferrioxidase responsible for reduction of non-heme iron in the duodenal lumen during intestinal iron absorption (McKie et al. 2001), is the only recognized member of the iron transport machinery that lacks an IRE in its mRNA. A recent study of the temporal relationship between Dcytb and Hif-2α during hypoxic stimulus in the enterocyte, revealed that both Dcytb and Hif-2α protein expression increase during the first (6–18) h of a hypoxic stimuli, although a significant change in hepcidin expression was evident only after 72 h of hypoxia (Latunde-Dada et al. 2011). As mentioned before, DMT1 and FPN1 exhibit transcriptional regulation by Hif-1α. Thus, the control of iron acquisition through the up-regulation of Dcytb and DMT1 by Hif-1α, coupled to the down-regulation of FPN1 by Hif-2α, may result in a concerted response of increased iron accumulation under hypoxic conditions.

The presence of Dcytb and SDR2 were recently reported in astrocytes, where they may have a limited role in iron accumulation by these cells since the reduction rate for ferric iron was substantially lower than the rate of cellular iron accumulation from 100 μM ferrous ammonium sulphate (Tulpule et al. 2010). Notoriously, there is no report on the presence of plasma membrane Dcytb in neurons. Perhaps the high concentration of ascorbate in the CSF, thereby maintaining NTBI in the 2+ state, makes unnecessary a membrane-bound iron reduction system in brain cells.

The Steap family of ferrireductases comprises the members Steap1, Steap2, Steap3, and Steap4 (Ohgami et al. 2006). Steap3 was described in endosomes of erythroid precursor cells, catalyzing the reduction of Fe^{3+} to Fe^{2+} prior to transport by DMT1 (Ohgami et al. 2005). In situ hybridization studies show that Steap2 is expressed in the brain (Ohgami et al. 2006). Whether Steap2 or other members of the Steap family has a role in iron uptake by neurons remains speculative.

Ferritin

Ferritin, a multimeric protein formed by 24 subunits of H and L monomers, is the only well-characterized

iron-storage protein in living organisms (Liu and Theil 2005). The 24 subunits of ferritin form a hollow cavity that can store as many as 4,500 Fe^{3+} atoms in the form of crystallized diferric oxo-hydroxyl complexes (Harrison and Arosio 1996). Ferritin plays a fundamental role in controlling the size of the cytoplasmic redox-active iron pool (Salgado et al. 2010). Although ferritin is expected to reduce this pool by storing iron, there is a dynamic exchange of iron between its ferritin-bound form and a cytoplasmic form amenable to transport by FPN1 (De Domenico et al. 2006). In addition, ferritin contributes to the pool of redox-active iron every time it is degraded (Mehlhase et al. 2005). H-ferritin, the subunit responsible for Fe^{2+} oxidation, is relatively poorly expressed in melanized dopaminergic neurons of the sustantia nigra pars compacta, as compared to neurons in other parts of the brain, but is strongly expressed in oligodendrocytes (Snyder and Connor 2009).

The SN of Parkinson's disease patients has a low content of ferritin compared to other brain areas (Dexter et al. 1991; Faucheux et al. 2002), and this ferritin has higher iron content when compared with ferritin of age-matched control individuals (Griffiths et al. 1999). The reasons for this low ferritin expression is unknown, but it could be due to increased Iron regulatory protein 1 (IRP1) activity (Faucheux et al. 2002). IRP1 in its active form binds to IRE elements present in the 5′ untranslated region of ferritin mRNA inhibiting its translation. In support of this notion, it was recently reported that inhibition of electron transport chain complex I, a common occurrence in Parkinson's disease (see ahead), also results in activation of IRP1.

Lack of active ferritin can cause neurodegeneration, as found in hereditary neuroferritinopathy, a neurodegenerative disease characterized by the increase of iron and ferritin in the extracellular spaces and cytoplasm of cells in the basal ganglia of affected individuals (Curtis et al. 2001). The clinical symptoms of neuroferritinopathy include severe movement disorders and the presence of nuclear and cytoplasmic ferritin inclusion bodies in glia and neurons throughout the CNS (Vidal et al. 2003). The molecular cause of neuroferritinopathy is nucleotide insertions in the L-ferritin gene, which results in low capacity of the sub-unit to assemble into 24-mer ferritin shell and the formation of heteropolymers with H-subunits that present a reduced capacity to incorporate iron in vitro (Cozzi et al. 2006).

It is noteworthy that uptake as the result of the endocytosis of exogenous ferritin has been acknowledged (Fisher et al. 2007). In the same vein, ferritin has been demonstrated to be an iron source for developing oligodendrocytes (Todorich et al. 2011), but its role as a putative iron donor to neurons has not been firmly established.

Neuromelanin

The most highly pigmented cells in the human brain are the dopaminergic neurons of the SN and the noradrenergic neurons of the locus coeruleus. The pigment is composed of neuromelanin, a polymer formed by oxidized metabolites of dopamine, containing a peptide component of about 15% (Zecca et al. 2002). Parkinson's disease is characterized by the preferential loss of neuromelanin-containing neurons of the SN (Kastner et al. 1992; Zecca et al. 2003). Thus, it is of relevance to assess the function of neuromelanin in the SN under physiological conditions and as a possible pathogenic element.

Neuromelanin avidly binds iron in its 3+ form, presenting high and low affinity binding sites (Double et al. 2003). Given that ferritin is poorly expressed in melanized dopaminergic neurons of the SN, as compared to neurons in other parts of the brain (Snyder and Connor 2009), neuromelanin is the main iron storage moiety in SN neurons. It is believed that the high affinity sites are protective as they sequester iron in a redox-inactive form, whereas iron in the low affinity sites is redox-active (Gerlach et al. 2008). Thus, under physiological conditions iron should safely bind to high affinity sites. For the contrary, when iron concentration increases above the high affinity binding capacity of neuromelanin, as is the case of increased age or pathophysiological conditions, iron binds to low-affinity binding sites in a redox-active form and the neurons become susceptible to oxidative damage.

The inverse relationship between iron and reduced GSH levels

The tripeptide GSH (γ-L-glutamyl-L-cysteinylglycine) is the most abundant and the main antioxidant agent in the central nervous system, where it reaches millimolar concentrations in the cytoplasm (Meister and

Anderson 1983; Dringen et al. 2000). In its redox cycling, GSH is present either in its reduced (GSH) form or its oxidized disulfide (GSSG) form. The ratio GSH/GSSG is a faithful reflection of the redox state of the cell (Schafer and Buettner 2001).

Iron accumulation induces the consumption of GSH and the production of GSSG (Fig. 3) (Núñez et al. 2004). After exposure to increasing concentrations of iron, SH-SY5Y dopaminergic cells show increased levels of ROS (Fig. 3a) and intracellular iron accumulation (Fig. 3b). The cells undergo a biphasic change in intracellular GSH levels, increasing at low (2–5 μM) iron concentrations and decreasing thereafter (Fig. 3c). Indeed, cell exposure to high iron concentrations (20–80 μM) markedly decreases GSH half-cell reduction potential, with the associated loss of cell viability at values more positive than −300 mV (Fig. 3d). These data support the hypothesis that a decrease in GSH levels is a consequence of the increased oxidative load produced by increased ROS and by the consumption of GSH by iron during its redox cycling. Nevertheless, increased iron and decreased GSH may be intertwined in a positive feedback loop, since in dopaminergic neurons the pharmacological reduction of GSH levels results in increased levels of TfR and an increased labile iron pool (Kaur et al. 2009).

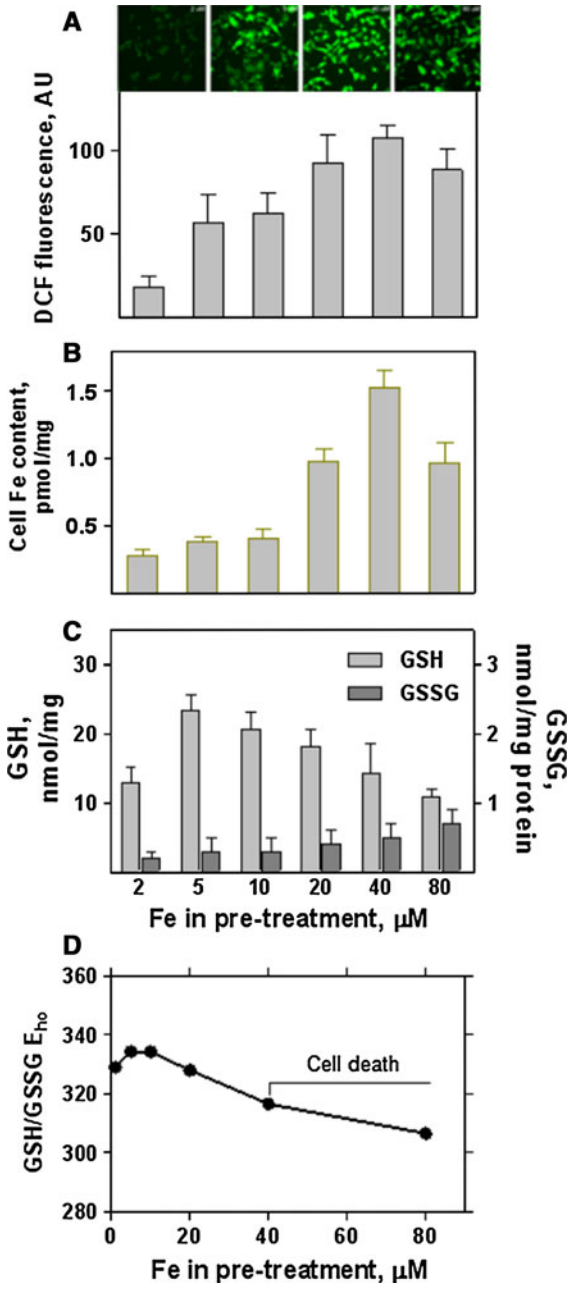
Iron accumulation, ROS and inflammation in Alzheimer's disease

Iron accumulation is a common feature of a number of neurodegenerative disorders of the central nervous system that include Parkinson's disease, Alzheimer's disease, Huntington's disease, Friedreich's ataxia, neuroferritinopathy and Amyotrophic Lateral Sclerosis (Jellinger 1999; Bartzokis et al. 2000; Sayre et al. 2000; Perry et al. 2003; Zecca et al. 2004; Berg and Youdim 2006; Wilson 2006; Weinreb et al. 2011).

Alzheimer's disease (AD) manifests as a dementia of slow progression characterized by the neuropathological findings of senile plaques (SP), extracellular insoluble aggregates of beta-amyloid, and neurofibrillary tangles (NFT), intracellular lesions consisting of paired helical filaments formed of hyperphosphorylated cytoskeletal protein tau (reviewed in (Davison 1987; Reynolds et al. 2007). Most AD cases are sporadic, whereas some rare familial forms are related to mutations in genes linked to the processing of the amyloid precursor protein (Yamada 2004). Available data suggest that SP and NFT represent two stages of response that take place in different brain regions (Braak and Braak 1991). In the neocortex, there is a predominance of SP, whereas in the entorhinal cortex and hippocampus there is a predominance of NFT (Yasha et al. 1997). In this respect, NFT correlates more closely with the clinical symptoms than SP (Duyckaerts 2004). Although the significance of SP aggregates remains ill-defined, current hypotheses propose that oligomeric and intracellular amyloid could mediate neurotoxicity through its ability to disrupt synaptic activity, cause calcium dyshomeostasis and even facilitate hyperphosphorylation of tau (LaFerla et al. 2007). The finding that Aβ-derived diffusible ligands promote tau phosphorylation suggests that tau pathology is secondary (Davis et al. 1999). Nevertheless, a large study that analyzed the prevalence of the amyloid and neurofibrillary lesions as a function of age showed that the NFT precedes SP by several decades (Braak and Braak 1991; Duyckaerts et al. 2009).

One of the more sought after questions that prevails current research in AD relates to the elucidation of the pathophysiological cascade of events leading to neuron dysfunction and death. It has been postulated that Aβ activation of astrocytes and oligodendrocytes upregulates pro-inflammatory cytokine expression and enhances the production of ROS, thus initiating an inflammatory cascade that leads to neuronal death (Johnstone et al. 1999; Meda et al. 2001; Ramirez et al. 2008; von Bernhardi 2007). In fact, a preeminent feature of the disease is the abundance of activated astrocytes and microglia in close proximity to the SPs (Itagaki et al. 1989). There is no certainty on whether inflammation precedes or is a consequence of neurodegeneration. Current evidence favors the hypothesis that microglia dysfunction induced by inflammation and aging-related cellular stress results in the release of further inflammatory stimuli that leads to neuronal dysfunction and death (von Bernhardi 2007).

Oxidative stress has been proposed as contributor to neuronal synaptic dysfunction and loss in AD (Joseph et al. 2005; Mancuso et al. 2007; Shi and Gibson 2007). Specifically, iron accumulation has been reported in brain regions affected by neurodegeneration (Connor and Benkovic 1992; Castellani et al. 2007). This observation has been revealed by post-mortem studies (Zecca et al. 2004) and confirmed by MRI studies (Falangola et al. 2005; Jack et al. 2005).



◀ **Fig. 3** Iron, ROS and GSH relationship**.** Dopaminergic SHSY-5Y cells were seeded and grown for 8 days in standard culture medium and then cultured for 2 days in medium containing 2, 5, 10, 20, 40, or 80 μM Fe prior to the assays. **a** ROS production determined by dichlorofluorescein (DCF) fluorescence. The upper part of the panel shows representative images of cells pre-cultured in 2, 5, 40, or 80 μM Fe. The lower panel shows the quantification of DCF fluorescence intensity in cells pre-cultured in the stated Fe concentrations. **b** Total iron content determined by atomic absorption spectroscopy performed as described (Mura et al. 2006). **c** GSH and GSSG content in cells pre-treated with the stated iron concentrations. **d** GSH/GSSG half-cell reduction potential of cells pre-treated with the stated iron concentrations. For experimental details of figures **a**, **c** and **d** see (Núñez et al., 2004)

There is a correlation between the degrees of iron accumulation and cognitive decline in AD subjects. As with NFT, hippocampal iron accumulation in AD subjects (evaluated by gradient-echo MRI) correlates well with cognitive decline assessed by the mini-mental state examination performance (Ding et al. 2009). In the hippocampus of AD subjects, iron is localized in NFT-containing neurons as well as in neuritic processes surrounding SP (Quintana et al. 2006). Concurrent with iron localization, a significant increase in products of lipoperoxidation (4-hydroxy-nonenal) has been also described in neurons containing NFT and neuritic processes in the periphery of SP (Markesbery and Lovell 1998; Sayre et al. 2000). Recent studies have demonstrated that molecules with the ability to bind iron are effective in slowing disease progression in AD models and patients.

Available evidence suggests that Tf-bound iron would not participate in iron accretion in AD. In quantitative terms, Tf is globally decreased in the brain of AD subjects and instead of being in its regular location in oligodendrocytes, it appears to be sequestered in SP (Connor et al. 1992); Tf receptor is highly expressed in the neocortex and hippocampus of AD brains but localized to capillary endothelium with only a weak expression in neurons (Jefferies et al. 1996). Thus, NTBI uptake could be the primary mechanism by which neurons acquire iron. Considering that iron accumulates in the brain with age (Bartzokis et al. 1997; Barnham and Bush 2008), that major neurodegenerative disorders such as AD and Parkinson's disease are characterized by elevated tissue iron (Thompson et al. 2001; Collingwood et al. 2005; Collingwood et al. 2008; Barnham and Bush 2008) and that excess iron is a strong promoter of oxidative stress, it is of relevance to investigate with more detail the potential role of iron homeostasis dysregulation in microglia and neurons in the inflammatory cascade discussed above.

Iron accumulation, ROS and inflammation in Parkinson's disease

A large body of evidence indicates that in Parkinson's disease iron accumulates in the dopaminergic neurons

of the SN pars compacta (Youdim et al. 1989; Hirsch et al. 1991; Gorell et al. 1995; Vymazal et al. 1999). Due to iron's capacity to catalyze the formation of free radicals, most likely iron accumulation contributes to the progression of events leading to neuronal death.

Considerable advances in the comprehension of the events that lead to neuronal death have been obtained with the use of the parkinsonian toxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (Langston et al. 1983). MPTP is a potent inhibitor of complex I of the mitochondrial electron transport chain, through which inhibition produces the death of dopaminergic neuron of the SN (Schapira et al. 1990; Schapira and Gegg 2011).

Neuronal death caused by MPTP or 6-hydroxydopamine (another inhibitor of complex I) intoxication is prevented by the pharmacologic or genetic chelation of iron (Kaur et al. 2003; Shachar et al. 2004; Youdim et al. 2004; Youdim and Buccafusco 2005; Zheng et al. 2010) or by dysfunction of the iron transporter DMT1 (Salazar et al. 2008). A recent study in mesencephalic dopaminergic neurons shows that low (0.25–0.5 μM) concentrations of MPP+, the active metabolite of MPTP, induces neuritic tree collapse without loss of cell viability (Gómez et al. 2011). This collapse was effectively prevented by decreasing iron supply or by the addition of antioxidants. Thus, it seems plausible that increased intracellular iron and ROS are involved in the early steps of dopaminergic neuron dysfunction, prior to cell death. At later times, a vicious cycle of iron accumulation, complex I dysfunction and ROS increase may result in uncontrolled oxidative damage and cell death.

ROS have a negative effect on complex I activity. Experiments with isolated synaptosomal mitochondria revealed that low concentrations of H_2O_2 decrease complex I activity by 10%. This relatively minor effect of H_2O_2 was additive to partial inhibition of complex I induced by low concentrations (5 nM–1 μM) of rotenone (Chinopoulos and Adam-Vizi 2001). Similarly, sub-mitochondrial particles exposed to $O_2^{\bullet -}$, H_2O_2, or $^{\bullet}OH$ presented decreased activity of NADH dehydrogenase, a marker of complex I activity (Zhang et al. 1990). Thus, an initial inhibition of complex I could generate a positive loop between ROS generation and further complex I inhibition.

Early post-mortem studies revealed decreased levels of GSH in degenerating SN of Parkinson's disease patients (Perry et al. 1982; Sofic et al. 1988; Sian et al. 1994), implicating that GSH depletion may play a major role in the neurodegenerative process. The question arises whether GSH depletion is an early event during the progression of the disease or a reflection of increased oxidative stress resulting from increased ROS and iron accumulation. Chronic submaximal inhibition of GSH synthesis in N27 dopaminergic cells produces 50% inhibition of mitochondrial electron transport chain complex I without causing cell death (Chinta and Andersen 2006). Thus, decreased level of GSH per se could inhibit mitochondrial function. Under this view, inhibition of complex I by decreased GSH levels results in increased electron leakage from the electron transport chain, increased ROS and iron accumulation. The question remains as to which of the three processes, decreased GSH levels, inhibition of complex I activity or iron accumulation, initiates the oxidative spiral. A reasonable assumption is that if one of them develops the other two will follow.

Fe–S cluster synthesis and its relevance to Parkinson's disease

Iron–sulfur (Fe–S) clusters are small inorganic cofactors formed by tetrahedral coordination of iron atoms with sulfur groups. The Fe–S clusters most commonly found in eukaryotes are 2Fe–2S and 4Fe–4S. Fe–S clusters are cofactors for proteins involved in many cellular processes, including electron transport, enzymatic catalysis and regulation and DNA synthesis (Lill and Muhlenhoff 2008). The proteins that contain Fe–S clusters in eukaryotes are present in mitochondria, endoplasmic reticulum, cytoplasm and nuclei (Lill et al. 2006; Sheftel et al. 2010).

The mitochondrion plays a central role in the generation and biology of Fe–S clusters since it holds the assembly machinery responsible for their synthesis (Lill et al. 2006). Fe–S cluster synthesis also occurs in the cytoplasm, albeit at a minor scale (Ye and Rouault 2010). Iron is transported inside the mitochondria by the transporter mitoferrin (Shaw et al. 2006). In eukaryotes, the Fe–S cluster assembly machinery of mitochondria comprises the cysteine desulfurase, Nfs1, which provides sulfur and Isu1, a protein that serves as a molecular scaffold for the assembly of the Fe–S cluster. Synthesis of the transiently bound Fe–S cluster on the Isu scaffold proteins requires reduced (ferrous) iron and the input of electrons, presumably to

generate sulfide anion from cysteine (Ye and Rouault 2010). The assembled cluster is transferred from the scaffold to recipient apoproteins. Finally, synthesized Fe–S clusters are either transferred to mitochondrial apo-proteins or exported into the cytoplasm by the Fe–S cluster export machinery. This machinery involves the ABC-B7 transporter, the antioxidant GSH and the sulfhydryl oxidase Erv1, which is also involved in protein import (Lill et al. 2006; Sheftel et al. 2010).

Mitochondria contain numerous Fe–S proteins essential for their function. Complex I has eight Fe–S clusters, succinate dehydrogenase (complex II) has three Fe–S clusters and ubiquinone: cytochrome c oxidoreductase (complex III) has one [2Fe–2S] cluster. Additionally, mitochondrial aconitase contains a 4Fe–4S cluster in its catalysis center (Beinert et al. 1983). Proteins involved in purine metabolism in the cytoplasm like xanthine oxidase and phosphoribosyl pyrophosphate amido-transferase contain two [2Fe–2S] and one [4Fe–4S] cluster, respectively (Unciuleac et al. 2004; Martelli et al. 2007). In the nucleus, proteins involved in DNA repair and replication contain Fe–S clusters (Sheftel et al. 2010).

Recent data from our laboratory indicate that inhibition of complex I by rotenone results in decreased synthesis of Fe–S clusters, as shown by the decreased activity of Fe–S cluster-containing enzymes such as cytoplasmic aconitase, mitochondrial aconitase, xanthine oxidase and glutamyl phosphoribosyltransferase as well as the activation of cytoplasmic IRP1 (Mena et al. 2011). As mentioned above, disassembly of the 4Fe–4S cluster in IRP1 alters the active site accessibility and determines IRP1 binding to target mRNAs (Wallander et al. 2006; Cairo and

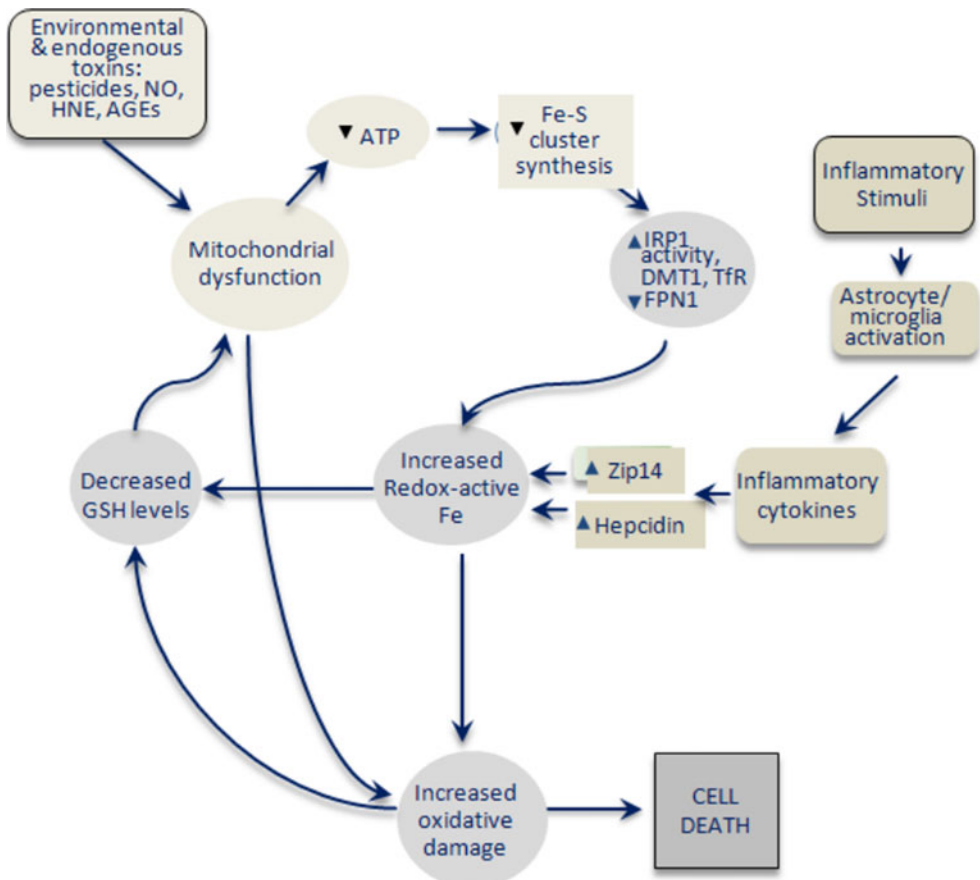


Fig. 4 A positive feedback loop resulting in uncontrolled oxidative load**.** Mitochondrial dysfunction results in decreased Fe–S cluster synthesis which leads to activation of IRP1, increased DMT1 and decreased FPN1 synthesis, iron accumulation, increased oxidative stress and increased GSH consumption. Decreased GSH produces further complex I inhibition. *NO* nitric oxide, *HNE* 4-hydroxynonenal, *AGEs* advanced glycation end products

Recalcati 2007). We think that decreased activity of complex I results, via decreased Fe–S cluster synthesis and the consequent activation of IRP1, in a false "low iron" signal that activates the iron uptake system. In consequence, diminished Fe–S cluster synthesis could play a fundamental role in the accumulation of iron observed in Parkinson's disease.

Defects in mitochondrial electron transport in other neurodegenerative diseases such as Alzheimer's disease, Huntington disease and Friedreich's ataxia (Brennan et al. 1985; Parker et al. 1994; Lodi et al. 1999), have raised the hypothesis that mitochondrial dysfunction is a common cause for a number of neurodegenerative and non-neurodegenerative diseases (Schapira 2006).

A positive feedback loop in the death of neurons in Parkinson's disease

We propose that inhibition of mitochondrial complex I by endogenous and/or exogenous toxins, and inflammatory processes produced by trauma or other causes, result in a vicious cycle of increased oxidative stress, increased iron accumulation and decreased GSH content (Fig. 4). In this scheme, neuronal death linked to complex I dysfunction is brought about by a positive feedback loop in which complex I inhibition results in decreased Fe–S cluster synthesis, IRP1 activation, increased DMT1 and TfR expression and iron accumulation. Complex I dysfunction and increased cellular iron result in decreased GSH levels. Both increased oxidative stress and low GSH levels further inhibit complex I activity. Another input to this cycle is contributed by inflammatory cytokines that induce hepcidin synthesis, which by inducing FPN1 degradation results in increased glial and neuronal iron content. Inflammatory cytokines also transcriptionally regulate DMT1, FPN1 and Zip 14 synthesis, and activate IRPs (see text). Central to this scheme is the deregulation of iron homeostasis since iron chelators effectively block cell death (Zhu et al. 2007; Kupershmidt et al. 2011; Weinreb et al. 2011).

Conclusions

A number of neurodegenerative diseases such as Parkinson's, Alzheimer's and Huntington, present diminished activity of mitochondrial complex I, iron accumulation, oxidative stress and inflammation. It is possible that the initiation of any one of these processes will initiate and enhance the others through the generation of a positive feedback loop that will produce apoptotic neuronal death. Intervention of this positive loop should result in prolonged life of the affected neurons. Still unanswered is the question of why SN pars compacta neurons are so particular prone to this deregulation.

Acknowledgments This work was financed by grant 1100599 from Fondo Nacional de Ciencia y Tecnología Chile, (FONDECYT) and by project ICM-P05-001-F from the Millennium Scientific Initiative, Ministerio de Economía, Chile.

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