Brain iron metabolism: Neurobiology and neurochemistry

Ya Ke\textsuperscript{a,1}, Zhong Ming Qian\textsuperscript{b,c,*}

\textsuperscript{a}Department of Physiology, Faculty of Medicine, The Chinese University of Hong Kong, NT, Hong Kong
\textsuperscript{b}Laboratory of Brain Iron Metabolism, Department of Applied Biology and Chemical Technology, Hong Kong Polytechnic University, Hong Kong
\textsuperscript{c}Department of Neurobiology, Nantong University, Nantong 226001, PR China

Received 4 January 2007; received in revised form 10 April 2007; accepted 26 July 2007

Abstract

New findings obtained during the past years, especially the discovery of mutations in the genes associated with brain iron metabolism, have provided key insights into the homeostatic mechanisms of brain iron metabolism and the pathological mechanisms responsible for neurodegenerative diseases. The accumulated evidence demonstrates that misregulation in brain iron metabolism is one of the initial causes for neuronal death in some neurodegenerative disorders. The errors in brain iron metabolism found in these disorders have a multifactorial pathogenesis, including genetic and nongenetic factors. The disturbances of iron metabolism might occur at multiple levels, including iron uptake and release, storage, intracellular metabolism and regulation. It is the increased brain iron that triggers a cascade of deleterious events, leading to neuronal death in these diseases. In the article, the recent advances in studies on neurochemistry and neuropathophysiology of brain iron metabolism were reviewed.

\textsuperscript{©} 2007 Elsevier Ltd. All rights reserved.

Keywords: Brain iron metabolism; Iron transport proteins; Iron uptake proteins; Iron release proteins; Iron regulatory protein; Iron storage proteins; Brain iron misregulation; Aging; Neurodegenerative diseases

Contents

1. Introduction ................................................................................ 150
2. Mechanisms of brain iron transport .................................................. 150
   2.1. Iron transport into the brain ...................................................... 150
   2.1.1. Iron transport across the blood–brain barrier ......................... 150
   2.1.2. Iron transport across the blood–cerebrospinal fluid barrier ........ 152
   2.2. Iron transport within the brain ............................................... 152
   2.2.1. Transferrin-bound and non-transferrin-bound iron in the brain .... 152
   2.2.2. Lactoferrin receptor, melatonintransferin and divalent metal transporter 1 and iron uptake by brain cells ...... 153
   2.2.3. The role of ceruloplasmin in brain iron transport: influx or efflux ................................................................. 155
   2.2.4. The possible role of ferroportin 1, hephaestin and heme oxygenase-1 in iron efflux from brain cells ......... 156
   2.3. Iron transport out the brain .............................................. 157

Abbreviations: AD, Alzheimer's disease; BBB, blood–brain barrier; CNS, central nervous system; CP, ceruloplasmin; CSF, cerebrospinal fluid; Dcytb, duodenal cytochrome b; DMT1, divalent metal transporter 1 (previously referred to as Nramp2 or DCT1); FLP, ferritin light polypeptide; FP1, ferroportin 1; HFE, hemochromatosis protein (the protein mutated in hereditary hemochromatosis); HO-1, heme oxygenase-1; Hp, Hephaestin; HSS, Hallerorden–Spatz syndrome; IF, interstitial fluid; IRE, iron responsive element; IRP, iron regulatory protein; Lf, lactoferrin or lactotransferrin; LfR, lactoferrin receptor; MTf, melanotransferrin; NTBI, non-transferrin-bound iron; PANK2, a novel pantothenate kinase gene; PD, Parkinson's disease; ROS, reactive oxygen species; SDR2, stromal cell-derived receptor2; sMTf, a soluble form of melanotransferrin; TCT, trivalent cation-specific transporter; Tf-Fe, transferrin-bound iron; Tf/TfR, transferrin/transferrin receptor.

* Corresponding author at: Laboratory of Brain Iron Metabolism, Department of Applied Biology & Chemical Technology, Hong Kong Polytechnic University, Hong Kong. Tel.: +852 3400 8673; fax: +852 2364 9932.
E-mail addresses: yake@cuhk.edu.hk (Y. Ke), bczmqian@polyu.edu.hk (Z.M. Qian).
1 Tel.: +852 2609 6780.

0301-0082/S – see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.pneurobio.2007.07.009
3. Brain iron misregulation and neurodegenerative diseases

3.1. Genetic factors and brain iron misregulation

3.1.1. Ferritin light polypeptide mutation in neuroferritinopathy

3.1.2. A novel pantothenate kinase mutation in Hallerorden–Spatz syndrome

3.1.3. Ceruloplasmin mutation in aceruloplasminemia

3.1.4. Iron regulatory protein 2 absence and neurodegeneration in mice

3.1.5. Hemochromatosis protein mutation and Alzheimer’s and Parkinson’s diseases

3.2. Nongenetic factors and brain iron misregulation

3.2.1. Iron transport (uptake and release) proteins

3.2.2. Iron regulatory proteins

3.2.3. Iron storage proteins

3.2.4. Heme oxygenase-1 and stromal cell-derived receptor 2

3.3. The role of aging for brain iron metabolism

4. Conclusion and perspectives

Acknowledgements

References

1. Introduction

Abnormally high levels of iron in the brain have been demonstrated in a number of neurodegenerative disorders (Aisen et al., 1999; Jellinger, 1999; Berg et al., 2002; Sadzadeh and Saffari, 2004), such as Hallerorden–Spatz syndrome (HSS) (Swaiman, 1991; Zhou et al., 2001; Hartig et al., 2006), Parkinson’s disease (PD) (Berg, 2006; Berg et al., 2006; Fasano et al., 2006a, b) and Alzheimer’s disease (AD) (Ong and Farooqui, 2005; Quintana et al., 2006). Oxidative stress, resulting from increased brain iron levels, and possibly also from defects in antioxidant defense mechanisms, is widely believed to be associated with neuronal death in these disorders (Qian et al., 1997a; Qian and Wang, 1998; Sayre et al., 1999; Andrews, 1999, 2000; Ke and Qian, 2003; Aracena et al., 2006; Berg and Hochstrasser, 2006; Shamoto-Nagai et al., 2006). However, two tightly linked key questions have not been completely answered. First, why does iron increase abnormally in some regions of the brain in these disorders? Second, is excessive iron accumulation in the brain an initial event that causes neurodegeneration or a consequence of the disease process (Ke and Qian, 2003; Lee et al., 2006)? During the past years, a considerable research effort has been devoted to addressing these two key issues. A number of studies, especially the new discovery of mutations in the genes associated with brain iron metabolism, have made important contributions to our understanding of homeostatic mechanisms of brain iron metabolism and the pathological mechanisms responsible for iron-associated neurodegenerative diseases. Although some relevant points remain to be clarified, the accumulated evidence demonstrates that misregulation in brain iron metabolism is one of the initial causes for neuronal death in some neurodegenerative disorders (Ke and Qian, 2003; Thomas and Jankovic, 2004; Kaur and Andersen, 2004; Casadesus et al., 2004). The errors in brain iron metabolism found in these disorders have a multifactorial pathogenesis, including genetic and nongenetic factors. The disturbances of iron metabolism might occur at multiple levels, including iron uptake and release, storage, intracellular metabolism and regulation (Ke and Qian, 2003). It is the increased brain iron that triggers a cascade of deleterious events, leading to neuronal death in these diseases. In the review, we summarized recent advances in studies on physiological and pathophysiological aspects of brain iron metabolism.
It is also possible that a small amount of iron might cross the BBB in the form of intact Tf-Fe complex by receptor-mediated transcytosis (Moos and Morgan, 1998, 2000; Morgan and Moos, 2002). In a number of other cell types including reticulocytes, there is a pathway for non-transferrin-bound iron (NTBI) transport (Qian and Morgan, 1990, 1991, 1992; Qian et al., 1996; Du et al., 2004). The existence of the pathway of NTBI transport across the BBB cells was also recently reported (Burdo et al., 2003; Deane et al., 2004). At present, it is unknown whether there is any difference between the nature of...
NTBI uptake by BBB endothelial cells and uptake by other cell types.

Currently, little is known about how the iron crosses the abluminal membrane of the BBB (Bradbury, 1997; Moos and Morgan, 1998) and then enters the interstitial fluid (IF) of the brain. Recent studies showed that ferroportin 1 (FP1)/hephaestin (Hp) and/or hephaestin-independent (FP1/CP) iron export systems might play a key role in Fe^{2+} transport across the basal membrane of enterocytes in the gut (Vulpe et al., 1999; Donovan et al., 2000; McKie et al., 2001). Based on the similarity of the process occurred in the enterocytes with that of iron transport across the blood–brain barrier (BBB), it has been suggested that FP1/Hp and FP1/CP iron export systems might have the same role in iron transport across the abluminal membrane of the BBB as in enterocytes (Fig. 1A) (Qian and Shen, 2001; Qian et al., 2002; Li et al., 2002; Li and Qina, 2002; Rouault and Cooperman, 2006). In the BBB, iron, possibly also in the form of Fe^{2+} as is found in enterocytes, crosses the basolateral membrane and enters the brain (Bradbury, 1997; Moos and Morgan, 1998; Qian and Shen, 2001; Qian et al., 2002). A number of recent studies have confirmed that the brain, including capillary endothelium of the BBB, neurons and astrocytes, has the ability to express FP1 and Hp (Burdo et al., 2001; Jiang et al., 2002; Jeong and David, 2003; Wu et al., 2004; Hahn et al., 2004; Qian et al., 2007) although one study did not find evidence of FP1 in brain capillary endothelial cells (Moos and Rosengren Nielsen, 2006). The existence of these proteins in the brain supports the above possibility although further studies on the relevant mechanisms are needed. Another proposed mechanism involved in iron transport across the abluminal membrane is the role of astrocytes. The astrocytes probably have the ability to take up Fe^{2+} from endothelial cells through their end feet processes on the capillary endothelia (Malecki et al., 1999; Oshiro et al., 2000).

2.1.2. Iron transport across the blood–cerebrospinal fluid barrier

Choroid plexus epithelial cells simultaneously constitute a barrier: the blood–cerebrospinal fluid (CSF) barrier (the blood–CSF barrier) for circulating plasma proteins from the brain (Giometto et al., 1990; Moos, 1996). The blood–CSF barrier has been anticipated to share some characteristics with those of the BBB on iron transport. A major difference between the choroid plexus (blood–CSF barrier) and the brain endothelium (BBB) is that the choroid plexus of the lateral and third ventricle synthesize Tf, which may be of importance for the transport of iron across the choroid plexus. Another difference between the BBB and blood–CSF barrier is that iron is found in the choroid plexus epithelial cells of both developing and adult brain (Moos, 2002). The iron content in these cells is accompanied by a profound expression of ferritin mRNA and protein. This may indicate that iron is present in the choroid plexus as NTBI, which is incorporated in ferritin, and it could result from detachment of iron from Tf following TIR-mediated endocytosis by choroid plexus epithelial cells (Moos, 2002). In addition, the presence of mouse stromal cell-derived receptor (SDR2), a homolog of duodenal cytochrome b (Dcytb), which is predicted to act as a ferric reductase, was recently detected in the choroid plexus and the ependymal cells lining the four ventricles in mice (Vargas et al., 2003). Given that SDR2 is the only known ferric reductase expressed in the choroid plexus and that DMT1 is also present in the choroid plexus (Gunshin et al., 1997), it is possible that iron reduction by this protein followed by DMT1 mediated absorption might be an alternative mechanism for iron transport across the BCB. The high rate of blood flow in choroid plexus has led to the hypothesis that the majority of iron enters the brain through the CSF. However, Ueda et al. (1993) found that peripheral intravenous injection of 59Fe into the CSF was significantly less than uptake into the cerebral hemispheres, cerebellum, and brainstem, which was in accordance with Crowe and Morgan’s observation (Crowe and Morgan, 1992). These studies indicate that the contribution of iron transported through the blood–CSF barrier to thebrain may be negligible as compared to that transported through the BBB. It is possible that the blood–CSF barrier is more useful for iron removal from brain other than iron transport into the brain. With the exception of iron transport across the BBB and blood–CSF barrier, iron transport into the brain bypassing the brain barrier is also suggested (Moos, 2002). The circumventricular organs that receive a plethora of neuronal projections, mainly from hypothalamic nuclei, have been suggested to play a role in iron transport in the developing brain. Some motor neurons that project to peripheral organs devoid of blood barrier express TIR and retrograde axonal transport iron into brain (Graeber et al., 1989; Moos, 1995). The physiological significance of bypassing the brain barrier iron transport still remains obscure.

2.2. Iron transport within the brain

2.2.1. Transferrin-bound and non-transferrin-bound iron in the brain

After the iron has been transported across the BBB or blood–CSF barrier, it is likely to bind quickly to the Tf secreted by the oligodendrocytes and choroids plexus epithelial cells (Espinosa de los Monteros et al., 1990; Bradbury, 1997; Moos and Morgan, 1998) (Fig. 1B). Data from several experiments reveal that the iron concentration exceeds that of the binding capacity of Tf in the CSF and IF. Because the affinity of Tf with iron is the highest, compared with other iron transporters (the equilibrium constant for formation of diferric transferrin is more than 10^{10} times that of ferric citrate), Fe^{3+} in CSF and IF will bind to Tf first. Unlike Tf found in blood, Tf in CSF and IF is fully saturated with iron. The excess iron will bind to other transporters. Hence, it is possible that there are two transport forms of iron in CSF and IF in the brain: Tf–Fe and NTBI (Bradbury, 1997; Moos and Morgan, 1998). The latter probably includes citrate-Fe^{3+} or Fe^{2+}, ascorbate-Fe^{2+}, and albumin-Fe^{2+} (Moos, 1998) and also Lf-Fe^{3+} and sMTf-Fe^{3+}. If the remaining NTBI in CSF is in the oxidized form, Fe^{3+}, it will be bound almost completely to citrate, because the complex has an equilibrium constant log K = 11.4. If it is present as Fe^{2+}, it will be bound rather more loosely to citrate with an equilibrium constant log K = 4.5. Low concentrations may be present in the
complex with ascorbate (log $K \sim 2$) and a finite concentration as free Fe$^{2+}$ ($10^{-8}$ to $10^{-7}$ M) (Bradbury, 1997; Moos and Morgan, 1998). The Tf-Fe or probably Lf-Fe and sMTf-Fe will be taken up by brain cells via TfR or LfR and GPI-anchored MTf-mediated processes, respectively (Malecki et al., 1999; Qian and Shen, 2001). NTBI will be acquired by neuronal cells or other brain cells, probably via DMT1 (iron carrier) or trivalent cation-specific transporter (TCT) (Attieh et al., 1999)-mediated mechanisms (Qian et al., 1999a; Ke and Qian, 2002). However, the relevant mechanisms have not yet been detailed.

The widespread distribution of TfR in neurons clearly indicates that neurons can acquire iron by means of TfR-mediated uptake of Tf-Fe (Dickinson and Connor, 1998; Qian et al., 1998, 1999b, 2000; Chang et al., 2003a). The concurrent expression of DMT1 (Burdo et al., 2001; Gunshin et al., 1997; Moos and Morgan, 2002, 2004b; Chang et al., 2005) in neurons suggests that internalization of Tf is followed by detachment of iron within recycling endosome and transport into the cytosol via DMT1. The presence of NTBI in brain CSF and IF suggests that neurons can also take up iron by a Tf-independent pathway. Oligodendrocyte is a myelin-forming cell type in the brain which has high iron need for their normal function. TfR-mediated endocytosis has been anticipated to be involved in Fe$^{3+}$ uptake in oligodendocytes (Roskams and Connor, 1992; Giometto et al., 1999). In addition, based on the recent identification of a ferritin receptor (FrR) predominantly on oligodendrocytes, it has been suggested that these cells may obtain iron via ferritin/FrR pathway (Hulet et al., 1999a;b; Hulet et al., 2000). It has yet to be determined whether oligodendrocytes can take up iron by interchangeing with the axon of vicinity neurons. In the astrocytes, the existence of TfR expression and TfR-mediated iron uptake has been reported (Qian et al., 1999b; Hoepken et al., 2004). However, little is known about the relevant molecular mechanisms.

2.2.2. Lactoferrin receptor, melanotransferrin and divalent metal transporter 1 and iron uptake by brain cells

2.2.2.1. Lactoferrin receptor. The presence of LfR on neurons and increased expression of LfR on iron-induced degenerative dopaminergic neurons in PD indicate that a Lf/LfR-mediated pathway may be involved in iron uptake by neurons. Lf is an 80-kDa iron binding glycoprotein belonging to the Tf family with a similar structure to Tf (Anderson et al., 1989; Lambert et al., 2005). The affinity constant of Lf for iron is 300 times greater than that of Tf and may retain iron under more acidic condition than Tf. Lf transport iron is receptor-mediated and the binding of Lf to its receptor is independent of its degree of iron saturation (Davidson and Lonnerdal, 1989). Lf is found in neurons, glial cells and microvasculature of the human brain (Aisen and Leibman, 1972), and is also synthesized within the brain (Fillebeen et al., 1999a). It crosses the BBB in an iron-saturated and native form (Fillebeen et al., 1999b, 2001). Lf-positive neurons have been found to be severely affected by the neurodegenerative process in PD patients, as indicated by a significant decrease in the number of immunolabeled neurons in all such cases (Qian and Wang, 1998). Increased Lf expression is also observed in the MPTP mouse model of PD (Fillebeen et al., 1999a). As a ligand of Lf, LfR, a monomeric 105 kDa glycoprotein that was originally identified only in monocytes and intestinal cells, is expressed in the brain. Immunohistochemical staining shows that LfR is localized on neurons, cerebral microvessels, and, in some cases, glial cells including astrocytes (Faucheux et al., 1995). LfR expression is apparently not regulated by intracellular iron levels (Bonn, 1996; Yamada et al., 1987) and has affinity for molecules with little relevance for iron. In the brains of PD patients, however, LfR immunoreactivity on neurons and microvessels is increased and more pronounced in those regions of the mesencephalon where the loss of dopaminergic neurons is severe. In the substantia nigra, the intensity of immunoreactivity on neurons and microvessels is higher for patients with higher nigral dopaminergic loss (Faucheux et al., 1995). These clinical and laboratory investigations suggest that the Lf/ LfR might play a role similar to that of Tf/TfR in brain iron transport under normal circumstances. If LfR and Lf levels are increased, intraneuronal iron might rise to pathological levels and could contribute to the degeneration of nigral dopaminergic neurons in PD, and possibly AD and other neurodegenerative diseases (Faucheux et al., 1995; Qian and Wang, 1998).

2.2.2.2. Melanotransferrin. MTf, also called p97, was first identified as a human melanoma tumor-associated antigen on the surface of melanoma cells and also can be expressed by a wide range of tissues and cultured cell types (Qian and Wang, 1998; Sekyere et al., 2005). This protein has only one iron-binding site and exists in two distinct molecular forms: a plasma membrane-associated GPI-anchored form and a soluble form (sMTf) in the serum or CSF. In all cases where cells have expressed GPI-anchored MTf, they have also expressed a soluble form. Studies using Chinese hamster ovary (CHO) cell lines (Jefferies et al., 1996) demonstrate that GPI-anchored MTf has the ability to bind and internalize iron into cells from Fe-citrate, but not from Tf-Fe. The internalization is temperature-sensitive, time-dependent, and saturated at a concentration of 2.5 μg/ml. The sMTf has an ability to donate iron to SK-Mel-28 melanoma cell by a process independent of TfR. A significant role of MTf in iron transport is also supported by recent studies on the binding affinity of iron to this molecule (Creagh et al., 2005). These results imply the existence of a novel route for cellular iron uptake that is independent of Tf/TfR. In an immunohistochemical study, this molecule has been found to be highly localized in the capillary endothelium in all brain tissues examined. The distributions of MTf and TfR are remarkably similar, but quite different from that of Tf. Clinical investigation (Jefferies et al., 1996; Yamada et al., 1999) shows that MTf is selectively expressed on reactive microglial cells in AD brains, and that expression is associated with amyloid plaques in post-mortem brain tissue. Also, the MTf level is significantly elevated in the CSF of AD compared with the CSF of subjects suffering from other neuropathologies (Kemmard et al., 1996; Yamada et al., 1999). The sMTf has been shown to be able to cross the BBB and it transports iron into the brain more efficiently than Tf (Moroo et al., 2003). Also, there is a significant correlation between increases in the serum iron.
concentration of MTf and the progression of AD. These findings suggest that GPI-anchored MTf may deliver iron across the BBB by a mechanism analogous to that mediated by the TIR, and play a role in iron uptake by microglial cells (Qian and Wang, 1998; Malecki et al., 1999). The dysregulation of MTf may be one of the causes of excess iron deposition in AD brain tissue. However, available information on the cellular distribution and functional characterization of this protein is currently very limited. The mechanism of homeostatic control of MTf expression in the brain and the cause of MTf over-expression in AD are unclear. In addition, Richardson and co-workers found that although GPI-anchored MTf and +MTf both can bind iron, but they cannot efficiently donate it to SK-Mel-28 melanoma cells (Richardson, 2000; Sekyere and Richardson, 2000) and to the brain (Richardson and Morgan, 2004). Also they reported that MTf expression is not regulated in a manner similar to TIR in response to cellular iron level (Richardson, 2000) and might be not essential for iron metabolism because of no differences in iron indices, tissue iron levels, hematolgy, and serum chemistry parameters between MTf –/– and wild-type (MTf +/+ ) mice (Sekyere et al., 2006). These data indicated that further study on a physiological role MTf in iron transport is needed.

2.2.2.3. Divalent metal transporter 1. DMT1 was first identified on the basis of its homology to Nramp1 in 1995 (Gruenheid et al., 1995). In 1997, Fleming et al. and Gunshin et al. independently identified DMT1 as the first mammalian transmembrane iron transporter. It is a widely expressed protein with 12 putative transmembrane-spanning domains and is responsible for the uptake of a broad range of divalent metal ions including Fe2+, Zn2+, Mn2+, Co2+, Cd2+, Cu2+, Ni2+ and Pb2+ (Fleming et al., 1997, 1998; Gunshin et al., 1997; Tandy et al., 2000; Li et al., 2003; Erikson and Aschner, 2006). The Fe2+ transport mediated by DMT1 is active and H+-dependent (Richardson, 2000) and might be not essential for iron metabolism because of no differences in iron indices, tissue iron levels, hematolgy, and serum chemistry parameters between MTf –/– and wild-type (MTf +/+ ) mice (Sekyere et al., 2006). These data indicated that further study on a physiological role MTf in iron transport is needed.

Findings on the co-localization of DMT1 (+IRE) with the early endosomal marker, EEA, also support that DMT1 (+IRE) species may function in the transport of divalent metals.

The existence of this protein in the brain has been well determined (Burdo et al., 2001, 2004; Moos and Morgan, 2004a,b; Ke et al., 2005; Huang et al., 2004). In addition to the expression in neurons (Roth et al., 2000), DMT1 is also found in a location able to influence iron transport into and out of the brain, like the epithelial cells of the choroid plexus, blood vessel endothelial cells, ependymal cells lining the ventricles as well as astrocytes associated with these vessels (Gunshin et al., 1997; Andrews et al., 1999; Burdo et al., 2001; Burdo and Connor, 2003). The cellular localization and functional characterization suggest that DMT1 might play a role in physiological iron transport in the brain (Gunshin et al., 1997; Burdo et al., 2001; Moos and Morgan, 2004a,b). A general correlation between levels of DMT1 and iron staining in the monkey basal ganglia was reported (Huang et al., 2004). Accumulated evidence has shown that neuronal survival to iron accumulation associates with decreased expression of the iron import transporter DMT1 (and increased expression of the efflux transporter IREG1) (Aracena et al., 2006). The presence of DMT1 on astrocytic endfeet in the cerebral cortex and hippocampus of monkeys suggests that astrocytes may take up iron from endothelial cells through DMT1 (Wang et al., 2001). Coexpression of Dcytb and DMT1 observed in cultured astrocytes implies that DMT1 is likely to mediate iron influx into astrocytes by the coordination with Dcytb (Jeong and David, 2003). It has also been reported that neurons of the substantia nigra have the ability to express SDR2 (Ponting, 2001; Moos and Morgan, 2004a). It remains to be investigated whether SDR2 serves as a ferric reductase to facilitate DMT1-mediated iron transport in neurons.

Studies (Gunshin et al., 1997; Tandy et al., 2000; Trinder et al., 2000; Yeh et al., 2000) have demonstrated that DMT1 expression in the small intestine are negatively regulated by iron status. In the heart, DMT1 expression is also negatively regulated by iron at the post-transcriptional level (Ke et al., 2003). Unlike in intestinal and heart cells, however, DMT1 expression in hepatocytes is positively regulated by iron at the post-transcriptional level (Trinder et al., 2000). In kidney, DMT1 protein expression is only slightly increased upon deprivation of dietary iron (Canonne-Hergaux and Gros, 2002). The results from LMTK-cells, a mouse fibroblast cell line, show that iron has no significant effect on DMT1 mRNA expression and protein level (War drop and Richardson, 1999). In RAW264.7 and J774 cells (mouse macrophage cell lines), DMT1 mRNA is not regulated in parallel to Tfr mRNA after modulation of intracellular iron levels (War drop and Richardson, 2000). The cell specificity of DMT1 expression also occurred in perinatal rat brain. Siddappa et al. (2003) found that
iron deficiency increased the percentage of neurons expressing DMT1 in the hippocampus and cerebral cortex, but had no effect on the percentage of cells with positive staining for the protein in other regions. Wang et al. (2006) reported that manganese has a role in up-regulation of DMT1 expression in choroidal epithelia of the blood–CSF barrier. It has also been reported that nitric oxide transcriptionally down-regulates DMT1 via NF-kappaB (Paradkar and Roth, 2006). Our recent study found that regulation of the expression of two isoforms of DMT1 is age-dependent and iron-independent in rat brain (Ke et al., 2005). These findings show that DMT1 expression in response to iron status is different in different cells or tissues and also in different developmental periods. It is also possible that the DMT1 expression might respond to iron only at a significant decrease (i.e., 40% above) of total iron content in the brain (Ke et al., 2005). At present, the mechanisms of its regulation on a molecular scale and also importance of DMT1 function at the level of the whole organism and the individual cell are only beginning to be understood (Mims and Prchal, 2005).

2.2.3. The role of ceruloplasmin in brain iron transport: influx or efflux

CP is a critical ferroxidase in the plasma of all vertebrate species. This protein is mainly synthesized in hepatocytes. Recent studies show that CP is also expressed in the mammalian central nervous system (CNS) (Klomp and Gitlin, 1996; Klomp et al., 1996; Patel and David, 1997; Mollgard et al., 1988; Salzer et al., 1998; Patel et al., 2000; Chang et al., 2005). Based on the observation that the ferroxidase activity of CP promoted iron incorporation into Tf, a role for CP in iron efflux was first suggested in the 1960s (Osaki et al., 1966). Since then, it is widely accepted that CP has an important role in iron release from cells (Richardson, 1999; Floris et al., 2000; Hellman et al., 2000; Hellman and Gitlin, 2002; Hahn et al., 2004). The data obtained from a study using an animal model of aceruloplasminemia (Harris et al., 1999) and some in vitro experiments (Young et al., 1997; Richardson, 1999; di Patti et al., 2004) support this suggestion. Clinical data on aceruloplasminemia reveal the possible role of CP in iron release from the brain cells (Miyajima et al., 1997; Gitlin, 1998; Miyajima et al., 1998). A recent study (Jeong and David, 2003), using astrocytes purified from the central nervous system of CP-null mice showed that GPI-CP is essential for iron release. It was found that the soluble CP (1 and 300 μg/ml) increased iron release by 9% and 50% respectively. Their data strongly suggested an iron-releasing function of CP.

However, the possibility that CP might play a role not only in iron efflux from, but also iron influx into the brain cells via its ferroxidase activity has been also suggested (Qian and Ke, 2001; Berg et al., 2002). This is supported by several pieces of recent works. The first is the location of CP in the brain. The expression of brain CP is not observed in all astrocytes but rather identified with a unique subpopulation of those glial cells predominantly surrounding the microvasculature (Klomp et al., 1996; Klomp and Gitlin, 1996). The CP located on these astrocytes is ideally positioned to oxidize effectively the highly toxic Fe²⁺ to Fe³⁺ (Salzer et al., 1998; Patel et al., 2000). This unique location implies that CP is necessary for Fe²⁺, after it crosses the abluminal membrane, to be oxidized to Fe³⁺. The latter can bind to transport carriers Tf (or Lf and MTf) in the CSF and IF, and is then acquired by neuron or other relevant brain cells. It shows that the unique location of CP is required for physiological iron uptake by brain neurons. The second piece of evidence is provided by data obtained from in vitro studies on the effect of CP on iron transport across cellular membrane. These studies were initiated to elucidate the mechanism of CP-mediated iron release from cells. However, the negative results were obtained. The addition of CP results in enhanced uptake rather than release of NTBI in different types of cells, including cultured neurons and astroglia (Mukhopadhyay et al., 1998; Attieh et al., 1999; Qian et al., 2001; Xie et al., 2002; Ke et al., 2006; Chang et al., 2007). The similarities between Fet3p and CP provide another indication. Studies in yeast demonstrate that iron uptake is dependent on the membrane protein Fet3p (in Saccharomyces cerevisiae) (Askwirth et al., 1994), which has been viewed as a CP homologue (Kaplan and O’Halloran, 1996; Askwith and Kaplan, 1998). A recent study demonstrated that injection of a soluble copper-containing yeast protein Fet3p can restore iron homeostasis in phlebotomized mice with a deletion of the CP gene (Harris et al., 2004). This finding and the similarities between Fet3p and CP suggest a possible role for the latter in mammalian cell iron uptake (Attieh et al., 1999). The final piece of evidence is the existence of spontaneous oxidation activity in the brain. It has been suggested that the rate of spontaneous oxidation (Fe²⁺ to Fe³⁺) is sufficient to manage the corresponding slow iron release rate. Only at higher release rates is the exogenous ferroxidase activity such as that provided by CP required (Young et al., 1997). In other words, under physiological conditions, the role of CP in iron release in the brain might not be important or a little amount of CP is enough to maintain normal iron levels of brain cells. Indeed, the role of CP in iron uptake by the brain cells is consistent with the function of CP as a ferroxidase. It is more reasonable to conclude that the physiological function of CP is provided by its ferroxidase activity. This activity plays a role in both iron efflux and iron influx (Qian and Ke, 2001).

If it is true that CP has a role in iron uptake by brain neurons, the question that needs to be answered is why lack of CP expression can induce excessive iron accumulation in neurons and some other brain cells, as found in patients with aceruloplasminemia (Gitlin, 1998). A possible explanation is that excessive intracellular iron is mainly due to increased NTBI uptake and partly decreased iron release. It is highly likely that the major role of CP in brain cellular iron balance might be “uptake”, and “release” plays a minor role. Under physiological conditions, brain cells obtain iron mainly from Tf (Tf-Fe³⁺). Most of iron (Fe³⁺), after crossing the BBB, will be oxidized to Fe³⁺ by the ferroxidase activity of CP and then bind to Tf before being acquired by brain cells. However, under pathological circumstances, the loss of CP (and thus ferroxidase activity) will make it impossible for most of Fe²⁺ to be oxidized to Fe³⁺. Accordingly, the amount of Fe³⁺ as
2.2.4. The possible role of ferroportin 1, hephaestin and heme oxygenase-1 in iron efflux from brain cells

2.2.4.1. Ferroportin 1. FP1 (Donovan et al., 2000), also known as IREG1 (McKie et al., 2000) or MTP1 (Abboud and Haile, 2000), is a newly discovered transmembrane iron export protein. It is expressed on the basolateral surfaces of duodenal enterocytes, and in macrophages of the spleen and liver and placental syncytiotrophoblasts (Abboud and Haile, 2000; Donovan et al., 2000). Functional studies demonstrate that FP1 plays a key role in Fe\textsuperscript{2+} transport across the basolateral membrane of enterocytes in the gut by a mechanism that requires an auxiliary ferroxidase activity of Hp or CP (Griffiths and Cox, 2000; Kaplan and Kushner, 2000) and is directly involved in the export of iron during erythrocyte-iron recycling by macrophages (Knutson et al., 2005). FP1 mutation has been increasingly recognized to be responsible for a new inherited disorder of iron metabolism, called “the FP1 disease,” which it was originally identified as an autosomal-dominant form of iron overload not linked to hemochromatosis (HFE gene) (Pietrangelo, 2004). It suggests that FP1 mutation may lead to a loss of protein function responsible for reduced iron export from cells in this disease. Although FP1 has a 5’ IRE sequence, the presence of an IRE-IRP-independent pathway in the regulation of FP1 expression has been shown in certain cells. The post-translational regulation of FP1 by liver-produced hormone hepcidin has recently been reported (Nemeth et al., 2004).

FP1 has been detected in most rat brain regions and cells, including the endothelial cells of the blood–brain barrier, neurons, oligodendrocytes, astrocytes, the choroid plexus as well as ependymal cells of the normal and IRP knockout mice (Burdo et al., 2001; Jiang et al., 2002; Wu et al., 2004). The expression of FP1 in brain cells implies that this protein may be involved in brain iron efflux that could be very significant in the context of protecting cells from iron-induced oxidative stress in the brain. As mentioned previously, given the presence of FP1 on endothelial cells, it is likely that FP1 serves as a molecular transporter of iron on the abluminal membrane of polarized endothelial cells of the BBB. The expression of FP1 in astrocytic foot processes further suggests a critical role for FP1 in brain iron uptake across the BBB. Recently, Aguirre et al. (2005) characterized the response of SH-SY5Y neuroblastoma cells and hippocampal neurons in a model of progressive iron accumulation. They found that iron accumulation killed a large proportion of cells, but a sub-population became resistant to iron. The surviving cells evoked an adaptive response consisting of increased synthesis of ferritin and FP1, and decreased synthesis of the iron import transporter DMT1. They also found that FP1 expression directly correlated with iron content in SH-SY5Y and hippocampal cells and that a high correlation was found between FP1 expression and the rate of iron efflux from SH-SY5Y cells. Obviously, the increased expression of FP1 is associated with neuronal survival of iron accumulation. The data provided further evidence for the physiological role of FP1 in iron export from brain cells.

2.2.4.2. Hephaestin. Hp is a copper-containing protein sharing close homology with CP. It was first identified in a study of the sex-linked anaemia (sla) mice, which are anemic as a result of impaired intestinal iron absorption (Vulpes et al., 1999). As a significant sequence identity to CP, Hp was predicted to be a ferroxidase and believed to facilitate iron export from cells by oxidizing ferrous to ferric. Its amine oxidase and ferroxidase activity have been shown in cultured cells and primary intestinal enterocytes (Chen et al., 2004). Insights into its function in cellular iron export have been obtained from the study of sal mice (Vulpes et al., 1999; Chen et al., 2003; Petrak and Vyoral, 2005). These mice are characterized by impaired basolateral iron transport leading to iron accumulation in the enterocytes and to systemic iron deficiency (Manis, 1971). Mutation of the Hp gene by deletion of two exons resulting in truncated protein missing 194 amino acids has been suggested response for the iron accumulation by reducing iron export from basolateral membrane of the enterocytes in these mice. The importance of Hp in iron export was also shown in recent studies in which sla mice were crossed with mice disrupted at the hereditary hemochromatosis locus, HFE. The mice carrying both the HFE and sla mutations were able to alleviate iron accumulation compared with homozygous mutant HFE/HFE mice, which accumulated very high levels of iron in the liver (Levy et al., 2000). Role of Hp in the brain iron transport, however, is far from understood. Our preliminary studies have shown that Hp has ability to be expressed in the different brain regions including the cerebral cortex, hippocampus, striatum and substantia nigra (unpublished data). In a recent study, Hahn et al. (2004) indicated that Hp is localized to Muller glia, retinal pigment epithelium and the blood–brain barrier. Deficiency of both CP and Hp leads to retinal iron overload and degeneration in CP and Hp double knockout mice. It is suggested that Hp may serve an essential function in retinal iron regulation by facilitating iron export from retinal cells by the cooperation with CP in the brain (Hahn et al., 2004).

2.2.4.3. Heme oxygenase-1. HO-1 is a 32 kDa stress protein that catalyzes the rate-limiting step in heme degradation, resulting in the formation of carbon monoxide, iron and biliverdin (Tenhunen et al., 1969; Ewing and Maines, 1991). The gene of this cellular stress protein is strongly induced by
dopamine, oxidative stress and metal ions in brain and other tissues (Schipper et al., 1999). HO-1 expression in the normal brain is confined to a small group of scattered neurons and neuroglia (Baranano and Snyder, 2001). HO-1 activity has been linked to the efflux of iron from cells for cellular protection under stress. The absence of HO-1 induced by the genetic deletion and the HO-1 inhibitor leads to iron accumulation in the liver and increased cellular iron in cultured fibroblasts, whereas HO-1 over-expression decreases cellular iron levels (Ferris et al., 1999). HO-1 transfection stimulates iron egress from cells, which is markedly diminished in cells from HO-1 knockout mice (Ferris et al., 1999). HO-1 is sequentially detected to co-localize with an ATP-dependent iron transporter (Baranano et al., 2000). While there exists ample evidence for HO-1 neuroprotection by exporting cellular iron in various animal and tissue cultures models of brain disease, a growing body of literature attests to the neuroendangering aspects of HO-1 activity by promoting mitochondrial iron deposition. Upregulation of HO-1 in astrocytes has been found to exacerbate intracellular oxidative stress and promote mitochondrial iron deposition in these cells (Schipper, 1999; Schipper, 2004a). In rat astroglia transfected with human HO-1 cDNA, mitochondrial iron trapping is abrogated by HO-1 inhibitors. Interestingly, over-expression of HO-1 protein is found in astrocytes and neurons cells indigenous to brain tissue affected by AD, PD and other aging-related neurodegenerative diseases (Schipper, 2004b; Castellani et al., 2004). Although there is debate on the exact role of HO-1 in these diseases, it has been widely proposed that over-expression HO-1 might contribute to pathological iron deposition and mitochondrial damage rather than to cellular protection by iron efflux under these conditions (see Section 3.2.4). Therefore, it is likely that HO-1 has a dual role in iron metabolism: it can offer neuroprotection by facilitating the cellular iron export in some stress conditions but exert a neuroendangering effect by promoting iron deposition in mitochondria in other circumstances.

2.3. Iron transport out the brain

As there is a constant influx of iron into the brain, there must be a way for iron to leave the brain to remain brain iron homeostasis. Knowledge about how iron leaves the brain is far from complete. Tf-Fe readily exits the brain via the venous system and returns to the systemic circulation through the arachnoid granulations (Bradbury, 1997; Rouault, 2001). Endothelial cells of the BBB also have a potential capacity to mediate the export of elemental iron from brain interstitium to the systemic circulation, although there is no evidence so far for direct export of iron from the brain (Rouault, 2001).

3. Brain iron misregulation and neurodegenerative diseases

3.1. Genetic factors and brain iron misregulation

The discovery of mutations in the genes encoding ferritin light polypeptide (FTL) in ‘neuroferritinopathy’ (or Ferritinopathy), a novel pantothenate kinase (PANK2) in HSS, and CP in aceruloplasminemia provides convincing evidence that “errors in iron metabolism do indeed have a key role” in neurodegenerative diseases (Qian and Shen, 2001; Rouault, 2001; Berg et al., 2002). Studies on a mouse line lacking iron regulatory protein 2 (IRP2) and recent findings on the increased

---

**Fig. 2.** Role of genetic factors-induced brain iron misregulation in the development of neurodegenerative disorders. Abbreviations: FTL, ferritin light polypeptide; HFE, hemochromatosis protein (the protein mutated in hereditary hemochromatosis); HSS, Hallervorden–Spatz syndrome; NBIA-1, neurodegeneration with brain-iron accumulation-1; PKAN, pantothenate kinase associated neurodegeneration; PANK2, a novel pantothenate kinase; Ft, ferritin; CoA, coenzyme A.
onset of AD and PD induced by one or more HFE gene
mutation also strongly support the conclusion that misregula-
tion in brain iron metabolism induced by genetic factors (gene
mutation or absence) is one of the initial causes in
neurodegenerative diseases (Fig. 2).

3.1.1. Ferritin light polypeptide mutation in
neuroferritinopathy

Curtis et al. (2001) reported a previously unknown,
dominantly inherited, late-onset basal ganglia disease, variably
presenting with extrapyramidal features similar to those of HD
or PD in a large family from Cumbria in the north west of
England. The patients have abnormal aggregates of iron and
ferritin in the brain, and low serum ferritin concentrations, due
to a mutation in the gene for FTL. Brain histopathology shows
widespread reddish discoloration of the basal ganglia. The
globus pallidus contains abundant iron-positive, roughly
spherical inclusions up to 50 µm in diameter, most of which
are also positive for ferritin. Many iron and ferritin positive
profiles are present throughout the forebrain and cerebellum.
The number and overall size of iron/ferritin elements greatly
exceeded those found in normal elderly individuals, although
they still follow the general distribution pattern for iron in the
normal aging human brain (Curtis et al., 2001).

Genetic analysis revealed an adenine insertion at position
460–461 that is predicted to alter the carboxy-terminal residues
of the gene product (Curtis et al., 2001; Vidal et al., 2004).
The authors named this disorder as ‘neuroferritinopathy’. Recent
studies showed that this ‘dominant adult-onset basal ganglia
disease’ (Levi et al., 2005) is not restricted to the UK and it has
been described in apparently sporadic cases (Chinnery et al.,
2003; Crompton et al., 2005; Levi et al., 2005). Chinnery et al.
(2003) described the first non-British family with a mutation in
the FLP gene causing neuroferritinopathy. The identification of
the same adenine insertion at position 460–461 of the FTL gene
was also found in French families. Recently, Maciel et al.
(2005) identified a missense mutation in the FTL gene
(474G > A; A96T) in a 19-year-old man with parkinsonism,
ataxia, corticospinal signs, mild nonprogressive cognitive
deficit, and episodic psychosis. However, this disease can be
diagnosed by a simple muscle or nerve biopsy without brain
biopsy, autopsy, or molecular genetic testing at present
(Schroder, 2005).

Ferritin has two major functions: intracellular storage and
detoxification of iron. It is composed of 24 subunits of two
types (heavy, H and light, L) which form a soluble, hollow
sphere. The H and L subunits are considered to play
complementary roles in iron storage. The H subunit has a
specific ferroxidase activity allowing rapid iron uptake and the
L is believed to be involved with the initiation and stabilization
of ferritin-iron core (Harrison and Arosio, 1996). In the brain,
ferritin is the major iron storage protein accounting for 1/3 to
3/4 of brain iron. The ferritin subunits are synthesized in the
neuronal cell body, and mature, assembled heteropolymers are
found within axons (Curtis et al., 2001; Rouault, 2001). Some
of the ferritin in distal axons and presynaptic terminals are
degraded within lysosomes, potentially releasing ferrous iron
into a region of the neuron in which proteins, such as
components of neurofilaments, are vulnerable to iron-binding
and oxidative damage (Rouault, 2001; Schroder, 2005).
Incorporation of mutant L subunits into ferritin heteropolymers
may partially compromise the structure and function of ferritin,
leading to the spontaneous release of highly toxic iron into the
axon or synapse, and hence oxidative stress and neuronal cell
death (Curtis et al., 2001; Rouault, 2001; Mir et al., 2005). The
highly polarized nature of the neuron, together with axonal
trafficking of iron-laden ferritin, may explain why significant
pathology is seen only in the nervous system (Rouault, 2001).
The abnormality in ferritin strongly indicates the primary
function of iron in the pathogenesis of this new neurodegen-
erative disease and a central role for iron metabolism in
neurodegenerative disorders (Crompton et al., 2002; Burn and
Chinnery, 2006). It also suggests that we may be able to treat
these patients with desferrioxamine (DFO) to try to chelate the
iron out of brain tissue (Senior, 2001).

The L subunit of ferritin might be essential for brain iron
homeostasis. Synthesis of both ferritin subunits (L and H) is
controlled by iron regulatory protein (IRP), which binds to the
iron-responsive element (IRE) in the 5’-UTR of the H- and L-
ferritin mRNAs. In a recent study, Kato et al. (2001) identified a
single point mutation (A49U) in the IRE motif of the H subunit
of ferritin mRNA in four of seven members of a Japanese family
affected by dominantly inherited iron overload. Their data
suggest that an increment in IRE affinity to IRP, induced by the
A49U mutation in the IRE of H-subunit, is responsible for
tissue (liver) iron deposition but no iron accumulation in the
brain. Animals that are heterozygous for the mutation in the H
subunit have <20% of H subunit of ferritin in the brain but
normal levels of iron (Ferreira et al., 2000). These studies and
the findings of neuroferritinopathy support that the L subunit of
ferritin is essential for brain iron homeostasis.

3.1.2. A novel pantothenate kinase mutation in
Hallerorden–Spatz syndrome

A second piece of evidence is provided by the study on HSS
by Hayflick and colleagues. HSS, also referred to as NBIA-1
(neurodegeneration with brain-iron accumulation-1) or PKAN
(pantothenate kinase associated neurodegeneration), is a
genetically neurodegenerative disease associated with iron
accumulation in the brain and a clinically heterogeneous
group of disorders (Swaiman, 1991; Egan et al., 2005). It
includes classical disease with onset in the first two decades,
dystonia, high globus pallidus iron with a characteristic
radiographic appearance and often either pigmented retino-
pathy optic atrophy (Angelini et al., 1992; Zhou et al., 2001;
Gregory and Hayflick, 2005; Hayflick, 2006). Individuals who
may not fit the diagnostic criteria for HSS, and yet have
radiographic or pathologic evidence of increased iron deposits
in the basal ganglia could be diagnosed with an atypical type of
HSS (Hayflick et al., 2001; Swaiman, 2001; Zhou et al., 2001;
Tofaris et al., 2007). Hayflick and colleagues (Zhou et al., 2001)
identified the genetic basis for this disorder. They demonstrated
that a novel pantothenate kinase gene, PANK2, is defective
(G411R) in this disease and proposed a mechanism of
secondary metabolite accumulation as the cause of high iron concentration in the basal ganglia.

Pantothenate kinase is an essential regulatory enzyme in the biosynthesis of coenzyme A (CoA). It catalyzes the cytosolic phosphorylation of pantothenate (vitamin B5), N-pantothenoyl-cysteine and pantetheine (Zhou et al., 2001; Johnson et al., 2004). Phosphopantothenate, the product of pantothenate kinase, normally condenses with cysteine in the next step in CoA synthesis. In HSS, phosphopantothenate is deficient due to a defect in pantothenate kinase (PANK2) that is localized to mitochondria of neurons in human brain (Hortnagel et al., 2003; Johnson et al., 2004; Kottbauer et al., 2005). This will block a metabolic pathway in the brain and cause cysteine accumulation, leading to an increase in N-pantothenoyl-cysteine and pantetheine in the brain. High cysteine level has been demonstrated in the globus pallidus (Perry et al., 1985; Jellinger, 2003). The well-known iron-chelating properties of cysteine might account for the observed regional iron accumulation. Cysteine undergoes rapid autooxidation in the presence of iron and then induces free radical reaction. In turn, iron-induced lipid peroxidation is enhanced by free cysteine, further stressing a cell with impaired membrane biosynthesis (Zhou et al., 2001; Johnson et al., 2004). Although PANK2 is not directly involved in iron metabolism, its absence may contribute to iron accumulation in the brain, leading to neuronal death via a free radical pathway (Rouault, 2001; Zhou et al., 2001; Hayflick, 2003, 2006). Hayflick and colleagues believe that this proposed mechanism may represent a common pathway in neurodegeneration since perturbed cysteine metabolism has been implicated in PD and AD (Heafield et al., 1990; Zhou et al., 2001). Currently, physiological functions of the PANK2 have not been fully understood. In addition, data about biochemical properties of PANK2 suggest that the inactivation of catalytic activity of this enzyme may not be the sole underlying cause of the PKAN (Zhang et al., 2006). Therefore, considerably more investigations on the physiological roles (other than its catalytic activity) of the wild-type PANK2 are required in order to relate it to the PKAN human disease or the phenotype associated with the PANK2 knock-out mouse model (Zhang et al., 2006).

Their recent work demonstrated that the PANK2 gene mutation (exon 5) is also associated with HARP (hypoprebetalipoproteinemia, acanthocytosis, retinitis pigmentosa, and pallidal degeneration), a rare syndrome with many clinical similarities to HSS (Ching et al., 2002). Racette et al. (2001) reported two patients with pathologically proven NBIA-1 with late onset (about age 85 years) and atypical presentations, and they concluded that NBIA-1 pathology can develop at any age, and that the phenotype should be expanded to include late-onset Parkinsonism. Yamashita et al. (2004) described a 24-year-old Japanese woman with PKAN showing homozygous N245S substitutions in the pantothenate kinase gene. A novel mutation was recently identified in exon 2 resulting in a change of histidine to tyrosine at amino acid position 173 (H173Y) in a patient from Pakistan (Saleheen et al., 2005). Rump et al. (2005) conducted diagnostic DNA analysis of the PANK2 gene in the Dutch patients with PKAN and found a novel 3-bp deletion encompassing the nucleotides GAG at positions 1142 to1144 of exon 5 in patients from four apparently unrelated families. One patient was compound heterozygous (also carried a novel nonsense mutation Ser68Stop) (Rump et al., 2005), Pellecchia et al. (2005) identified 12 mutations in the PANK2 gene, five of which were new, in 16 patients with PKAN. Mancuso et al. (2005) reported a family of French Canadian and Dutch ancestry with hereditary ferritinopathy (neuroferritinopathy) and a novel mutation (C insertion at nt646-647 in exon 4) in the ferritin light chain gene. These accumulated data support the notion of genetic heterogeneity in the HSS/NBIA syndrome (Thomas et al., 2004). PANK2 mutations might be associated with all cases of classic Hallervorden–Spatz syndrome and one third of cases of atypical disease (Hayflick et al., 2003; Shevell, 2003). The discovery of this gene defect suggests that the drugs to deliver phosphopantothenate to cells and bypass the defective enzyme and gene induction procedures may be able to prevent or treat these diseases (Senior, 2001; Zhou et al., 2001; Gordon, 2002).

3.1.3. Ceruloplasmin mutation in aceruloplasminemia

The further evidence for the primary role of iron in neurodegenerative diseases comes from clinical observation on aceruloplasminemia. Aceruloplasminemia is an autosomal recessive disorder and the clinical triad of this disease includes retinal degeneration, diabetes mellitus, and neurological symptoms in the form of ataxia, blepharospasm, dystonia, tremor, parkinsonism, and chorea in association with cognitive dysfunction and dementia (Miyajima et al., 1987, 2002; Miyajima, 2003; Hatanaka et al., 2003). Clinical and pathologic studies on patients with aceruloplasminemia revealed a marked accumulation of iron in affected parenchymal tissues in conjunction with an absence of circulating serum CP (Gitlin, 1998; Grisoli et al., 2005). The most striking and unique feature is the progressive neurodegeneration of the retina and basal ganglia in association with the excessive iron accumulation in these tissues and cells (Harris et al., 1998). It has shown that basal ganglia, thalamus, and dentate nucleus of the cerebellum present brownish pigmentation due to iron deposition and severe iron deposition in the glial cells and neurons in association with a decrease in the neurons in the affected areas, with caudate and putamen being the most affected from microscopic examination (Miyajima, 2003; Thomas and Jankovic, 2004). Histopathological examination in autopsied aceruloplasminemia brains shows that GFSBs (grumose or foamy spheroid bodies) form in clusters at the ends of perivascular astrocytic foot processes. Both the deformed astrocytes and the GFSBs contained ferric iron are intensely immunolabelled with antibodies against the ferritin and manganese superoxide dismutase (Mn SOD) (Oide et al., 2006). Molecular genetic analysis of this disease reveals inherited mutations in the exons 3, 7, 13, 15, 18 of the CP gene on Chromosome 3q23-24 (Harris et al., 1995; Yoshida et al., 1995; Kaplan and O’Halloran, 1996; Kawanami et al., 1996; Logan, 1996; Takahashi et al., 1996; Miyajima et al., 1999; Takeuchi et al., 2002; Hellman et al., 2002; Thomas and Jankovic, 2004). The presence of neurological symptoms in
patients with aceruloplasminemia is unique among the known inherited and acquired disorders of iron metabolism. A number of studies have demonstrated that CP is an important iron transport protein critical for physiological iron homeostasis in the brain and neuronal survival in the retina and basal ganglia. Although the precise role of CP in the process of iron transport across the membrane of brain cells (iron uptake or release) has not been conclusively determined (see Section 2.2.3), the physiological importance of this protein in brain cell iron balance has been well established. Therefore, mutation in CP gene might function as an initial cause, leading to misregulation of brain iron metabolism. Intracellular iron is abnormally increased due to increased non-transferrin-bound iron uptake and/or decreased cell iron release. It is followed by free radical formation and neurodegeneration in some relevant regions in the brain (Miyajima et al., 1998, 2001, 2002; Yoshida et al., 2000). Observation by Miyajima et al. (1997) demonstrated that treatment with the iron chelator DFO can decrease brain iron stores, prevent progression of the neurological symptoms, and reduce plasma lipid peroxidation in aceruloplasminemia. The finding suggests that early treatment with this chelator may be useful in such patients to diminish central nervous system iron accumulation and to prevent or ameliorate neurological symptoms associated with neurodegeneration. It also implies that iron misregulation induced by CP gene mutation is a key cause in the pathogenesis of this disorder.

### 3.1.4. Iron regulatory protein 2 absence and neurodegeneration in mice

This conclusion is also strongly supported by the findings from a mouse line lacking IRP2. Rouault and co-workers (LaVaque et al., 2001; Smith et al., 2004; Cooperman et al., 2005) reported that targeted deletion of the gene encoding IRP2 or IRP2/IRP1 could cause misregulation of iron metabolism and neurodegeneration in mice. They detected iron accumulation in neuronal cell bodies in numerous gray matter areas, including portions of the thalamus, the deep cerebellar nuclei and colliculi. The regulation and management of iron at the cellular level is primarily by two proteins found in most cells throughout the body: TIR and ferritin. By controlling the level of expression of these two proteins, the cell can determine the amount of iron acquired (proportional to the number of membrane TIR) and sequestered (proportional to the cytoplasmic level of ferritin). In most types of cells, the coordinated control of TIR and ferritin by cellular iron occurs at the post-transcriptional level and is mediated by cytoplasmic RNA binding proteins, known as the iron regulatory proteins (IRPs) (Klausner et al., 1993; Bonkovsky et al., 1996; Hentze and Kuhn, 1996; Qian and Wang, 1998; Qian, 2002). The same elegant system that exists in extraneural organs has been demonstrated in brain cells for regulating iron concentration and availability (Hu and Connor, 1996). There are two distinct but highly homologous proteins, IRP1 and IRP2. Both act as key regulators of cellular iron homeostasis (Cairo and Pietrangelo, 2000; Eisenstein, 2000; Ho et al., 2001; Roy et al., 2002). IRP-2 is structurally and functionally similar to IRP-1, but does not assemble a cluster nor exhibits aconitase activity (Cairo et al., 2002). These proteins sense cytosolic iron levels and modify expression of TIR and ferritin via interactions with iron responsive elements (IREs) according to the needs of individual cells. A recent study demonstrated that IRP2 dominates regulation of mammalian iron homeostasis (Meyron-Holtz et al., 2004). Rouault and co-workers (LaVaque et al., 2001) demonstrated that the absence of IRP2 leads to misregulation of iron metabolism in the intestinal mucosa as well as the central nervous system and that over-expression of ferritin is a prominent feature in tissue that is adversely affected by IRP2 absence. The mice developed a movement disorder characterized by ataxia, bradykinesia and tremor. More importantly, they found that significant accumulation of iron in white matter tracts and nuclei throughout the brain precedes the onset of neurodegeneration and movement disorder symptoms by many months (LaVaque et al., 2001). Furthermore, they (Smith et al., 2004) found that mice that are homozygous for a targeted deletion of IRP2 and heterozygous for a targeted deletion of IRP1 (IRP1+/− IRP2−/−) develop a much more severe form of neurodegeneration, characterized by widespread axonopathy and eventually by subtle vacuolization in several areas, particularly in the substantia nigra. These findings show that misregulation of iron metabolism induced by the absence of IRP2 and/or IRP1 is an initial cause for neurodegeneration rather than a consequence of the disease process in this mouse line.

### 3.1.5. Hemochromatosis protein mutation and Alzheimer’s and Parkinson’s diseases

Recent findings that HFE mutation is closely associated with onset of AD and PD further support this viewpoint. HFE protein is a membrane protein that can influence cellular iron uptake (Arredondo et al., 2001; Griffiths et al., 2001; Beutler, 2006). Mutations in the HFE gene are most commonly associated with type 1 hereditary hemochromatosis, which leads to iron overload disease in homozygotic and some heterozygotic individuals. Historically, the brain was thought to be unaffected by the peripheral iron accumulation seen in hereditary hemochromatosis. However, this view has been challenged by recent observations. A number of studies suggest that carrying an HFE mutation is a risk factor or genetic modifier for AD or PD (Lee and Connor, 2005; Connor and Lee, 2006). Moalem et al. (2000) reported that the presence of mutant HFE alleles may put apolipoprotein E4 (APOE4)-negative men at increased risk of developing familial AD. Sampietro et al. (2001) presented evidence that AD patients possessing one or two copies of the H63D mutation develop AD on average 5 years earlier than those with wild-type HFE protein. Also, Pulliam et al. (2003) further revealed in their recent study the association of HFE mutations with neurodegeneration and oxidative stress in AD and correlation with APOE. HFE protein has recently been localized to brain vasculature, choroid plexus and ependymal cells that line the ventricles (Connor et al., 2001), as well as to the reactive astrocytes and neurons in the brains of patients with AD (Zecca...
et al., 2004a,b). These findings strongly support the idea that HFE mutation-associated iron imbalance in the brain contributes to AD, and disrupted iron metabolism may be a primary risk factor for neurodegeneration and oxidative stress in AD. Case reports of PD and parkinsonism in association with hemochromatosis have also been published (Nielsen et al., 1995; Buchanan et al., 2002; Costello et al., 2004; Papanikolaou et al., 2004; Thomas and Jankovic, 2004). Dekker et al. (2003) studied the role of mutations in the hemochromatosis gene HFE in PD and other Parkinsonism (non-PD PS) in two population-based series. The first series consisted of 137 patients with PD and 47 with non-PD PS, and the second of 60 patients with PD and 25 with non-PD PS. In the first series, PD patients were significantly more often homozygous for the C282Y mutation than controls ($P = 0.03$). Patients with non-PD PS in both series were more often carriers for the C282Y mutation than controls ($P = 0.009$, $P = 0.006$, respectively). Their data suggest that the HFE mutation (C282Y) increases the risk of Parkinson’s disease and other parkinsonism (non-PD PS). Recently, Russo et al. (2004) reviewed the seven published cases of movement disorders associated with the HFE mutation as well as data concerning brain iron deposition. They suggested that movement disorders are rare in association with hereditary hemochromatosis, and that such patients should be thoroughly investigated for another cause for their movement disorder. Currently, it is too early to conclude whether HFE mutations indeed contribute to pathological brain iron deposition and earlier manifestation of symptoms in AD and PD patients. Further investigations are needed.

### 3.2. Nongenetic factors and brain iron misregulation

In some cases, brain iron misregulation might be not due to gene mutation or absence but result from the disrupted expression of brain iron metabolism proteins induced by nongenetic factors. These currently undetermined factors might disrupt normal control mechanisms of protein expression (Qian and Wang, 1998; Qian and Shen, 2001; Ke et al., 2003; Ke and Qian, 2003) and lead to iron imbalance in the brain, and then induce oxidative stress and neuronal death in some neurodegenerative disorders (Fig. 3).

#### 3.2.1. Iron transport (uptake and release) proteins

As discussed in the preceding paragraphs, a number of proteins, including TLR, LfR, MTf and DMT1, might have a role in iron uptake by brain cells although physiological importance of these proteins in brain cell iron balance needs to be addressed. Clinical data show that there is increased expression of some iron ‘uptake’ proteins in certain neurodegenerative disorders. The over-expression of LfR/Lf in PD and MTf in AD has been reported and associated with the abnormal accumulation of iron in the brain and the subsequent development of neurodegeneration in PD (Leveugle et al., 1994, 1996; Faucheux et al., 1995; Bonn, 1996) or AD (Jefferies et al., 1996, 2001; Kennard et al., 1996; Yamada et al., 1999; Kim et al., 2001; Ujiie et al., 2002). The increased Lf expression found in PD brain has also been demonstrated to occur in the brain of AD and other neurodegenerative disorders (Kawamata et al., 1993; Rebeck et al., 1995). Based on the cellular localization of DMT1 and its functional characterization (Fleming et al., 1997; Gunshin et al., 1997), it has been proposed that the misregulation of DMT1 is likely to contribute to the etiology of certain neurodegenerative diseases (Gunshin et al., 1997). It has been reported that DMT1 is highly expressed in the neurons of the substantial nigra in PD that coincidentally correlates to the iron abnormally deposition in the same area (Andrews et al., 1999). A recent study demonstrated that the expression of DMT1 (and iron regulatory proteins) is significantly increased in the rat hippocampus after kainate induced neuronal injury. This could lead to increased iron influx into the brain areas undergoing neurodegeneration, and might

![Fig. 3. Hypothetical scheme for the possible role of nongenetic factors-induced mis-expression of iron metabolism proteins in the development of some neurodegenerative disorders. Abbreviations: LfR, lactoferrin receptor; Lf, lactoferrin; PD, Parkinson’s disease; p97, melanotransferrin; AD, Alzheimer’s disease; NDs, neurodegenerative disorders; DMT1 (DCT1 or Nramp2), divalent metal transporter1; CP, ceruloplasmine; Al, aluminum; IRP, iron regulatory protein; IRE, iron regulatory element; TLR, transferrin receptor; Ft, ferritin; HO-1, heme oxygenase-1; SDR2, stromal cell-derived receptor 2.](attachment:image.png)
be a factor contributing to neuronal damage after the initial excitotoxic injury (E. Huang et al., 2006; Y. Huang et al., 2006). These data suggest that disruption of DMT1 expression may be involved in the increased iron accumulation in PD or some other types of neurodegeneration.

On the other hand, the decreased expression of iron ‘release’ proteins has also been considered as a possible cause for excessive iron accumulation in some neurodegenerative disorders. The defective CP activities in patients with Alzheimer’s disease and Parkinson’s disease have been reported although it is not known whether these defects are associated with major disturbances in iron homeostasis (Boll et al., 1999; Torsdottir et al., 1999; Vassiliev et al., 2005; Hochstrasser et al., 2005). The clinical findings imply that nongenetic factors may have disrupted the expression of brain iron transport proteins, leading to abnormally increased or decreased expression of iron ‘uptake’ or ‘release’ proteins, excessive iron accumulation and neurodegeneration in some regions of the brain. This hypothesis provides a reasonable explanation for how iron is deposited in high quantities in the brain cells in some neurodegenerative disorders where gene mutation has not been found. However, available information on the cellular distribution and functional characterization of these proteins in the brain is limited. Very little is known about the mechanisms involved in homeostatic control of these proteins’ expression in the brain and the said nongenetic factors that can disrupt the normal cycle of expression. Although physiological roles have been suggested for FP1, Heph and HO-1 in cell iron efflux in the brain; however, very limited information on their functional mechanisms is available. Some studies that underscore a possible role for over-expression of LfR/Lf in PD have been reported (Berg et al., 2001). Therefore, further investigations are needed before a conclusion can be reached.

3.2.2. Iron regulatory proteins

In addition to brain iron transport proteins, the possible role of a disruption in the IRPs in the development of AD has been suggested. Pinero et al. (2000) examined IRPs in normal and AD brains and found that IRP/IRE complex formed in some AD brain tissue were more stable. Increased stability in this complex would result in an increase in TfR stability and a decrease in ferritin production. Therefore cells in the AD brain might take up more iron without the ability to store the iron, leading to iron accumulation in the cells of AD brains. Aluminum might have a role in raising the ability of IRP to bind to the IRE by interfering with the iron-catalyzed oxidation process (Yamanaka et al., 1999). A possible mechanism for disrupting IRP/IRE interactions in AD has been suggested (Thompson et al., 2001). In addition, Smith et al. (1998) found that alteration of IRP2 level was associated with intraneuronal lesions, including neurofibrillary tangles, senile plaque neuritis and neuron path (Curtis et al., 2001), the abnormal behavior of ferritin in AD and PD had been reported. Connor et al. (1995a,b) found variations in ferritin levels between young adult, aged adult, AD and PD brains. With increasing age, an increase in H and L ferritin was observed that coincided with an age-related increase in iron. In PD and AD, however, there was no concomitant increase in ferritin with the iron increase (Connor et al., 1995a,b; Thompson et al., 2001). Ferritin isolated from the brains of these patients had a higher iron content than did ferritin isolated from control human brains (Fleming and Joshi, 1987; Griffiths et al., 1999; Bartzokis et al., 2004). This data suggests that iron levels are increased in the brains of AD and PD patients’ relative to ferritin levels. The rise in levels of iron without a concomitant change in ferritin provides a source or iron for free radical generation. Indeed, ferritin has been suggested as a source of iron for the production of reactive oxygen species in diseases related to oxidative stress (Jellinger et al., 1995; Double et al., 1998). For example, 6-hydroxydopamine, a hydroxylated form of dopamine, nitric oxide metabolites resulting from head trauma or stroke can release ferrous iron from ferritin by a simple outer sphere electron transfer (Reif, 1992; Jellinger et al., 1995). The ferrous iron that has been released can then react with hydrogen peroxide, a by-product of dopamine metabolism and autoxidation, producing hydroxyl radicals and resulting in subsequent cellular damage (Reif, 1992; Jellinger et al., 1995). Thus, increased loading of ferritin in PD and AD may provide a source of iron and encourage free radical generation and subsequent neuronal damage. However, little is known so far about the factors that change the properties of ferritin in iron loading. Further investigation is needed to elucidate this issue.

3.2.3. Heme oxygenase-1 and stromal cell-derived receptor 2

A putative role has been proposed for the chronic over-expression of HO-1 in the pathological iron deposition and mitochondrial damage in AD, PD and other aging-related NDs (Schipper et al., 1999, 2006; Schipper, 1999, 2000, 2004a,b; Atamna, 2004; Song et al., 2006). As mentioned previously, HO-1 is a 32 kDa stress protein that degrades heme to biliverdin, free iron and carbon monoxide and has a dual role. In response to oxidative challenge, induction of HO-1 may protect cells by degrading pro-oxidant metalloporphyrines and appears
to facilitate iron efflux from the cell. A recent study showed that HO-1 expression in microglia induced by astrocytes might have a role in preventing excessive brain inflammation (Min et al., 2006; Shih et al., 2006). However, HO-1 may also exacerbate oxidative stress by the release of free ferrous iron during heme degradation and promote iron sequestration and mitochondrial insufficiency. Heme oxygenase-1 (HO-1) mRNA and protein are confined to small populations of scattered neurons and neuroglia. The HO-1 gene in neural tissues is exquisitely sensitive to upregulation by a host of pro-oxidant and other noxious stimuli.

Schipper (2004b) found that HO-1 immunoreactivity was greatly enhanced in neurons and astrocytes of the hippocampus and cerebral cortex of AD subjects and is co-localized to senile plaques and neurofibrillary tangles and HO-1 staining was augmented in astrocytes and decorates neuronal Lewy bodies in the Parkinson nigra. The HO-1 gene is also upregulated in glial cells within multiple sclerosis plaques, in the vicinity of human cerebral infarcts, hemorrhages and contusions, and in various other degenerative and nondegenerative human CNS disorders. Also, the upregulation of HO-1 induced by cysteamine, dopamine, b-amyloid, IL-1b and TNF-a is followed by mitochondrial sequestration of non-transferrin-derived 55Fe in cultured rat astroglia (Schipper, 2004b). In these cells and in rat astroglia transfected with the human HO-1 gene, mitochondrial iron trapping is abrogated by HO-1 inhibitors (Schipper, 2000). HO-1 induces the expression of the mitochondrial antioxidant enzyme MnSOD in astroglia. MnSOD induction in these cells can be abrogated by antioxidant administration indicating that HO-1 may promote intracellular oxidative stress in astroglia (Frankel et al., 2000).

Based on these findings, a model for the role of HO-1 in pathological iron deposition, mitochondrial insufficiency, and oxidative injury in brain aging and neurodegenerative diseases has been proposed (Schipper, 2004b,c). According to this model, extracellular pro-oxidant stressors and intrinsic aging processes might upregulate HO-1 in astrocytes, liberating heme-derived free Fe\(^{2+}\) and carbon monoxide. The latter generates intraglial oxidative stress that promotes opening of mitochondrial permeability transition pores and influx of NTBI into the mitochondrial matrix. The redox-active iron can then augment oxidative damage to the electron transport chain machinery, resulting in a vicious spiral of mitochondrial ROS generation and injury (Schipper, 2004c). Schipper believes that this progressive state of astroglial mitochondrial heteroplasmia endangers nearby neuronal constituents. The mitochondrial ferrous iron catalyzes the bioactivation of protoxins to potent neurotoxins. The latter may be extruded to the extracellular space and exert direct dystrophic effects on susceptible neuronal targets. Diminished ATP levels accruing from glial bioenergetic failure may further predispose neurons to degeneration by augmenting extracellular glutamate concentrations (excitotoxicity) and curtailting glutathione delivery to the neuronal compartment. Neuronal degeneration stimulates microglial activation, resulting in the release of ROS, nitric oxide, and proinflammatory cytokines. The latter further induces the HO-1 gene in indigent astroglia, completing a self-sustaining loop of pathological cellular interactions that perpetuates oxidative damage and mitochondrial insufficiency within senescent and degenerating neural tissues. Genetic and environmental risk factors may confer disease specificity by superimposing unique pathological signatures on this “core” lesion. In the cases of AD and PD, for example, b-amyloid deposition in the hippocampus and dopamine release from dying nigrostriatal projections feed into the pathological cascade by stimulating HO-1 activity in regional astroglia. Progressive derangement of astrogial iron mobilization and mitochondrial insufficiency would then drive the primary neurodegenerative process regardless of its etiology.

In addition, SDR2, a member of the cytochrome b-561 family including Dcytb, was recently demonstrated in humans and mice (Ponting, 2001; Vargas et al., 2003). Based on findings from sequence analysis, it is predicted that SDR2 is a catecholamine-regulated ferric reductase active in the brain. It has recently been detected in the choroid plexus and in the ependymal cells lining the four ventricles of mouse, through in situ hybridization analysis (Vargas et al., 2003). Cytochrome b-561 is found to preferentially express in neural tissues including the substantia nigra, which suffers from degeneration of dopaminergic neurons in PD (Vargas et al., 2003). Possible roles of SDR2 or cytochrome b-561 dysfunction in the progression of neurodegenerative disorders have been suggested (Ponting, 2001; Vargas et al., 2003). At present, no information is available on the function and mechanism of SDR2 or cytochrome b-561 in brain iron metabolism or on the involvement of SDR2 dysfunction in neurodegenerative diseases.

### 3.3. The role of aging for brain iron metabolism

Changes in iron metabolism with age are well known (Connor et al., 1992; Martin et al., 1998; Bartzokis, 2004; Cass et al., 2007). The cellular and molecular mechanisms that regulate iron transport, uptake, and utilization are complex and interconnected (Levenson and Tassabehji, 2004). A number of studies showed that many of these mechanisms may well be affected by the ageing process. There is increasing evidence that iron progressively accumulates in the brain with age (Hardy et al., 2005) and that oxidative stress-induced by the increased iron can cause neurodegenerative diseases (Zecca et al., 2004a,b; Aracena et al., 2006). The process of ageing-induced iron accumulation in the brain is quite specific and involves the accumulation of iron-containing molecules in certain cells, particularly in brain regions that are preferentially targeted in neurodegenerative diseases such as AD and PD (Bartzokis et al., 2004; Zecca et al., 2004b). Ferritin gene expression is developmentally regulated (Levenson and Fitch, 2000; VanLandingham and Levenson, 2003; Zucca et al., 2006) with gender differences (Bartzokis et al., 2007). Hallgren and Sourander (1958) demonstrated that concentrations of non-haem iron (mostly ferritin) increase in the putamen, motor cortex, prefrontal cortex, sensory cortex and thalamus during the first 30–35 years of life, and variable changes are observed in older individuals. The data reported by Connor et al. (1995a,b) and Zecca et al. (2001) showed that level of H-ferritin
in the frontal cortex, caudate nucleus, putamen, substantia nigra, and globus pallidus is higher in older individuals than in younger controls. The increased L-ferritin is observed only in the substantia nigra and globus pallidus. Chowers et al. (2006) demonstrated that transferrin is upregulated in retinas from patients with age-related macular degeneration. Regions of the brain that are associated with motor functions (extrapyramidal regions) tend to have more iron than non-motor-related regions. These regions at birth have little iron; however, with ageing they accumulate significant amounts (Koeppen, 1995; Palomo et al., 2003).

The brain is unique among organs (Zecca et al., 2004a). The concentration of iron in various regions of the brain varies greatly. Also, changes in iron contents with age are regionally specific. Iron homeostasis in the brains of older individuals is accomplished more efficiently in the locus coeruleus than in the substantia nigra. Zecca et al. (2001, 2004a,b) found that ageing can induce a linear increase in iron concentration in the substantia nigra, whereas the iron concentration in the locus coeruleus is lower. H- and L-ferritin concentrations in the substantia nigra increase with age, whereas those in the locus coeruleus remain lower and invariant. In individuals over 80 years of age, many extraneuronal iron deposits are present in the substantia nigra, but few iron deposits are observed in the locus coeruleus (Zecca et al., 2004a). Although the concentration of the neuromelanin–iron complex in neurons of the substantia nigra and locus coeruleus increases linearly during life, the slope of the accumulation curve is steeper in the substantia nigra than in the locus coeruleus (Zecca et al., 2001). At the cellular level, more iron staining is observed in the microglia and astrocytes of the cortex, cerebellum, hippocampus, basal ganglia and amygdala. Ferritin immunoreactivity in these cells is also stronger in older individuals than in younger subjects (Connor et al., 1990). Iron accumulation in microglia might stimulate the activation of these cells in the neuroinflammatory processes that contribute to AD and PD.

Assessment of iron concentrations by noninvasive methods, such as MRI, has also revealed an age-related increase in the non-haem iron concentration in the nucleus caudatus, putamen and globus pallidus (Bartokis et al., 1997; Martin et al., 1998; Ogg et al., 1999). The increased iron concentrations in certain brain regions could result from the altered vascularization that is observed during ageing and in neurodegenerative diseases (Brun and En gland, 1986; Faucheux et al., 1999; Snowdon, 2003). Immunohistochemical and ultrastructural findings related to the BBB in the blood vessels of the cerebral white matter in aged dogs suggest that age-related morphological changes in capillaries of the white matter are associated with BBB dysfunction, leading to the exudation of serum constituents, including harmful substances (e.g., iron), thereby causing tissue damage by oxidative injury (Morita et al., 2005). The increase in iron concentrations in neurons, astrocytes and microglia, which normally have low iron contents up to middle age, is typically present in regions such as the cortex, hippocampus and substantia nigra, which are particularly susceptible to the neuropathological changes that characterize AD and PD (Jellinger et al., 1990).

All of the above data, either from post-mortem tissue, noninvasive methods or animal studies, showed that brain iron concentrations appear to increase with age. The increased iron in the brain, induced by ageing, might contribute to some neurodegenerative diseases such as AD and PD. However, before we can make a final conclusion on the relationship among iron, ageing and neurodegeneration, more investigations are absolutely needed on the age trend of iron concentrations in different regions and the cellular distribution of iron in the brain and more information on effects of ageing on proteins and other molecules that are involved in iron metabolism is also needed.

4. Conclusion and perspectives

How iron homeostasis is maintained in the brain is an area of increasing interest in the fields of neurobiology, neurochemistry, neurology and neuropharmacology. Understanding the mechanisms involved in brain iron homeostasis is critical for elucidating the pathophysiological mechanisms responsible for excess iron accumulation in the brain as well as for developing pharmacological interventions that can disrupt the chain of pathological events occurring in neurodegenerative diseases caused by iron accumulation. In some neurodegenerative diseases, however, deregulation of iron metabolism in the brain might not be the initial cause but a secondary consequence of the pathophysiological processes of neurodegenerative diseases. It is possible for some neurodegenerative diseases to be initially induced by the defects in antioxidant defense mechanisms, i.e. decreased Atox 1 (a copper transport protein) gene expression (Kelner et al., 2000), reduced availability of glutathione (GSH, an important neuroprotectant for midbrain neurons) and other antioxidant substances in the brain. Changes in the integrity of the BBB due to altered vascularization of tissue or inflammatory events could be another initial cause. It is also possible for some disorders to be initiated by the interactions with amyloid precursor protein–heme oxygenase (APP-HO) (Takahashi et al., 2000), or multiple factors or other unknown causes. However, neuronal death by any initial cause could lead to large amounts of iron release and increased ROS formation, even though the initial causes of the neuronal injury may be completely unrelated to the deregulation of brain iron metabolism proteins (Jellinger, 1999; Qian and Shen, 2001; He et al., 2003; Tang et al., 2000; Xie et al., 2003; Andersen, 2004). Therefore, iron and iron-induced oxidative stress might be a common mechanism involved in the development of neurodegenerative diseases. The available data strongly support this hypothesis (Miyajima et al., 1997; Jellinger, 1999; Richardson, 2004; Casadesus et al., 2004; Youdim et al., 2004). Based on this hypothesis, therefore, therapeutic efforts should be devoted to reducing brain iron levels and inhibiting the generation of ROS (Ke and Qian, 2003; Doraaiswamy and Finefrock, 2004; Cass et al., 2007). A few recent studies showed that iron chelation may be an effective therapy for prevention and treatment of these diseases (Kaur and Andersen, 2002; Kaur et al., 2003; Cole, 2003; Shachar et al., 2004; Liu et al., 2005).

New findings reviewed in the preceding paragraphs have made important contributions in this area. Taken as a whole, it
seems appropriate to conclude that brain iron misregulation is an initial cause of neuron death in some neurodegenerative diseases (Ke and Qian, 2003; Thomas and Jankovic, 2004; Kaur and Andersen, 2004). Abnormally increased iron levels in the brain could result from disruption in the expression of brain iron metabolism proteins. This disruption in expression might be a result of either genetic causes, as found in patients with neuroferritinopathy, HSS as well as aceruloplasminemia, or non-genetic factors, which can disrupt normal control mechanisms of some iron metabolism proteins expression or properties (Ke and Qian, 2003) although these factors are currently unknown. Disturbances of iron metabolism might occur at multiple levels, including iron uptake and release, storage, intracellular metabolism and regulation. However, it should be pointed out that many relevant questions need to be clarified. The precise roles and mechanisms of iron transport proteins in brain iron homeostasis have not been completely understood. It is also unknown whether there are other molecules such as carbon monoxide (CO) and heme oxygenase-2 (HO-2) that play a role in brain iron metabolism (Wu and Wang, 2005; E. Huang et al., 2006; Y. Huang et al., 2006). The brain has multiple regions with specialized metabolic requirement and a variety of cell types with different iron requirement. The uneven distribution of iron in the brain implies that the distribution of these proteins in different types of brain cells and different brain regions is different. A detailed analysis of cellular and regional distribution of these proteins is necessary. How the expression of brain iron transport proteins is controlled in the brain under physiological circumstances and what genetic and non-genetic causes may lead to misregulation of brain iron metabolism are two other key questions that need to be addressed.

Our preliminary data show that brain has the ability to express hepcidin (unpublished), a recently discovered hepatic peptide (Krause et al., 2000; Nicolas et al., 2001, 2002; Park et al., 2001). It is not known whether hepcidin has a physiological role in brain iron homeostasis. This putative iron-regulatory hormone may be a central player in the communication of body iron stores to the intestinal iron absorption (Frazer et al., 2002; Hugman, 2006; Deicher and Hörl, 2006). A number of recent studies showed that this peptide regulates intestinal iron absorption, maternal–fetal iron transport across the placenta, and probably also affects the release of iron from hepatic stores and from macrophages involved in the recycling of iron from hemoglobin. It was also reported that hepcidin can regulate cellular iron efflux by binding to ferroportin and inducing its internalization (Nemeth et al., 2004). The absence of hepcidin expression in mice progressively develops multivisceral iron overload: plasma iron overcomes transferrin binding capacity, and NTBI accumulates in various tissues (Nicolas et al., 2001; Fleming and Syl, 2001, 2002). The existence of hepcidin in the brain suggests a possible function in regulation of brain iron metabolism. Further studies to describe the detailed location, possible physiological function of this peptide in the brain might be necessary. In addition, all genes involved in iron transport and metabolism have not yet been identified (Roy and Andrews, 2001). Therefore, identification of all unknown genes of iron metabolism is also important. A better understanding of all of these important aspects will greatly improve our knowledge of brain iron metabolism as well as the role of the disruption of brain iron homeostasis in the development of neurodegenerative disorders.

Acknowledgements

The studies in the laboratories were supported by Research Grants from The Chinese University of Hong Kong Faculty of Medicine (Direct grant: 4450226), The Hong Kong Polytechnic University (I-BB8L and GU-384) and Competitive Earmarked Grants of The Hong Kong Research Grants Council (CUHK466907 – KY).

References


Erikson, K.M., Aschner, M., 2006. Increased manganese uptake by primary culture of rat substantia nigra and its synthesis by the human microglial CHME cell line is upregulated by tumor necrosis factor alpha or 1-methyl-4-phenylpyridinium treatment. Brain Res. Mol. Brain Res. 96, 103–113.


Hajjar, R., 2005. Recent advances in the understanding of iron homeostasis in mammalian systems. J. Nutr. 135 (10), 2611S–2615S.

Hajjar, R., 2005. Recent advances in the understanding of iron homeostasis in mammalian systems. J. Nutr. 135 (10), 2611S–2615S.

Hajjar, R., 2005. Recent advances in the understanding of iron homeostasis in mammalian systems. J. Nutr. 135 (10), 2611S–2615S.

Hajjar, R., 2005. Recent advances in the understanding of iron homeostasis in mammalian systems. J. Nutr. 135 (10), 2611S–2615S.

Hajjar, R., 2005. Recent advances in the understanding of iron homeostasis in mammalian systems. J. Nutr. 135 (10), 2611S–2615S.

Hajjar, R., 2005. Recent advances in the understanding of iron homeostasis in mammalian systems. J. Nutr. 135 (10), 2611S–2615S.

Hajjar, R., 2005. Recent advances in the understanding of iron homeostasis in mammalian systems. J. Nutr. 135 (10), 2611S–2615S.
Ke, Y., Qian, Z.M., 2002. DMT1: a newly discovered mammalian iron transport
Kaur, D., Yantiri, F., Rajagopalan, S., Kumar, J., Mo, J.Q., Boonplueang, R.,
Kaur, D., Andersen, J.K., 2002. Ironing out Parkinson’s disease: is therapeutic
Kato, J., Fujikawa, K., Kanda, M., Fukuda, N., Sasaki, K., Takayama, T.,
Kaplan, J., Kushner, J.P., 1996. Iron metabolism in eukaryotes: Mars and
Johnson, M.A., Kuo, Y.M., Westaway, S.K., Parker, S.M., Ching, K.H.,
Ji, B., Maeda, J., Higuchi, M., Inoue, K., Akita, H., Harashima, H., Suhara, T.,
Kwon, T., Kang, S.S., Lee, D.W., 2006. Iron dysregulation and neurode-
Nramp2 gene: characterization of the gene structure, alternative splicing,
Leventon, C.W., Fitch, C.A., 2000. Effect of altered thyroid hormone status on
rat brain ferritin H and ferritin L mRNA during postnatal development.
iron regulatory mechanisms. Ageing Res. Rev. 3 (3), 251–263.
Leveugle, B., Spik, G., Perl, D.P., Bouras, C., Fillit, H.M., Hof, P.R., 1994. The
iron-binding protein lactotransferrin is present in pathologic lesions in a
variety of neurodegenerative disorders: a comparative immunohisto-
Leveugle, B., Faucheux, B.A., Bouras, C., Nillesse, N., Spik, G., Hirsch, E.C.,
Agid, Y., Hof, P.R., 1996. Cellular distribution of the iron-binding protein
lactotransferrin in the mesencephalon of Parkinson’s disease cases. Acta
Neuropathol. (Berl.) 91, 566–572.
atol. 18 (2), 265–276.
Li, H.Y., Qin, Z.M., 2002. Transferrin/transferrin receptor-mediated drug
Li, H.Y., Sun, H.Z., Qin, Z.M., 2002. Drug delivery and targeting: role of
transferrin/transferrin receptor system. Trend Pharmacol. Sci. 23 (5), 206–
209.
Li, H.Y., Sun, H.Z., Qin, Z.M., 2003. Membrane-inserted conformation of
transmembrane domain 4 of divalent metal transporter. Biochem J. 372 (Pt
3), 757–766.
Lis, A., Barone, T.A., Paradkar, P.N., Plunkett, R.J., Roth, J.A., 2004. Expres-
sion and localization of different forms of DMT1 in normal and tumor
and other metal chelation therapeutics in Alzheimer disease. Biochim.
Maciel, P., Cruz, V.T., Constante, M., Inestra, I., Costa, M.C., Gallati, S., Sousa,
misssense mutation in FTL causing early-onset bilateral pallidal involve-
cause iron overload in chromosome 1q-linked juvenile hemochromatosis. Nat. Genet. 36, 77–82.


