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Brain Iron Metabolism

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Brain iron uptake is regulated by the expression of transferrin receptor 1 in endothelial cells of the blood-brain barrier. Transferrin-bound iron in the systemic circulation is endocytosed by brain endothelial cells, and elemental iron is released to brain interstitial fluid, likely by the iron exporter, ferroportin. Transferrin synthesized by oligodendrocytes in the brain binds much of the iron that traverses the blood-brain barrier after oxidation of the iron, most likely by a glycophosphosinositide-linked ceruloplasmin found in astrocytic foot processes that ensheathe brain endothelial cells. Neurons acquire iron from diferric transferrin, but it is less clear how glial cells acquire iron. In aging mammals, iron accumulates in the basal ganglia, and iron accumulation is believed to contribute to neurodegenerative diseases, including Parkinson and Alzheimer disease. Here we consider the possibility that iron accumulations, which are often thought to facilitate free radical generation and oxidative damage, may contain insoluble iron that is unavailable for cellular use, and the pathology associated with iron accumulations may result from functional iron deficiency in some diseases.
Semin Pediatr Neurol 13:142-148 © 2006 Elsevier Inc. All rights reserved.

KEYWORDS blood brain barrier, transferrin receptor, ferroportin, GPI-linked ceruloplasmin, Parkinson disease

In mammals, iron is essential for the functions of many enzymes and prosthetic groups, including heme and iron-sulfur clusters. In the last decade, many of the proteins required for uptake of dietary iron from the duodenum, transport of iron in the serum, uptake of iron by individual cells, and retrieval of iron from senescent red cells have been identified. In the intestinal mucosa, a proton-coupled ferrous iron transporter, DMT1[1] (previously DCT1[2] or Nramp2[3]), is located on the apical membrane of the duodenal epithelium, the site of dietary iron uptake, along with a reductase known as Dcytb that is thought to reduce ferric (Fe3+) to ferrous (Fe2+) iron[4] for transport by DMT1. On the basolateral membrane, an iron exporter known as ferroportin[5] (also known as IREG1[6] or MTP1[7]) exports iron to the circulation aided by the membrane-bound ferroxidase hephaestin.[8] Duodenal mucosal cells also express a dedicated heme transporter, HCP1, that transports intact heme across the apical epithelium,[9] and intact heme may also be transported across the basolateral membrane into the systemic circulation, although there is no direct evidence for basolateral heme transport.[10,11] Ferroportin, Dcytb, and HCP1 messenger RNA levels increase in iron-deficient animals, implying that these intestinal iron-uptake proteins are partially regulated at the transcriptional level.[12] When iron enters the mammalian circulation, it binds tightly to serum transferrin (Tf),[13] and cells acquire iron from serum by expressing transferrin receptor 1 (TfR1), which binds iron-loaded Tf[14] and internalizes the Tf-TfR complex in endosomes. Acidification of the endosome facilitates the release of ferric iron, and endosomal reductases[15] generate ferrous iron for transport into the cytosol by endosomal DMT1.

The cytosolic iron pool is highly regulated because it is an important source of iron for numerous cytosolic and nuclear iron proteins and is also the likely source from which mitochondria and other organelles derive iron. In developing erythroid cells, iron may bypass the cytosolic pool, moving directly from endosomes into mitochondria,[16] but in most cells, it appears that the iron needed by organelles is absorbed from the cytosol. A duplicate homologous pair of regulatory proteins known as iron regulatory proteins 1 and 2 (IRP1 and IRP2) sense cytosolic iron levels and regulate expression of genes that affect cytosolic iron levels, including expression of TfR, which increases iron uptake, and ferritin, which reduces

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Supported by the intramural program of the National Institute of Child Health and Human Development.

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1071-9091/06/$-see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.spen.2006.08.002

cytosolic iron levels by sequestering iron. IRP1 registers cytosolic iron levels through an iron-sulfur "switch" mechanism, whereas the highly homologous IRP2 undergoes iron-dependent degradation in iron-replete cells. In iron-depleted cells, IRPs bind to RNA stem-loop elements found in the transcripts of ferritin, TfR1, ferroportin, mitochondrial aconitase, and erythroid ALA synthase, decreasing translation of proteins that would lower cytosolic iron levels by sequestering, using, or exporting iron, while simultaneously stabilizing the TfR transcript and thereby allowing increased TfR1 synthesis and iron uptake.[17,18,19]

In mammals, hepatocytes coordinately regulate absorption of dietary iron and reutilization of iron after senescent red cells are phagocytosed and digested by macrophages by secreting a regulatory peptide hormone, hepcidin. Hepatocytes gauge total-body iron stores by integrating information derived from iron uptake and signaling pathways, and they secrete hepcidin in direct proportion to hepatocytic iron stores. Macrophages regulate the return of iron to the circulation by regulating expression of ferroportin, which appears to be the sole mammalian iron exporter.[20] In the iron-replete liver, hepcidin transcription increases, and secreted hepcidin binds to ferroportin on the plasma membrane of duodenal epithelial cells and macrophages throughout the body, causing internalization and lysosomal degradation of ferroportin.[21] Decreased expression of intestinal ferroportin reduces intestinal iron absorption, and decreased expression of ferroportin in macrophages simultaneously reduces return of iron to the circulation. By decreasing iron absorption from the intestine and increasing macrophage iron sequestration, hepcidin causes a decrease in serum iron levels.[22]

The question of how hepatocytes regulate hepcidin transcription is a key issue in systemic iron homeostasis. To correctly assess iron levels, hepatocytes require function of several other proteins, including a second liver-specific transferrin receptor, TfR2,[23] the expression of which is not regulated by iron. In addition, appropriate hepatocytic hepcidin expression requires a TfR-binding protein, HFE[24] and hemojuvelin,[25] a coreceptor for bone morphogenetic protein that activates hepcidin transcription by using SMAD transcription factors.[26] Mutations in TfR2, HFE, and hemojuvelin (HJV) cause systemic iron overload by impairing normal hepcidin expression, and mutations in these genes cause different types of hereditary hemochromatosis.[27] Hepcidin deficiency and systemic iron overload also develop in mice that lack the transcriptional coactivator, SMAD 4, a member of the SMAD family of transcription factors responsible for signal transduction in the transforming growth factor (TGF)-β–signaling pathway.[28]

Thus, the iron homeostasis of most mammalian tissues served by the systemic circulation, including bone marrow, kidney, muscle, and liver, is governed by a regulatory system in which the liver secretes hepcidin, which in turn coordinates iron metabolism by regulating iron absorption and reutilization in the multiple tissues served by the systemic circulation. The hepcidin-regulatory system ensures that Tf-bound iron is sufficiently abundant to meet tissue nutritional needs, whereas individual cells appropriately regulate iron uptake mainly by altering TfR1 expression.

There are 3 sites in the mammalian body that are excluded from the liver-dependent macroregulatory axis: the central nervous system (CNS), testis, and retina. Each site is separated from the systemic circulation by a tight epithelial barrier analogous to that of the mammalian duodenum. Iron that enters any of these compartments must cross an apical and basolateral membrane before it disperses to various iron-consuming cells on the other side of the epithelium. The barrier that separates the CNS from the systemic circulation is known as the blood-brain barrier.[29]

How Does Iron Cross the Blood-Brain Barrier and Move Within the CNS?

For many years, it was believed that iron entered the brain mainly during infancy before the blood-brain barrier matured. However, in the last decade, it has become apparent that brain-iron uptake is mediated by endothelial TfR expression in the blood-brain barrier of adult animals, and this TfR expression on the luminal endothelial surface is regulated by the iron status of the CNS. The blood-brain barrier is an unusual structure composed of endothelial cells, a basal lamina, pericytes, and astrocytic foot processes. In other parts of the body outside the CNS, fenestrations of the endothelial cells that line blood vessels allow serum substances to easily pass into interstitial fluids of tissues. However, in the CNS, endothelial cells are joined by tight junctions, and substances that enter the CNS must use dedicated endothelial transport systems.[29] An important key to blood-brain barrier formation is the interaction between astrocytes, star-shaped cells distributed throughout the CNS that extend long processes that ensheathe blood vessels, and endothelial cells, which are induced to polarize by contacts with astrocytes. Unlike other blood vessel endothelia, the endothelial cells of the blood-brain barrier express receptors and proteins on the luminal endothelial membrane side of the systemic circulation that differ from those that are expressed on the abluminal membrane, which is surrounded by astrocytic foot processes, neuronal processes, and brain interstitial fluid. Pericytes found within the endothelial basement membrane near the endothelial tight junctions that seal the blood-brain barrier are crucial to barrier formation.[30] In animals that cannot express the platelet-derived growth factor receptor, pericytes are dysfunctional and the blood-brain barrier does not form correctly.[31]

The first step of iron entry into the CNS is mediated by TfR1 expressed on the luminal membrane of the endothelial cell (Figure 1).[32-34] Initially, it was thought that Tf that entered the cell with the aid of the TfR could be transcytosed and released from the abluminal membrane.[35] However, radiolabeling of Tf with ^{60}Fe iron showed that transferrin-bound iron enters the CNS, whereas systemic Tf is excluded from brain interstitial fluid.[33,36] The import of iron from endosomes is generally believed to depend on DMT1, but there

is some disagreement about whether DMT1 is expressed in the endothelial cells of the blood-brain barrier.[37,38] Once iron has been imported into the cytosol of endothelial cells, it can then be exported into the CNS by ferroportin, which has been immunohistochemically detected in the blood-brain barrier, although its exact membrane location has not been ascertained by immunoelectron microscopy.[39] The end-foot processes of astrocytes that contribute to the blood-brain barrier express a special form of the ferroxidase, ceruloplasmin. Through alternative splicing, astrocytes generate ceruloplasmin that attaches to the membrane via a glycophosphosinositide (GPI) linkage.[40] The linkage of ceruloplasmin to membranes near its site of activity may allow the CNS to express ample ceruloplasmin without increasing the concentration of soluble proteins in brain interstitial fluid. Importantly, GPI-linked proteins can transfer to neighboring membranes, and perhaps GPI-linked ceruloplasmin facilitates ferroportin activity of endothelial cells by oxidizing newly released ferrous iron and allowing it to bind to the Tf in brain interstitial fluid.

In the CNS, Tf is synthesized and secreted mainly by oligodendrocytes, cells found throughout the CNS that elaborate the myelin sheathes around axons, and Tf transports iron throughout the CNS, and although the cells of the CNS are closely packed together, fluid moves in the interstitial areas between cells primarily by convection or bulk flow rather than simple diffusion.[41] Notably, in patients with aceruloplasminemia, iron accumulates in astrocytic foot processes in deposits known as "grumose foamy spheroid bodies."[42] Absence of ceruloplasmin or the related ferroxidase, hephaestin, at the blood-brain barrier would mean that ferrous iron would not be oxidized to ferric iron, the form that extracellular Tf binds, and the Tf secreted by oligodendrocytes could not transport iron to other sites in the brain interstitium. Glial cells such as astroglia are believed to have a metal import system that does not depend on Tf.[43,44] Thus, the absence of ceruloplasmin could allow excess ferrous iron to accumulate near astrocytic foot processes, and uptake of ferrous iron by nonspecific metal transport pathways in astrocytes could lead to the observed iron overload in the astrocytic foot processes of aceruloplasminemic patients.

Although blood vessels throughout brain parenchyma could theoretically permit the return of iron from the CNS interstitial fluid to the circulation, the well-established topology of TfR expression in the blood-brain barrier suggests that parenchymal blood vessels permit iron entry into the CNS, whereas iron likely exits the brain by crossing the arachnoid membrane in the arachnoid granulations and entering the venous drainage system.[32] The cerebrospinal fluid that fills the ventricles is elaborated by the choroids plexus, which consists of capillary tufts that protrude into ventricular spaces and are covered by a tightly connected layer of epithelial cells. Tf synthesized within the CNS does not return to the systemic circulation, most likely because egress from the CNS involves crossing the arachnoid membrane, a tight epithelial cell layer across which substances must be transported before they can reenter the systemic circulation.[32,45]

Diferric Tf is likely the main source of iron for neurons in the CNS because both TfR1 and DMT1 are highly expressed in neurons.[46,47] Whether TfR1 is expressed in astrocytes,[48] oligodendrocytes,[49] and microglia[50] has been debated,[46] but it is important to remember that proteins expressed at low levels may be difficult to detect, and results may be difficult to interpret correctly. In addition, TfR2, a second TfR that is not regulated by iron,[23] is expressed in the brain according to the unigene expression profile for human TfR2, but it is not known which cells express TfR2 in the CNS. HFE, hephaestin,[51] and HJV[52] are also expressed in brain, but their expression patterns are also not yet well defined. Interestingly, HFE-/- mice have been reported to have a mild movement disorder,[53] and some patients with HFE hemochromatosis appear to be more likely to develop Parkinson disease.[54,55] Hepcidin is expressed in the spinal cord,[56-58] but the cell of origin is unknown. Interestingly, mutations in TfR2, HFE, HJV, and hepcidin are not known to cause diseases of the CNS, even though mutations in these genes cause iron overload in the periphery. The absence of apparent brain iron overload in mouse models of hemochromatosis[53] as well as in most humans with HFE-related hemochromatosis implies that regulation of brain iron homeostasis differs from the regulation of peripheral iron homeostasis. However, there are important similarities between peripheral and CNS regulation because TfR expression increases in brain endothelial cells of iron-deficient animals,[33] leading to appropriately increased iron uptake in the CNS of iron-deficient animals.

Iron that crosses the abluminal membrane of the blood-brain barrier endothelial cell binds to Tf in interstitial fluid, (Figure 1) but brain Tf concentrations are about 10% of serum Tf concentrations, and measurements of interstitial iron concentrations imply that the Tf may be highly saturated by iron; significant amounts of nontransferrin-bound iron (NTBI) may exist.[59] NTBI is rarely found in serum because Tf is in great excess, and NTBI can be uniquely damaging, perhaps because it can enter cells through unregulated channels. NTBI may be an important iron source for cells such as oligodendrocytes that do not express much TfR1.[43] It is also possible that NTBI enters astrocytes and neuronal processes and is transported across the brain by trafficking of intracellular metalloproteins. Astrocytes are connected by gap junctions, which can allow transport of ions between cells.[60] Manganese, which is similar to iron in many of its properties, can be directly imported by astrocytes.[44] Manganese transport in the olfactory system has been used to trace neuronal tracts that include multiple synapses.[61,62] If manganese can be transported along neuronal tracts, it is also theoretically possible that iron can be similarly transported, although experimental evidence does not support this possibility.[63] In addition, ferritin is present in the axons of normal neurons in the retina[64,65] and in the brain,[66] implying that there may be a trafficking mechanism that permits ferritin to carry iron from the neuronal cell body to the synapse. Ferroportin is present in synaptic vesicles, suggesting that ferrous iron may be released into synapses.[39] It is not clear how post-synaptic neurons or glial cells would take up elemental iron, but expression of DMT1 on the plasma membrane is a good candidate

for uptake of non-transferrin bound iron, as it can function at neutral pH[67] as well as in acidified endosomes.[2]

Iron Redistribution With the Brain

After initial uptake in the brain, iron redistributes into various areas of the brain,[68,69] but the mechanisms by which iron redistributes in the brain are poorly understood. Studies in hypotransferrinemic mice suggest that redistribution of iron depends on the presence of intact Tf in brain interstitial fluid.[70] As previously stated, Tf synthesized by oligodendrocytes does not return to the systemic circulation,[71] presumably because CNS Tf cannot cross the tight epithelial cells of the arachnoid granulations in which fluid and ions exit the brain and return to the systemic circulation.[29]

During the aging process, iron accumulates in the substantia nigra, and iron accumulation during aging may contribute to the development of Parkinson and Alzheimer disease.[72,73] However, one of the most important unknowns about iron redistribution is that it is not known which cells accumulate iron within various regions of the brain. Increased iron levels have been measured repeatedly in the substantia nigra of Parkinson patients, but it is not clear whether a change in cellular composition, such as an increase in microglia or the small macrophages of the brain, accounts for the increase. In general, identifying cell types that accumulate iron in the brain is much more challenging than in other tissues because astrocytes, oligodendrocytes, and neurons have long processes, and it can be hard to discern where one cell ends and another begins.

Once acquired, it appears that the brain can conserve iron very well because brain iron contents decrease minimally in adult animals that develop severe systemic iron deficiency on a low-iron diet,[74] implying that mechanisms are in place to allow the brain to efficiently conserve its iron.[32] Although little is known about potential mechanisms for iron export from the brain, the fact that the arachnoid membrane contains tight junctions implies that egress of iron can be a regulated process, analogous to brain iron uptake. However, it is not known if the arachnoid membrane expresses TfR, DMT1, ferroportin, and other iron metabolism proteins. Immunoelectron microscopy and localization of these proteins in the arachnoid could greatly aid in formulation of hypotheses about brain iron homeostasis.

Misregulation of Brain Iron Metabolism and Neurodegeneration

In addition to the observation that iron accumulates abnormally in the brain of patients with sporadic Parkinson and Alzheimer diseases, abnormal accumulations of iron are also found in diseases with known genetic causes, including aceruloplasminemia,[75] neurodegeneration with brain iron accumulation,[76] and neuroferritinopathy.[77] The usual hypothesis associated with these diseases is that abnormal accumulations of iron lead to increased formation of reactive oxygen species because ferrous iron reacts with endogenously generated hydrogen peroxide to yield damaging hydroxyl radicals.

Although it is possible that excess iron is the culprit, another intriguing possibility is that the iron observed in pathologic sections represents iron that is unavailable for use in normal cellular metabolism, and mislocalization of iron results in functional iron deficiency. An example of misregulation of iron metabolism resulting in functional iron deficiency occurs in animals with targeted deletions of IRP2.[78] In these animals, abnormal accumulations of ferric iron were detected in the cell bodies of oligodendrocytes and in their extensions. Axonal degeneration was present in areas in which increased ferric iron was detected by iron stains, and it initially seemed likely that the iron detected within the axonal framework, which was mainly sequestered in ferritin, could be a cause of axonal degeneration, with loss of neurofilament structures and collapse of the axon. However, on more rigorous analysis performed by using electron tomography, it turned out that the increased iron detected within degenerating axons was mainly contained in invaginations of oligodendrocytic processes into the space that should have been occupied by axonal structures.[67] The study confirmed that ferritin is present in normal axons, but it also revealed that increased ferritin in the axonal shaft of IRP2-/- animals was within oligodendrocyte processes. Total brain iron was not statistically increased,[78] and the nonheme iron content of IRP2-/- anamils may be decreased (Rouault, TA, unpublished). Thus, increased ferric (3+) iron staining detected in the Prussian blue reaction may be misleading because it may represent aggregates of unavailable ferric iron in cells that lack sufficient ferrous iron. IRP2-/- animals have an iron-insufficiency anemia[79,80] associated with increased protoporphyrin IX levels. The iron-insufficiency anemia of IRP2-/- mice is probably caused by expression of inadequate levels of TfR on developing erythroid precursor cells to allow sufficient iron uptake for hematopoiesis. In addition, overexpression of ferritin likely results in the sequestration of iron making it unavailable for use in normal metabolism.[79] Thus, it is worth considering whether iron insufficiency associated with inappropriate sequestration of iron within ferritin or the ferritin breakdown product, hemosiderin, is a common problem in diseases in which "iron overload" has been reported. In human patients, measurements of cerebrospinal fluid iron and Tf saturations may be informative about brain iron status, but this procedure is very difficult to accomplish in mice because mice have so little cerebrospinal fluid and brain interstitial fluid. Although there are no reports of its detection, it would be interesting to measure hepcidin levels in the cerebrospinal fluid and brain interstitial fluid of humans and other mammals.

Future Directions

The axis that governs brain iron homeostasis has been partially described because it seems clear that endothelial TfR determines how much iron the brain will absorb. However, it

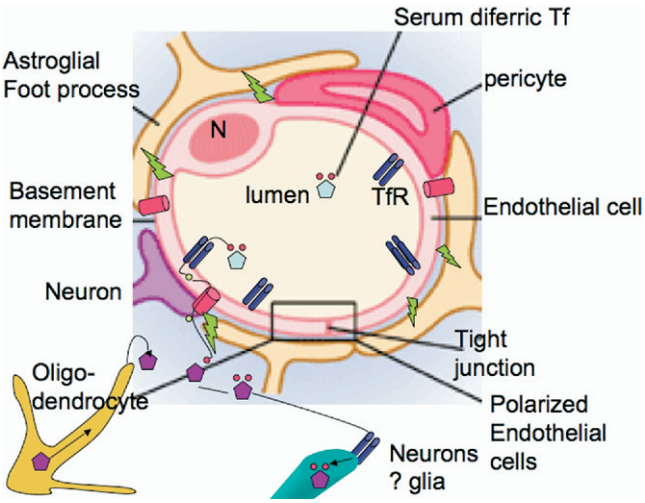

Figure 1 The blood-brain barrier is composed of endothelial cells joined by tight junctions surrounded by a basement membrane in which pericytes are found and which is in close apposition with astrocytic foot processes. TfR1 is expressed on the lumenal membrane of endothelial cells, which have nuclei (N) and which likely express ferroportin (cylinders). Astrocytic foot processes express GPI-linked ceruloplasmin (lightening). On endocytosis of the Tf-TfR complex, ferric iron (dots) is reduced to ferrous iron (pale dot); exported to cytosol, most likely by DMT1; exported from the cell, presumably by ferroportin; and oxidized to ferric iron by GPI-linked ceruloplasmin within brain interstitium. Transferrin synthesized by oligodendrocytes in the brain binds ferric iron, and neurons and probably many other brain cells acquire iron by expressing the TfR, althoughTfR expression is low in nonneuronal cells. To exit the brain interstitial fluid and cerebrospinal fluid, iron must cross the arachnoid membrane (not shown), a tight epithelial layer that brain Tf does not cross. (Modified with permission from Francis K, Van Beek J, Canova C, et al: Innate immunity and brain inflammation: The key role of complement. Expert Rev Mol Med 2003:1-19, 2003.)[86] (Color version is available online.)

is unclear how the brain and spinal cord gauge iron status and communicate this information to the blood brain barrier. To identify candidates for this role, the cells that express TfR2 should be identified because cells that express TfR2 could be functionally similar to hepatocytes. In addition, identifying the site of hepcidin synthesis by *in situ* hybridization could also facilitate identification of important CNS iron-sensing cells. It is likely that the mechanisms that maintain homeostasis in the systemic circulation will be recreated with some modifications in the CNS (ie, a cell analogous to the hepatocyte will gauge iron availability and will secrete hepcidin according to iron status).

The mechanisms by which iron moves and accumulates in the brain require elucidation. Are there intracellular trafficking pathways? Why does iron accumulate in regions of the brain such as the globus pallidus and substantia nigra? Do the basal ganglia function as iron repositories in the central nervous system? The highest levels of iron in the brain are found in the globus pallidus, followed by the putamen, substantia nigra, and caudate nucleus.[32] Notably, iron concentrations in these brain regions are comparable to iron concentrations in the mammalian liver, a recognized iron-storage tissue that supplies iron to tissues served by the systemic circulation. A possible reason for high iron concentrations in the substantia nigra is that tyrosine hydroxylase,[81] crucial in dopamine synthesis, is an iron enzyme.[82] The substantia nigra appears to be particularly vulnerable to iron deficiency,[83] which can impair dopamine production and cause motor problems in adults.[84]

Can iron export from the brain be accurately measured and is export regulated? Are TfR1, TfR2, hemojuvelin, and HFE expressed on the arachnoid membrane? Does NTBI in the brain have a significant function, and, if so, how does the brain protect itself from the reactivity and toxicity of unbound ferrous and ferric iron?

The fact that iron misregulation is often observed in neurodegenerative diseases underscores the need to understand basic brain iron homeostasis. Application of high-resolution imaging technologies such as electron energy-loss spectroscopy[85] and electron tomography[67] may permit identification of the cell type, intracellular location, and bioavailability of iron deposits in patients with Parkinson disease, NBIA, and aceruloplasminemia. High-resolution imaging of iron deposits in patient samples before the onset of end-stage disease could help to guide future research on iron misregulation and neurodegeneration by focusing attention on the cells and organelles that accumulate iron early in disease.

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