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REVIEW

Brain iron pathways and their relevance to Parkinson's disease

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Abstract

A central role of iron in the pathogenesis of Parkinson's disease (PD), due to its increase in substantia nigra pars compacta dopaminergic neurons and reactive microglia and its capacity to enhance production of toxic reactive oxygen radicals, has been discussed for many years. Recent transcranial ultrasound findings and the observation of the ability of iron to induce aggregation and toxicity of α-synuclein have reinforced the critical role of iron in the pathogenesis of nigrostriatal injury. Presently the mechanisms involved in the disturbances of iron metabolism in PD remain obscure. In this review we summarize evidence from recent studies suggesting disturbances of iron metabolism in PD at possibly different levels including iron uptake, storage, intracellular metabolism, release and post-transcriptional control. Moreover we outline that the interaction of iron with other molecules, especially α-synuclein, may contribute to the process of neurodegeneration. Because many neurodegenerative diseases show increased accumulation of iron at the site of neurodegeneration, it is believed that maintenance of cellular iron homeostasis is crucial for the viability of neurons.

Keywords: auto-oxidation, iron metabolism, oxidative stress, Parkinson's disease, α-synuclein aggregation, transcranial ultrasound.

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A pivotal role of iron in the pathogenesis of Parkinson's disease (PD) has been emphasized because of it's capacity to enhance the production of oxyen radicals and accelerate neuronal degeneration (Riederer et al. 1988; Sofic et al. 1988; Jenner et al. 1992; Dexter et al. 1993; Youdim et al. 1993; Gerlach et al. 1994; Wang et al. 1995; Gerlach and Riederer 1996; Gerlach et al. 1997; Griffiths et al. 1999; Youdim et al. 1999; Shoham and Youdim 2000).

Iron is increased in the substantia nigra (SN) in Parkinson's disease (PD; Sofic et al. 1988; Dexter et al. 1989; Riederer et al. 1989, 1992; Griffiths et al. 1999); until recently, however, standard structural neuroimaging techniques were unable to demonstrate elevated iron levels in the SN of PD patients (Olanow 1992; Antonini et al. 1993; Ye et al. 1996). Only the development of more sophisticated MRI-techniques made reliable assessment of the iron increase in PD possible (Gorell et al. 1995; Ryvlin et al. 1995; Bartzokis et al. 1999).

Several studies have suggested that transcranial ultrasound (TCS) may also demonstrate increased iron content in the SN of patients with PD, even prior to the development of the disease symptoms (Becker et al. 1995; Berg et al. 1999a, 2001). Transcranial ultrasound is a new, easily applicable ultrasound technique enabling the two dimensional visualisation of the brain parenchyma. Parkinson's disease

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Abbreviations used: AGEs, advanced glycation endproducts; IREs, iron-responsive elements; IRPs, iron-regulatory proteins; Lf, Lactotransferrin; MHC, major histocompatibility complex; MTf, melanotransferrin; MTfR, melanotransferrin receptor; NM, neuromelanin; PD, Parkinson's disease; SN, substantia nigra; TCS, transcranial ultrasound; TF, transferrin; TfR, transferrin receptor.

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patients exhibit a substantially increased echogenicity of the SN (Becker et al. 1995; Berg et al. 1999a, 2001). In over 90% of PD patients, the SN is superimposed by extended white signals (increased echogenicity) which predominate contralateral to the clinically more affected body side. The underlying reason for the elevated tissue echogenicity has been evaluated in postmortem studies, revealing a close correlation between echogenicity of the SN and tissue iron content (Berg et al. 2001). Animal experiments con®rmed that iron substantially increases tissue echogenicity (Berg et al. 1999b).

Increased echogenicity of the SN has been detected not only in PD patients but also in 8.6% of healthy adults. [^{18}F]DOPA PET studies performed in 10 of these healthy adults revealed a reduced [^{18}F]DOPA uptake of the putamen and caudate nucleus below the standard deviation of a control group in 50±60% of these subjects. A reduction in [^{18}F]DOPA uptake suggests a subclinical alteration of nigral neurons in these healthy subjects with SN hyperechogenicity. Thus a phenotype of SN hyperechogenicity characterizes both patients with PD and subjects with subclinical nigral injury, which might proceed to typical PD later in life (Berg et al. 1999a, 2001) dependent upon other, possibly environmental, factors (Spencer and Butter®eld 1995).

These ®ndings and the interaction of iron with α-synuclein (Hashimoto et al. 1999; Paik et al. 1999; Münch et al. 2000; Osterova-Golts et al. 2000) have reinforced the critical role of iron in the pathogenesis of nigrostriatal injury and suggest that nigral iron elevation in PD may underlie a basic pathogenetic mechanism, rather than re¯ecting a secondary degenerative phenomenon. Therefore, the way iron is distributed and metabolised within the brain and possible alterations in this system in PD deserve further investigations.

In this review we summarize the pathways of brain iron metabolism, including iron uptake, storage, release, intracellular metabolism and post-transcriptional control and discuss evidence for alterations of iron metabolism within these pathways.

Iron uptake

Transferrin (Tf) and transferrin receptor (TfR)

This bilobal glycoprotein of approximately 80 kDa with its two homologous domains both containing one high-af®nity Fe(III)-binding site (Ponka 1999) is the most important iron transport protein in humans (Fig. 1). Most studies on transferrin (Tf) within the brain focus upon its role in iron uptake via the transferrin receptor (TfR), although it is also involved in intracellular iron processing and possibly in iron ef¯ux (Aisen et al. 1999; Ponka 1999).

Normally, cellular iron acquisition from Tf is accomplished by glycoprotein-receptor mediated endocytosis, via the TfR, which is likely to be identical in all cell types (Ponka 1999). The transferrin receptor is thought to play a central role in the regulation of cellular iron content, as iron levels are controlled by regulating the level of expression of the TfR. In spite of the iron increase occuring in the SN of PD patients, evidence from recent studies argue against the hypothesis that iron uptake by the Tf/TfR pathway is increased in the SN of PD patients.

The most striking argument comes from immunohistochemical studies, demonstrating a signi®cant loss of TfR-binding (about 60%) similar to the degree of neuronal loss (about 80%; Morris et al. 1994), and a decrease in Tf-binding sites on perikarya of melanized neurons in the SN of Parkinsonian brains (Faucheux et al. 1997). In 6-hydroxydopamine lesioned rats, a decrease of tyrosine

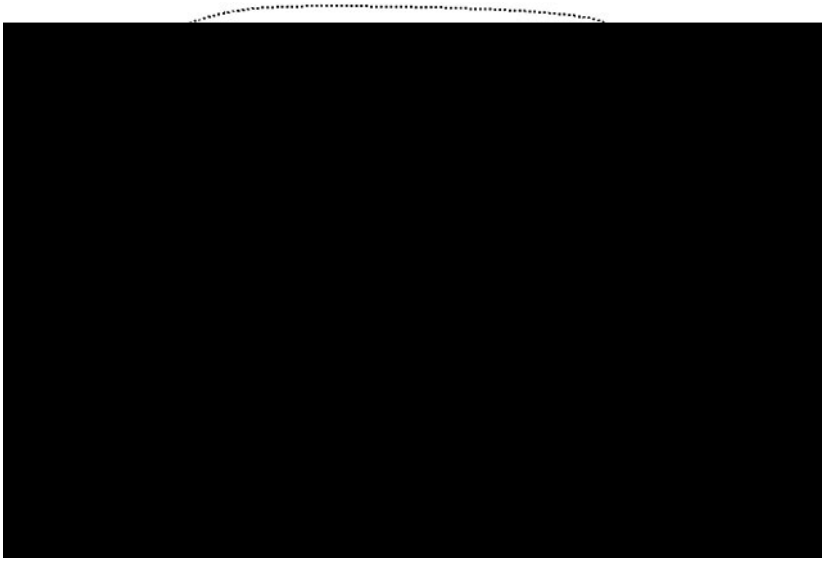

Fig. 1 Pathways of iron metabolism with iron uptake mediated by transferrin (Tf) and the transferrin receptor (TfR), in possible interaction with the hemochromatosis gene-product (HFE), melanotransferrin (MTf) and the melanotransferrin receptor (MTfR), lactoferrin (Lf) and the lactoferrin receptor (LfR), divalent cation transporter (DCT1) and stimulator of iron transport (SFT). Iron storage takes place in ferritin and neuromelanin (NM). Iron release is mediated by ascorbate, ATP, catecholamines, Tf, ceruloplasmin (Cp) and heme oxygenase 1 (HO-1). Intracellular iron metabolism involves, e.g. DCT1, SFT, Cp and HO-1. Iron related proteins (IRPs) are involved in the post-transcriptional control of genes mediating iron transport and storage.

hydroxylase- and TfR positive cells in the SN can be demonstrated (He et al. 1999). Thus accumulation of iron in the SN in PD does not seem to depend upon increased Tf or TfR. Moreover iron distribution within the brain does not primarily seem to be dependent on iron acquisition of the cells via TfR as iron levels do not correspond to TfR or Tf levels and the localization of Tf in the brain coincides poorly with its receptors (Dwork et al. 1988; Hill 1988; Morris et al. 1992). In old rats subjected to dietary iron de®ciency, brain iron levels are lowered but neuronal TfR mRNA remains unaffected, while these parameters are unchanged in iron overloaded rats (Moos et al. 1999). Additionally, atransferrinemia leads to an elevated tissue iron content (Hayashi et al. 1993) suggesting that a loss of Tf and TfR binding capacity rather than an excess might lead to iron accumulation in the cell. This observation and results from animal experiments (Banks et al. 1988; Ueda et al. 1993; Takeda et al. 1998) have led to the hypothesis that the decrease of Tf and/or it's receptor might stimulate iron uptake via non-Tf pathways, which could also play a role in the pathogenesis of PD.

Proteins with similar distribution as the TfR and possible interaction with the Tf/TfR-system

Melanotransferrin (MTf)

The structure of MTf and the distribution of its receptor (melanotransferrin receptor, MTfR) is similar to Tf and TfR, respectively (Rothenberger et al. 1996). The two different molecular forms (one soluble, the other attached to the plasma membrane) have one iron binding site and provide an alternative, presumably energy-dependent route for iron transport (Baker et al. 1992; Alemany et al. 1993; Kennard et al. 1995; Fig. 1). An association with neurodegenerative disorders has been assumed because an increased expression of MTf has been detected in reactive microglia in Alzheimer's disease (Jefferies et al. 1996). In brain tissue of PD, however, MTf expression is reported to be unchanged (Jefferies et al. 1996; Kennard et al. 1996), although the number of patients surveyed in these studies was small.

Hemochromatosis gene and gene product (HFE)

Positional cloning of this transmembrane glycoprotein has revealed a structural homology to major histocompatibility complex (MHC) class I proteins (Lebron et al. 1998). In the healthy cell, iron overload is prevented by both the association of HFE with TfR (which decreases TfR af®nity for Tf; Feder et al. 1998) and the ability of HFE to enhance dissociation of Tf from it's binding site at the receptor (Lebron et al. 1998; Fig. 1). The loss of HFE repression of transferrin uptake is therefore thought to contribute to iron overload in some tissues in hemochromatosis (Feder et al. 1998). Moreover, HFE is thought to regulate intracellular iron because its expression markedly decreases levels of ferritin and modestly increases levels of TfR in HeLa cells (Gross et al. 1998). Mutations in the HFE gene may thus lead to an increase in cellular ferritin and iron levels (Feder 1999). In a mouse model of hemochromatosis (beta2-microglobulin knock out, Santos et al. 1996), however, an abnormal accumulation of iron was demonstrated in the liver, while the level of brain iron was virtually normal (Moos et al. 2000). In humans, in contrast, iron accumulation within the brain by mutations of HFE has been described. Although reports are rare and iron accumulation of the brain is by no means a leading sign of this disorder, autopsy (Sheldon 1928; Cammermeyer 1947; Miyaski et al. 1977), neuroimaging (Nielsen et al. 1995; Berg et al. 2000) and clinical ®ndings (Nielsen et al. 1995; Demarquay et al. 2000) underscore a role of HFE in iron metabolism in the human brain. However, whether there is any relevance for iron accumulation within the SN in PD remains to be elucidated.

Lactotransferrin (Lf) and lactotransferrin receptor (LfR)

Lactotransferrin (Lf; also referred to as lactoferrin), an 80-kDaA glycoprotein belonging to the Tf family with a Tf-similar structure (Anderson et al. 1989), has been localized in human brain to neurons, glial cells and microvasculature (Aisen and Leibman 1972; Fig. 1). Lf crosses the blood±brain-barrier in an iron saturated and native form (Fillebeen et al. 1999b) and is also synthesized within the brain (Fillebeen et al. 1999a). Iron binds more avidly to Lf than to Tf (Birgens 1991), and in contrast to Tf, the binding of Lf to its receptor is independent of its degree of iron saturation (Davidson and Lonnerdal 1989). In addition, LfR expression is not regulated by intracellular iron (Yamada et al. 1987). In this absence of intracellular feedback the expression of LfR could be launched out of control (according to Faucheux et al. 1995). Moreover, Lf itself might directly enhance the generation of oxygen radicals (Ambruso and Johnston 1981; Faucheux et al. 1995; Fillebeen et al. 1999b). Immunohistochemical studies of PD patients revealed an increase of lactoferrin receptors (LfR) on SNpc neurons and microvessels (Faucheux et al. 1995), the degeneration of predominantly Lf-positive neurons and a higher Lf immunolabeling in a quantitative analysis of surviving neurons of the SNpc (Leveugle et al. 1996). These ®ndings may argue for the relevance of Lf/LfR in SN iron accumulation and the subsequent degeneration of dopaminergic neurons in PD.

On the other hand, several lines of evidence underscore the potential protective properties of Lf and suggest that the increase in LF/LfR is secondary to the iron accumulation in the SN. Lf inhibits the formation of hydroxyl radicals and reduces the auto-oxidation of membranes (Britigan et al. 1991). Moreover, in the MPTP mouse model of PD, Lf expression and levels of other antioxidants were found to be

elevated (Fillebeen et al. 1999b). Thus, Lf may also have antioxidative properties in PD, acting as an iron scavenger and antioxidant. To date, it is unclear whether changes observed in the LF/LfR system are of primary nature or secondary to other pathological processes.

Divalent cation transporter (DCT1), metal transporter protein (MTP1) and stimulator of iron transport (SFT)

DCT1, a 561-amino-acid protein with 12 putative membrane-spanning domains is one of the two isoforms of the divalent metal transporter 1 (DMT1) (Nramp2 and DCT1) (Roth et al. 2000) and is ubiquitously expressed (Gunshin et al. 1997). With its broad substrate spectrum, DCT1 mediates active proton-coupled transmembrane transport including the transport of iron not only across the cell membrane but also out of the endosomes (Andrews 1999; Fig. 1). The finding of duodenal up-regulation of DCT1 by dietary iron deficiency (Gunshin et al. 1997) and increased duodenal expression in hemochromatosis (Fleming et al. 1999) indicates an important role for this protein in iron metabolism. The ubiquitously expressed mRNA of DCT1 has been found only in moderate amounts in neurons (but not in glial cells) of the SN (Gunshin et al. 1997; Roth et al. 2000). Because of its role in physiological iron transport (Conrad et al. 1999), defects in this membrane protein have been suggested to play a crucial role in brain iron imbalance and neuronal death in neurodegenerative diseases (Gunshin et al. 1997).

Recently the metal transporter protein 1 (MTP1) has been isolated and characterized. This protein is one of a number of MTPs expressed in the CNS. Involvement in iron uptake, intracellular iron metabolism and iron exporting has been described, with overexpression of MTP1 leading to intracellular iron depletion (Abboud and Haile 2000).

In contrast to the broad spectrum of DCT1, the transmembrane protein stimulator of iron transport (SFT) is relatively selective for the uptake of iron into the cell and export of iron out of endosomes (Gutierrez et al. 1997; Gutierrez and Wessling-Resnick 1998). SFT mediates both the uptake of Fe^{2+} and the uptake of Fe^{3+} and is involved in Tf dependent and Tf independent iron uptake (Yu and Wessling-Resnick 1998; Fig. 1). An increased expression in hemochromatosis patients implies an association with intracellular iron metabolism (Gutierrez et al. 1998).

The physiological functions of these iron transporters and their relevance for PD require further study. Present data suggesting that these iron transporters are responsible for nigral iron accumulation in PD are preliminary and unconvincing.

Iron storage

Ferritin

Knowledge regarding the subcellular distribution of iron within the brain is scarce, although it is known that subcellular iron is bound to various proteins, amongst which ferritin is most important within the SN (Dexter et al. 1991; Hallgren and Sourander 1958; Riederer et al. 1989; Fig. 1). This ubiquitous protein consists of an iron core and a shell of 24 subunits, which are assembled into two different molecules: L-ferritin (19 kDa) and H-ferritin (21 kDa), the proportions of which vary between tissues (Theil 1987). H-ferritin takes up and releases iron more rapidly than L-ferritin (Drysdale 1988; Cairo et al. 1991). In contrast to Tf, ferritin levels in the human brain are higher in the nuclei of the extrapyramidal system, notably in the non-neuronal cells of the SN (Connor et al. 1990; Connor and Benkovic 1992). The cellular ferritin level is regulated by iron at the post-transcriptional level (Hentze and Kuhn 1996). In contrast to L-ferritin, synthesis of H-chain ferritin can be increased independent of changes in iron levels (Arosio et al. 1991). Other substances, such as NO and cytokines, may also influence the transcriptional or post-transcriptional synthesis of ferritin (Mascotti et al. 1995).

Oligodendrocytes, microglia and astrocytes contain high levels of ferritin and the number of ferritin-positive astrocytes increases with advancing age (Connor et al. 1990). To date, little is known about the localization of ferritin in neurons. Immunostaining for ferritin in neurons of the cortex of a subhuman primate has been described (Connor et al. 1994), however, absence of ferritin protein in the SN pars compacta neurons has been reported in mice (Moos et al. 2000). This might be due to species differences. On the other hand technical factors need to be taken into account: Neurons immunostain primarily for H-, rather than L-ferritin, whereas microglia mainly contains L-ferritin and oligodendrocytes immunolabel for both L- and H-ferritin (Connor et al. 1994). Thus the scarcity of knowledge regarding the possible localization of ferritin within neurons of the basal ganglia may result from the use of a polyclonal antibody which binds preferentially to L-ferritin in many of these studies (Kaneko et al. 1989; Jellinger et al. 1990; Connor and Benkovic 1992).

In PD, the number of ferritin-immunoreactive microglial cells in the SN increases dramatically with many reactive microglial cells located in close proximity to melanin-containing or degenerating neurons (Jellinger et al. 1990). This might be of importance for the pathogenesis of PD because iron release from ferritin induced by activated microglia has been demonstrated (Yoshida et al. 1995) and a contribution of iron released from ferritin to free radical-induced cell damage has been shown (Double et al. 1998).

The binding capacity of ferritin influences levels of unbound cytosolic iron and may also play a role in free radical formation. Ferritin is able to bind up to 4500 iron atoms (Halliwell and Gutteridge 1986). Ferritin cores in the SN of PD patients, however, are reported to be denser and contain more iron than those in the SN of healthy subjects

(Grif®ths et al. 1999). An increased loading of ferritin may encourage free radical generation and subsequent neuronal damage (Double et al. 1998; Grif®ths et al. 1999). Alternatively, the denser loading of the cores might be the result of an attempt to protect the cell from iron induced oxidative damage.

Neuromelanin

Recent studies con®rm that the number of pigmented and non-pigmented dopamine neurons is constant in normal aging while in PD there is a preferential loss of pigmented neurons (Mann and Yates 1974, 1993; McGeer et al. 1977; Gibb and Lees 1991; Pakkenberg et al. 1991; Muthane et al. 1998; Kubis et al. 2000). Melanized neurons are also the main target of pathogenetic mechanisms in PD (Hirsch et al. 1988). The insoluble black-brown granular pigment neuromelanin (NM) is synthesized via the oxidation of cytosolic catechols (Bridelli et al. 1999; Sulzer et al. 2000). NM is an excellent binder of metal ions, especially iron (Ben-Shachar et al. 1991). Synthetic dopamine derived melanin has two iron binding sites, a high and a low af®nity site (Ben-Shachar et al. 1991). Similar binding properties have been observed for the binding of iron by neuromelanin in the substantia nigra pars compacta dopaminergic neurons (Jellinger et al. 1992) and recently with neuromelanin in vitro (Double et al., unpublished data). Thus, increases of heavy metal content in regions containing NM may result in alterations in the local distribution and reactivity of such metals (Good et al. 1992; Swartz et al. 1992; Gerlach et al. 1995). Of all the inorganic components, iron has been shown to be present in highest concentrations within NM (Zecca and Swartz 1993; Zecca et al. 1992, 1994; Fig. 1). Moreover, the iron content of NM in the SN of normal subjects aged 70±90 years has been shown to account for 10±20% of the total iron contained in the SN (Shima et al. 1997; Zecca et al. 2001). In PD patients, iron levels of NM are higher than in controls (Good et al. 1992; Jellinger et al. 1992). This is of special interest as it appears that the amount of iron bound to NM determines whether this molecule acts as a cytoprotectant which sequesters redox active metal ions within the cell or whether, in the presence of excess iron, it promotes the formation of reactive oxygen radicals (Ben-Shachar et al. 1991; Wesemann et al. 1994; Zareba et al. 1995; Zecca et al. 1996; Double et al. 1999), the latter being readily suppressed by iron chelators (Ben-Shachar et al. 1991). Iron is normally toxic to cells in a dose dependent manner. In the presence of synthetic dopamine-melanin, however, iron is reported to mediate damage to dopaminergic neurons at low concentrations (Mochizuki et al. 1997). This phenomenon has been associated with the ability of melanin to reduce ferric iron to the ferrous form, thus stimulating hydroxyl radical production (Pilas et al. 1988). These data were obtained using synthetic melanin produced from the oxidation of dopamine, thus con®rmation of these ®ndings in human NM is necessary to elucidate the in¯uence of NM on hydroxyl radical production and its possible role in PD. However, it still should be noted that melanin is a two edged sword that may act as both an antioxidant as well as a pro-oxidant depending on the availability of transitional metals (Youdim et al. 1994). Under normal circumstances, wherever melanin is formed and deposited it acts as a protective antioxidant agent (skin, retina, pancreas, etc.). However, in the presence of divalent metals, especially iron and copper, it may promote the formation of reactive hydroxyl radicals and other reactive oxygen species (Youdim et al. 1994).

Another interesting ®nding is that the structure of neuromelanin in PD may be different from that in normal brain (Lopiano et al. 2000). PD neuromelanin may foster the release of iron to the cytoplasma pool and the catalysis of radical production (Double et al. 1999; Lopiano et al. 2000).

A similar role in the enhancement of lipid oxidation has recently been reported for aluminium (Meglio and Oteiza 1999), which is also bound to human NM. This result was again obtained using synthetic melanin and must therefore be considered with caution. However, aluminium concentrations are especially high in NM of the SN in PD patients (Good et al. 1992) where it may potentiate iron toxicity (Xie et al. 1996; Savory et al. 1999).

Further lines of evidence stressing the possible role of NM in the pathogenesis of PD comes from the observation that NM accumulates other toxic substances, such as MPP^+, the neurotoxic metabolite of MPTP (D'Amato et al. 1986). Species with NM-containing dopaminergic neurons are more susceptible to MPTP toxicity, suggesting a causative role of NM in neurotoxin-induced neurodegeneration (Mochizuki et al. 1993, 1997; Gerlach and Riederer 1996) Moreover, NM may facilitate the toxic in¯uence of other endo- or exotoxins in so far as it binds organic molecules that may react with metal ions or the products of their reactions (Swartz et al. 1992).

Iron release

Iron export is mediated by various molecules, such as ascorbate and ATP, as well as catecholamines which bind iron and pass through the cell membrane (Fig. 1). Distinct proteins control the release of iron from the cell and an alteration in one or more of these proteins might lead to iron accumulation within the cell. Proteins involved in iron uptake, such as Tf, are also involved in iron ef¯ux from the cell. For example, it has been demonstrated that iron ef¯ux in Caco-2 cells and human liver cells is partly dependent on Tf (Young et al. 1997; Takeda et al. 1998; Nunez and Tapia 1999).

Ceruloplasmin (Cp)/ferroxidase

This abundant glycoprotein contains $>95\%$ of plasma copper

(Klomb and Gitlin 1996). In addition to participating in the acute-phase response of in¯ammation and antioxidant systems (Fitch et al. 1999), Cp acts as a copper-dependent ferroxidase, which may impact iron metabolism and homeostasis. Cp is critical for iron export from some cell types, converting ferrous to ferric iron and accelerating iron incorporation into apotransferrin (Harris et al. 1995, 1999; Kaplan and O'Halloran 1996; Askwith and Kaplan 1998). The absence of Cp in aceruloplasminemia results in iron accumulation in the basal ganglia associated with neuronal degeneration in these tissues and the retina (Klomb and Gitlin 1996; Harris et al. 1995). Cp can also increase iron uptake by iron de®cient cells independently of Tf (Mukhopadhyay et al. 1998; Attieh et al. 1999).

The redox state of the cell is affected by this protein because Cp has antioxidant properties related to its ferrioxidase activity and contributes to antioxidant defence by scavenging H_2O_2. In the absence of apoferritin, however, Cp may also function as a pro-oxidant (Samokyszyn et al. 1989).

Cp does not cross the blood±brain barrier, but is expressed in glial cells adjacent to the brain microvasculature and pigmented dopaminergic neurons in the SN (Moshkov et al. 1979). Loef¯er et al. have also suggested local Cp synthesis in SN-neurons (Loef¯er et al. 1996). The synthesis of Cp is transcriptionally regulated by iron supply and may be enhanced by in¯ammation and oxidative stress (Loef¯er et al. 1996).

Several lines of evidence implicate Cp in the pathogenesis of PD: Ferroxidase activity is copper dependent (Loef¯er et al. 1996; Boll et al. 1999). Nigral copper levels are, however, decreased in PD, while CSF copper levels are reported to be either normal or increased (Jimenez-Jimenez et al. 1998). In accordance with the latter ®nding, ferroxidase activity in the CSF of untreated PD patients is reported to be decreased (Boll et al. 1999). Moreover, studies by Loef¯er and colleagues revealed an increase in Cp content in different brain areas, including the SN, in PD patients (Loef¯er et al. 1996), while CSF Cp concentrations are normal (Loef¯er et al. 1994) and serum Cp levels are reported to be reduced (Torsdottir et al. 1999).

Heme oxygenase 1 (HO-1)

The gene of this cellular stress protein, mediating the catabolism of heme to biliverdin in brain and other tissues is strongly induced by dopamine, oxidative stress and metal ions (Schipper 1999). In the brain it is primarily expressed in the astroglia and, when up-regulated, HO-1 promotes mitochondrial iron deposition in these cells (Schipper 1996, 1999). HO-1 protects cells by degrading pro-oxidant metallopophyrines and appears to facilitate iron ef¯ux from the cell (Fig. 1). In some animal models and in a child with complete HO-1 de®ciency underexpression or mutation of HO-1 has been shown to lead to cellular iron accumulation (Ohta et al. 2000). Under other circumstances, HO-1 may exacerbate oxidative stress by the release of free ferrous iron during heme degradation (Schipper 1995, 1999; Schipper et al. 1995). Interestingly, transfection of human HO-1 cDNA into rat astroglia induces the expression of the mitochondrial antioxidant enzyme MnSOD. MnSOD induction in these cells was abrogated by antioxidant administration indicating that HO-1 may promote intracellular oxidative stress in astroglia (Frankel et al. 2000). An immunohistochemical study has revealed increased HO-1 immunoreactivity in the neuropil of the PD nigra and intense immunostaining in Lewy bodies within affected dopaminergic neurons (Schipper et al. 1998). The proportion of SN astroglia expressing HO-1, however, is signi®cantly greater in PD compared with control brain (Schipper et al. 1998). As HO-1 immunostaining is faint or absent in other brain regions, an association with the pathogenetic process of PD seems plausible. It remains to be established, however, whether the observed changes are of a primary nature or represent a secondary phenomenon, mediated by, for example, oxidative stress.

Intracellular iron metabolism

HO-1 is not only responsible for iron export, but also for the intracellular sequestration of iron by glial mitochondria (Schipper et al. 1999). Most proteins involved in iron uptake into the cell participate in intracellular iron traf®cing (Fig. 1). Tf, for example, mediates intracellular transport of Fe^{3+}, while DCT1 (also known as Nramp2) and SFT promote iron export from endosomes (Gruenheid et al. 1999; Tabuchi et al. 2000). In addition, ferrireductases are suggested to play a pivotal role in intracellular iron metabolism because they are necessary for converting Fe^{3+} to Fe^{2+}. One ferrireductase has recently been identi®ed to be up-regulated in hemochromatosis (Patridge et al. 1998). The number and function of the ferrireductases and other possible associations with neurodegenerative diseases remain to be elucidated. Other mitochondrial iron transportes like frataxin and ATB-binding cassette 7 (ABC7) have become subjects of major interest as mutations in the genes encoding for these proteins are associated with severe neurological diseases like Friedreich's ataxia or X-linked sideroblastic anemia with ataxia. The precise role of these proteins in iron metabolism is not clear yet. Evidence from the yeast homologue of frataxin suggests that by the sequestration and regulation of the bioavailability of iron it may play an important role in the export of iron out of the mitochondrium (Radisky et al. 1999; Adamec et al. 2000). ABC7 seems to be involved in the iron export out of mitochondria, too, as mutations in the ABC7 gene seem to prevent export of Fe/S clusters generated within the mitochondria for the formation of cytoplasmatic Fe/S-proteins (Bekri et al. 2000). However, although there is some

evidence of mitochondrial disturbance in PD (Janetzky et al. 1994), there is no evidence for involvement of these proteins up to now.

Post-transcriptional control of iron homeostasis

Iron regulatory proteins (IRPs)

Iron levels within the cell are tightly regulated by the activity of cytosolic iron-regulatory proteins (IRPs) 1 and 2 which bind to RNA motifs named iron-responsive elements (IREs) (Hentze and Kuhn 1996). IREs are found in the mRNAs of genes responsible for iron metabolism; ferritin, the transferrin receptor, erythroid aminolevulinic acid synthase and mitochondrial aconitase (Haile 1999). DCT1 has also been proposed as a target for IRPs (Gunshin et al. 1997). IRPs regulate gene expression post-transcriptionally by binding to the IRE (Gunshin et al. 1997; Fig. 1). Iron hereby regulates the RNA-binding activity of IRP1: In cells with low iron content IRP1 binds IREs with high affinity while in cells with high iron content iron converts apo-IRP1 into the non-mRNA-binding form. (Aisen et al. 1999). IRP2 is regulated by iron as an iron-dependent oxidation mechanism leads to proteolysis by the proteasome (Aisen et al. 1999).

In addition to iron, changes in the redox state of the cell can affect the binding of IRPs to the IREs, indicating an important link between these iron regulating cascades and oxidative stress (Hentze and Kuhn 1996; Haile 1999; Hanson and Leibold 1999; Ponka 1999; Kim and Ponka 2000; Theil 2000). The main iron storage protein, ferritin and the iron transporter, transferrin are tightly regulated by the IRPs: In the case of high intracellular iron levels IRPs facilitate increased translation of ferritin mRNA (Klausner et al. 1993) thus limiting the pro-oxidant activity of iron (Cairo et al. 1998). Iron uptake is regulated by the IRPs protecting TfR mRNA from degradation (Hentze and Kuhn 1996), if intracellular iron level is low. High levels of ferritin within the SN in PD and the decrease of TfR activity are concordant with the expected function of IRPs in an iron abundant environment. As discussed above, however, a decrease in TfR binding sites might possibly lead to other, less well-regulated, mechanisms of iron uptake Whether this alteration might also be influenced by IRP regulation remains to be established. Moreover, several studies indicate marked alterations in the regulation of iron homeostasis under specific physiologic conditions, raising the possibility of additional regulatory mechanisms (Mikulitis et al. 1999; Garate and Nunez 2000).

Interaction of iron with α-synuclein

The association of mutations in α-synuclein and familial PD (Polymeropoulos et al. 1997; Krüger et al. 1998) and the accumulation of α-synuclein in Lewy bodies (Spillantini et al. 1997, 1998; Markopoulou et al. 1999) suggest that α-synuclein plays an important role in the pathophysiology of PD. Recently it has been shown, that iron-related oxidative stress induces aggregate formation of α-synuclein in vitro (Hashimoto et al. 1999; Paik et al. 1999). Moreover, in neuroblastoma cells stimulation of the production of intracellular aggregates that contain α-synuclein and ubiquitin by iron and an increased vulnerability to iron in cells overexpressing α-synuclein was demonstrated (Osterova-Golts et al. 2000).

Immunohistochemically, postmortem analyses of patients with incidental Lewy body disease have demonstrated that the crosslinking of α-synuclein may be promoted by advanced glycation endproducts (AGEs; Münch et al. 2000). AGE formation starts with the reaction of the amino groups of proteins and reducing sugars. This glycation leads to protein-bound Amadori products, which by subsequent dehydrations, oxidations and rearrangements form AGEs (Münch et al. 2000). The study of Münch et al. (2000) shows that AGEs are markers of iron-induced oxidative stress, as their formation is accelerated by free iron, and that they may be involved in the crosslinking of Lewy bodies. Moreover, by exerting oxidative stress on cells AGEs themselves may also serve as disease promoting factors (Münch et al. 2000).

These observations link the multiple findings of an impaired iron metabolism with new findings of the participation of intracellular aggregates in the pathogenesis of PD.

Summary

Iron is increased in the SN of PD patients. It remains to be elucidated whether elevated iron levels antedate injury of pigmented neurons or are a consequence of neuronal degeneration. Recent ultrasound studies argue in favour of the hypothesis that iron is of primary importance in the pathogenesis of PD. Evidence from numerous studies suggest disturbances of iron metabolism in PD at multiple levels, including iron uptake, storage, intracellular metabolism, release and post-transcriptional control. Imbalance in one or more of these iron regulation mechanisms could result in chelatable (free) iron accumulation within the cells, thus enhancing toxic effects and promoting cell death. Although so far it has not been possible to measure free iron levels in substantia nigra of PD, iron toxicity could operate at several levels. Free iron can induce oxidative stress via the Fenton reaction and it may promote the auto-oxidation of dopamine to its isoquinoline metabolites generating superoxide and hydrogen peroxide, which in turn can generate reactive hydroxyl radicals. Finally, iron-related oxidative stress may promote α-synuclein aggregation

linking the pathogenetically important role of iron with histopathologcial hallmarks of PD.

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