BioMetals **16:** 63–75, 2003.

Brain iron uptake and homeostatic mechanisms: An overview

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Published on line: September 2002

Abstract

Timely and adequate iron acquisition by the brain is essential to normal neurological function. Despite the numerous cognitive and neurological impairments that are associated with disruptions in brain iron acquisition, including both too much and too little iron, the mechanism and regulation of the mechanisms by which the brain acquires iron are poorly understood. In this article, we review the current state of knowledge regarding expression of iron transport proteins in the brain, brain iron uptake and discuss why a model for brain iron uptake must take into consideration the potentially competing influences on the endothelial cell between the status of iron in the brain versus the systemic iron status.

Introduction

Iron is abundant in the brain and has a distinct regional and cellular pattern of distribution (Connor & Menzies 1995). The reason for this heterogenous distribution probably involves differences in oxidative metabolism and in neurotransmitter concentration. Because of the high concentration of iron in the brain, iron transport proteins can also be expected to be abundant. However, there should also be regional and cellular variability in the expression of these transporters. Because the brain is regionally and cellularly compartmentalized in regard to function and hence regional shifts in metabolic activity, iron delivery to this organ has challenges not encountered in other organs. In addition, the brain presents challenges to iron transport because of the blood-brain barrier. Cells in the brain, unlike the cells in other organs, do not have direct access to plasma borne iron. The signal from a cell in the brain that it requires iron must be sent, presumably, to endothelial cells lining the blood vessels in the brain and iron must be released from these cells into the brain extracellular fluid. Alternatively, iron in the extracellular space could come from the cerebrospinal fluid; although it is difficult to consider how regional differences in iron accumulation could arise in this scenario. Furthermore, the expression of transferrin receptors on the brain microvasculature is compelling evidence for regional regulation of iron uptake. The mechanisms of brain iron uptake and their regulation will be considered in this review. Furthermore, we examine the evidence that regulation of brain iron uptake is not only regionally selective but developmentally sensitive. For example, iron rich brain regions such as the substantia nigra do not become iron rich until adolescence in humans (Sourander & Hallgren 1958) and between 60 and 75 days of age in the rat (Roskams & Connor 1994). Finally, we discuss the numerous neurological diseases that are associated with either too much or too little brain iron concentrations.

During postnatal development of the rat, the amount of iron in the brain is highest at birth, declines over the first two weeks of life and then begins to increase. The amount of iron in the brain during this time can be influenced by diet and alcohol consumption (Miller *et al.* 1995). The importance of sufficient and timely iron delivery to the brain has been repeatedly demonstrated. Iron in the brain is used as a cofactor in ATP and DNA synthesis, as it is in the rest of the body, but the brain also has unique requirements for iron such as in neurotransmitter and myelin synthesis. These latter activities are regionally and developmentally regulated and thus are particularly vulnerable to disruptions in iron delivery during critical periods of

development. The ability of these systems to recover from insufficient iron delivery during the critical periods of development is an important area that has not been studied in sufficient detail (Erikson *et al.* 1997). Recent evidence suggests that both loss and recovery of iron levels following supplementation occurs in a region specific and age dependent manner. There are numerous studies reporting cognitive and motor impairment in children and adults who are iron deficient (Deinard *et al.* 1986; Tu *et al.* 1994; Cook *et al.* 1994) and learning impairments at a young age have been demonstrated in both laboratory animals (Yehuda *et al.* 1986) and in humans (Palti *et al.* 1985) that were iron deficient. Restless Leg Syndrome (RLS) is a sensory disorder characterized by akathisia, an uncontrollable, irresistible urge to move the limbs, with a night predilection to these symptoms. MRI studies (Allen *et al.* 2001) and CSF analysis (Earley *et al.* 2000) have shown that a significant percentage of patients who present with RLS have low levels of iron in the brain.

Brain iron accumulation in humans increases dramatically until the third decade of life and then reaches a plateau (Hallgren & Sourander 1958; Bartzokis *et al.* 1997). However, in many neurological diseases (discussed later), excess iron accumulation is frequently observed in specific brain regions that are associated with decreased function and cell loss. Damage associated with too much iron is presumably due to the direct relationship between iron and oxidative injury. The neurological diseases in which iron accumulation is abnormal are presented in Table 1.

Neurodegenerative diseases

These diseases and the association with iron have recently been reviewed in detail (Pinero & Connor 2000). A summary of these disorders is included in this review. In Alzheimer's Disease, iron is associated with neuritic plaques, the hallmark pathological change in AD. The key component of neuritic plaques is amyloid and the production of amyloid may be influenced by cellular iron status (Mantyh *et al.* 1993; Tanzi & Hyman 1991). Furthermore, the deposition of the toxic fragments of amyloid is enhanced by iron (Atwood *et al.* 1998). Also, in AD, iron accumulates in the brain at a pace that is faster than ferritin production. This relative excess in iron compared to ferritin occurs in those regions of the AD brain that undergo the most degeneration (Connor *et al.* 1992b) and would increase the likelihood of oxidative injury (Connor, 1992a).

Another prominent neurological disease in which iron accumulation is excessive relative to the amount of ferritin expression is in Parkinson's Disease. This relationship of iron to ferritin is specifically altered in the substantia nigra and striatum which are areas that undergo cell loss in this disease (Earle, 1968; Dexter *et al.* 1989; Dexter *et al.* 1991; Sofic *et al.* 1988). A consistent histopathological finding in Parkinson's Disease is the aggregation of a-synuclein into Lewy bodies. This aggregation is promoted by iron, specifically for the mutated form of α-synuclein associated with familial Parkinson's Disease (Ostrerova-Golts *et al.* 2000).

In neuroferritinopathy, a disease with similar extrapyramidal symptoms as Huntington's and Parkinson's diseases, abnormal aggregates of ferritin and iron are present in the basal ganglia (Curtis *et al.* 2001; Sequeira 2001).

Deficiencies in Fe-S dependent enzyme activities, in cytochrome complexes I, II, and III, and in aconitase activity have been associated with Friedrich's Ataxia (FA), secondary to a mutation in the *frataxin* gene (Rotig *et al.* 1997). Increased levels of iron are also present in the dentate nucleus of affected cerebelli in FA (Waldvogel *et al.* 1999). Frataxin appears to be responsible for iron export from mitochondria. The specific accumulation of iron in the basal ganglia of this disorder and the motor impairment associated with this disorder are taken as indication of the dynamic nature of iron flux in mitochondria and of possibly elevated activity of mitochondria and iron in this brain region. Indeed the iron rich areas of the brain all have in common a role in motor activity. Even within the cerebral cortex, the motor cortex has higher iron concentrations than other cortical areas (Connor *et al.* 1990).

In Hallervorden-Spatz disease, there are large amounts of iron in the globus pallidus and substantia nigra pars reticulata (Swaiman 1991). It has recently been discovered that a novel mutation in pantothenate kinase (PANK) may lead to accumulation of cysteine which binds iron and the cytsteine-iron complex accumulates (Zhou *et al.* 2001) accounting for the iron accumulation seen with this disorder.

In Huntington's chorea, iron accumulates in the basal ganglia prior to the onset of clinical symptoms (Bartzokis *et al.* 1999) suggesting that iron accumulation is part of the disorder and not a consequence of cell loss. Cells transfected with the mutant form of the

Table 1. Neurological diseases and their associated alterations in iron status.

Neurological disease	Involvement of iron
Tardive dyskinesia	Increased striatal iron
Hereditary hemochromatosis	Increased iron in choroid plexus and pituitary, basal ganglia Presence of mutation influences onset/incidence of AD
Aceruloplasminemia	Increased basal ganglia and CSF iron
Restless legs syndrome	Decreased plasma and CSF ferritin Increased CSF transferrin Decreased iron in substantia nigra and putamen
Huntington's disease	Increased iron in striatum, occurs presymptomatically
Freidreich's ataxia	Increased mitochondrial iron in striatum and cerebellum Mutation in frataxin
Multiple sclerosis	Loss of ferritin receptors in periplaque white matter Increase ferritin in CSF Iron in oligodendrocytes exacerbates oxidative stress Treatment with chelators or antioxidants decreases symptoms and pathology in animal models
Parkinson's disease	Iron accumulation in substantia nigra, striatum, and in neuromelanin-containing cells Decreased levels of ferritin in substantia nigra, caudate, putamen
Alzheimer's disease	Iron accumulation in brain regions associated with neurodegeneration Ferritin decreased in areas that accumulate iron Increased iron in amyloid plaques Changes in Iron Regulatory Protein (IRP) distribution and activity TfR decreased in hippocampus
Hallervorden-Spatz	Iron accumulation in globus pallidus, substantia nigra
Neuroferritinopathy	Iron and ferritin accumulation in the basal ganglia Low serum ferritin levels

huntingtin protein have abnormalities in the trafficking of transferrin (Hilditch-Maguire *et al.* 2000).

A report exists that in Multiple Sclerosis, the prototype of demyelinating diseases, iron accumulates in the basal ganglia (Bakshi *et al.* 2001). However, it is more likely that the oligodendrocytes, iron rich cells in the white matter, are the target in Multiple Sclerosis. Oxidative injury is hypothesized as a major component of Multiple Sclerosis. Both iron chelation and anti-oxidants have been shown to limit the clinical symptoms and pathological changes in a mouse model of MS (Forge *et al.* 1998). Other demyelinating diseases such as Pelizaeous-Merzbacher (Koeppen *et al.* 1988; Jaeken *et al.* 1984), central pontine myelinolysis (Gocht & Lohler 1990) and progressive rubella panencephalitis (Valk & van der Knaap 1991) reportedly are associated with changes in iron and transferrin CNS content.

Mechanisms for brain iron uptake

In most areas of the brain, the plasma membranes of vasculature endothelial cells are in close apposition to one another, forming tight junctions. These tight junctions are also present in the endothelial cells lining the vasculature of the testes, but do not exist elsewhere in the periphery of the body. To enter the brain, iron must

pass through the endothelial cells lining the vasculature, or the ependymal cells that line the ventricles. In a few select regions where the blood-brain-barrier is lacking, iron can enter the brain directly. The blood brain barrier (BBB) was thought to have developed to protect the brain from large hydrophilic compounds that may have potentially harmful effects. While this function may spare the brain from unwanted effects, it also necessitates the presence of specific transporters for compounds that the brain needs to perform its daily functions. These transporters have already been described for compounds such as glucose (Boado & Pardridge 1990; Rahner-Welsch *et al.* 1995) and insulin (Pardridge *et al.* 1985). In this review, we will discuss transport of iron into the brain from the blood and CSF, and the transporters that play a role in facilitating this transport.

Transferrin and transferrin receptor

Non-heme iron circulates in the blood mainly in the form of transferrin-ferric iron (Tf-Fe) complexes. In other parts of the body, Tf-Fe can be transported from the vasculature paracellularly, due to the lack of endothelial cell tight junctions. But, as mentioned above, the tightness of the blood brain barrier precludes any significant paracellular transport. A specific transferrin receptor (TfR) is necessary to accept the Tf-Fe complex from the blood.

Transferrin receptors on brain endothelial cells were first demonstrated by injection of OX-26, a mouse anti-transferrin receptor antibody, intravenously into the rat (Jefferies *et al.* 1984). This study showed that transferrin receptors are present at the luminal surface of the rat brain blood vessels, while vasculature staining of peripheral rat organs with the OX-26 antibody was not seen. Transferrin receptor was also localized to the human BBB using the B3/25 human transferrin receptor monoclonal antibody in the same study. There is a high density of TfR expression on the brain microvasculature in the adult brain, 6–10 times higher than the expression on the brain parenchyma (Kalaria *et al.* 1992). The kinetics of transferrin binding to isolated human brain capillaries has been evaluated (Pardridge *et al.* 1987). It has been suggested that both aluminum (Roskams & Connor 1990) and gallium (Murphy & Rapoport 1992) enter the brain by utilizing the extant system for iron uptake. A promising approach to exploit the Tf-TfR system in an attempt to deliver trophic factors into the brain to prevent or retard neurodegeneration has been undertaken, (Friden *et al.* 1993; Pardridge *et al.* 1994), but these studies have been hampered by our general lack of understanding of iron/transferrin transport in and through endothelial cells lining the brain vasculature.

One of the first studies designed to address this question involved perfusion of ^{125}I-Tf into the rat brain (Fishman *et al.* 1987). A pulse of ^{125}I-Tf was followed by a chase period, in which there was a decrease in radioactivity associated with the endothelial cells and an increase in radioactivity associated with the non-vascular elements of the brain. This finding seems to argue for transcytosis of a Tf-Fe complex. However, this study did not determine whether or not the iron bound to the radiolabeled transferrin was transported into the brain parenchyma or if it was removed and remained in the vasculature. Other studies using radiolabeled Tf contradict the above findings. Analysis of ^{125}I-Tf perfused into the rat brain showed only 10% of the initial perfused radioactivity was associated with the brain (Roberts *et al.* 1991). This small pool of remaining radioactivity is assumed to be either ^{125}I-Tf that was transcytosed into the brain or ^{125}I-Tf that was not recycled in the endothelial cells and thus remained in the vasculature.

A subsequent study showed that iron is transported across the blood-brain barrier at a greater rate than transferrin (Banks *et al.* 1988). It was found that the brain to blood ratio was 14.7 ± 0.9 μl/g brain for radiolabeled iron versus 11.7 ± 0.6 μl/g brain for radiolabeled transferrin. The iron saturation level of transferrin used in this study was not clear. For transferrin fully saturated with iron, which is the preferred form of Tf by the TfR, one might expect a 2:1 stoicheometry if the iron-transferrin complex is transported across the blood brain barrier intact. Because whole brain was analyzed for radioactivity after application of peripheral iron and/or transferrin, it may be that transport of some iron-transferrin complex is taking place at the circumventricular organs, resulting in misleading transferrin/iron ratios. These organs are served by vasculature that does not have the tight junctions seen in the rest of the brain, leading to the possibility of paracellular transport.

Studies combining transferrin-horseradish peroxidase (Tf-HRP) complexes with electron microscopic evaluation have found Tf-HRP in coated vesicles within the vascular endothelial cells, although no evidence of Tf-HRP transcytosis was seen (Roberts *et al.* 1992). These findings would seem to argue for removal of iron from Tf within the endothelial cell. However, HRP-conjugated compounds may not

be transported within cells in the same manner as naturally occurring complexes. Furthermore a previous study demonstrated HRP permeation into the brain only at the circumventricular organs that are not protected by the BBB (Broadwell & Brightman 1976).

The hypotransferrinemic (*hpx*) mouse is an important model in discerning the mode of iron transport across the BBB. This mouse has less than 1% of normal circulating Tf levels in its blood (Bernstein 1987) due to a defect in splicing of Tf precursor mRNA (Huggenvik *et al.* 1989). These animals die within the first week of life unless they are supplemented with serum or purified Tf. Although the hpx mice also have no endogenous brain transferrin, peripheral supplementation with human apo-Tf is sufficient for normal delivery of iron to the brain and for a normal oligodendrocytic Tf and iron staining pattern (Dickinson & Connor 1995). This result indicates that Tf crosses the BBB. The integrity of the BBB in these animals is reportedly normal (Ueda *et al.* 1993).

Iron uptake into the developing brain prior to the formation of the BBB is a separate consideration. In the rat brain, there is no evidence of histologically stainable iron at embryonic day 10 (E10). But by E14, iron-containing endothelial cells line the blood vessels in the brain (Moos 1995). The iron content in these endothelial cells, as indicated by the Perl's-DAB histochemical reaction, decreases to reach a low point by P15. Closure of the blood-brain barrier generally occurs during the first few weeks of life in the rat, with some regional variation (Dobbing 1968). The decrease in endothelial cell iron staining coinciding with the formation of the BBB may represent increased iron movement into the brain from the endothelial cells. During the first two weeks of life, many processes requiring large amounts of energy are at their peaks, including replication of glial cells, myelinogenesis and mitochondriagenesis. Uptake of iron and transferrin in the rat both peak at approximately day 15. This latter study used ^{125}I-Tf-^{59}Fe (Taylor & Morgan 1990) and reported a slow accumulation of transferrin occurs, but this accumulation is much less than that of iron. This latter observation indicates that the majority of iron that reaches the brain is removed from transferrin within the endothelial cell, whereas the majority of transferrin is recycled back into the plasma. It is possible that the mechanism of Tf-Fe transport changes during development, which may explain the evidence for Tf transcytosis seen by Fishman's group (Fishman *et al.* 1987) noted above. This proposed mechanism change during development may be triggered by or reflected in the decrease in stainable iron in endothelial cells as noted above.

Endogenous brain transferrin

The brain is the only organ in which the mRNA for Tf increases after birth (Thomas *et al.* 1989). This postnatal increase coincides with and is dependent upon the maturation of oligodendrocytes (Bartlett *et al.* 1991). Oligodendrocytes, the main transferrin producing and iron staining cells in the rat brain, do not appear until postnatal day 10 (Connor & Fine 1987). Oligodendrocytes are responsible for 95% of the Tf in the brain (Connor *et al.* 1987). The other source of Tf mRNA in the brain is the choroid plexus, and Tf mRNA is present in this organ at least by postnatal day 7 (Connor *et al.* 2001). The original hypothesis was that Tf made in the brain obtained iron as it was transported from the endothelial cells into the brain interstitial fluid. However, it has recently been reported that Tf in oligodendrocytes is not secreted (de Arriba Zerpa *et al.* 2000). Brain Tf levels have been shown to increase in animals fed an iron deficient diet, but the source of the Tf is not known to be solely from the brain (Pinero *et al.* 2000). Clearly the concept of movement of iron within the brain and across the BBB requires revisiting.

As mentioned, the choroid plexus contains Tf mRNA, and Tf protein has been shown to be secreted from this organ. Secretion of Tf into the ventricles may be an important delivery mechanism for iron to the rest of the brain. This is especially true during development, when the requirement for iron in the brain is high. In the pig (Cavanagh *et al.* 1982) and sheep (Cavanagh *et al.* 1983), CSF transferrin levels are highest at one month gestation, and transferrin is also found in the human fetal choroid plexus (Mollgard *et al.* 1979). A review of iron status in the CSF has shown that previous measurements of iron and transferrin concentration vary widely (Bradbury 1997). Most of these measurements indicate that transferrin in the CSF may be fully saturated. Sampling and analysis of CSF and brain tissue after peripheral ^{125}I-Tf-^{59}Fe injection indicates that a percentage of iron in the CSF and possibly brain interstitial space occurs as non-transferrin bound iron (Moos & Morgan 1998a). Compounds in the CSF that may also be responsible for transport of iron in the fully transferrin saturated state include citrate (Haerer 1971), ascorbate (Spector *et al.* 1977), and ferritin (Fehling & Qvist 1985; Moos & Morgan 1998a; Earley *et al.* 2000).

Because the total CSF volume is replaced approximately every 8 h, both iron and iron carrying compounds are subject to non-specific, rapid removal from the ventricles. After injection of ^{125}I-Tf-^{59}Fe into the rat ventricle, only 2.5% remains after 4 hours (Moos & Morgan 1998b). Within the brain parenchyma, the level of ^{125}I-Tf declined steadily after intra-cerebroventricular (ICV) injection to less than 1% of dose after 24 h, while ^{59}Fe was retained at 18% of the initial dose even after 72 h. This indicates that iron may be removed from transferrin within the ependymal cells lining the ventricles before it is transported into the brain. We have found that ependymal cells contain high levels of DMT1 (Burdo *et al.* 2001), supporting the idea of iron transport across ependymal cells. ICV injections of ^{125}I-Tf-^{59}Fe into seven day old rats led to a greater retention of radiolabled transferrin in both the CSF and the brain parenchyma as compared to adults, while radiolabeled iron was retained in the parenchyma to a higher degree than in the adult rats (Moos & Morgan 1998b). The greater retention of transferrin in the CSF of young rats probably is a result of lower turnover of CSF as compared to the adults.

The high rate of blood flow and thus Tf-Fe through the choroid plexus has led to the hypothesis that the majority of iron enters the brain through the CSF. This possibility was investigated by Ueda *et al.* who found that uptake of peripherally injected ^{59}Fe into the CSF was significantly less than uptake into the cerebral hemispheres, cerebellum, and brainstem (Ueda *et al.* 1993). Although the choroid plexus may be more densely vascularized than other areas of the brain, the sheer size of the rest of the brain in comparison seems to allow more iron to accumulate directly into the brain parenchyma through the vasculature than what is directed into the CSF. We propose that the CSF is useful for general distribution (and perhaps removal) of iron in the brain whereas the Tf receptors on the vasculature direct regional uptake.

There is a possibility for non-transferrin bound iron (NTBI) transport across the BBB and ependymal cells, particularly when Tf is saturated as occurs peripherally in pathological states such as hemochromatosis (Bacon *et al.* 1993; de Valk *et al.* 2000). Inhibition of transferrin receptors in mouse at levels sufficient to block iron uptake in the spleen blocks only 75% of brain iron uptake (Ueda *et al.* 1993). Using the hpx mouse model, we have shown that iron can be delivered to the choroid plexus in the absence of detectable levels of serum transferrin. However, after 1 week the iron is only detectable in the choroid plexus indicating that transferrin is necessary to move iron from the choroid plexus into the brain or that brain iron uptake other than at the level of the choroid plexus is dependent upon transferrin. These latter two ideas are not mutually exclusive (Malecki *et al.* 1999).

Divalent metal transporter 1

Divalent Metal Transporter 1 (DMT1) is responsible for movement of iron out of duodenal lumen (Canonne-Hergaux *et al.* 1999; Fleming *et al.* 1999), and more importantly to the CNS, acts to transport iron out of endosomes. (Gunshin *et al.* 1997; Burdo *et al.* 2001). This transporter is predominantly found in a location able to influence iron transport into and out of the brain, namely ependymal cells that line the ventricles, blood vessel endothelial cells and astrocytes associated with these vessels The presence of DMT1 within the endothelial cells lining the blood vessels suggests that iron can be removed from the endosome within the endothelial cell. Whether this iron is for use by the endothelial cell or is then transported into the brain remains to be determined. The presence of DMT1 on astrocytes also indicates an involvement for this protein on iron transport into the brain.

Astrocytes have end-feet that envelop the vasculature (Xu & Ling 1994) and DMT1 has a polar expression in astrocytes; it is found only in the process associated with the BBB and occasionally into the soma of the astrocytes (Figure 1). To investigate the role of DMT1 in brain iron transport, the Belgrade (*b*) rat, which has a defect in DMT1 (also known as NRAMP2) (Fleming *et al.* 1998), has been examined. This rat has a hypochromic, microcytic anemia (Sladic-Simic *et al.* 1969) with only 20% normal levels of iron uptake into reticulocytes (Edwards *et al.* 1978). We have shown that iron staining is decreased in *b* rat brains compared to normal (Burdo *et al.* 1999) and the decrease in iron status is also detectable with MRI (Zywicke *et al.* 2002). ^{59}Fe uptake in Belgrade rat brains is only 10% that of normal, while ^{125}Tf uptake is 40% that of normal after peripheral injection of ^{125}I-Tf-^{59}Fe (Farcich & Morgan 1992). The point at which the DMT1 defect affects iron uptake into brain (BVECs or astrocytes) is yet to be determined.

Melanotransferrin (P97)

A homologue of transferrin, p97, has also been reported to be present in the brain (Rothenberger *et al.*

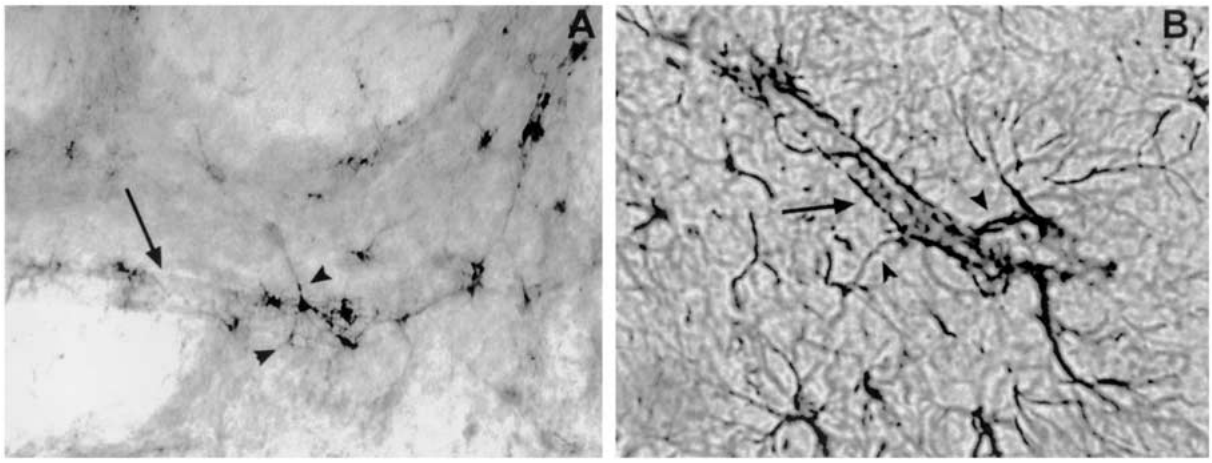

Fig. 1. (A) This is a photomicrograph of DMT1 staining in the rat brain. DMT1 reaction product is found in the blood vessel (arrow) and in the glial processes (arrowhead) that are associated with the blood vessels. (B) This photomicrograph is of glial fibrillary acidic protein (GFAP) immunostaining of the adult rat brain. This micrograph shows the abundance of astrocytes relative to that seen with DMT1 immunostaining. Furthermore, GFAP reaction product can be seen in all processes of the astrocytes including those associated with blood vessels (arrow), as opposed to the DMT1-positive processes in Figure A which are normally only associated with a blood vessel.

1996; Yamada *et al.* 1999). Transport of iron on transferrin-like molecules may also take place at the BBB. Melanotransferrin, or p97, has been localized to the capillary endothelia of the brain (Rothenberger *et al.* 1996). This molecule is structurally similar to transferrin, but has an independent mechanism for iron uptake. Its distribution suggests that it may play a role in uptake of iron into the brain. The protein p97 has also been suggested for use as a marker for Alzheimer's Disease. Reactive microglia associated with senile plaques contain increased levels of p97 (Jefferies *et al.* 1996; Yamada *et al.* 1999), and serum levels of p97 are also increased in the disease (Kim *et al.* 2001).

Hfe

Hfe is a membrane protein that can influence cellular iron uptake (Arredondo *et al.* 2001; Griffiths *et al.* 2001). Hfe has recently been localized to brain vasculature, choroid plexus and ependymal cells that line the ventricles (Connor *et al.* 2001). 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 historical view has been challenged (Connor *et al.* 2001). Indirect measurements of iron using MRI found increased iron in the lentiform nucleus in individuals with hemochromatosis (Berg *et al.* 2000). Carrying one or more of the Hfe mutations has recently been reported to influence the onset of Alzheimer's Disease (Moalem *et al.* 2000; Sampietro *et al.* 2001) and to increase the severity of gliomas (Martinez di Montemuros *et al.* 2001). The increase in onset of AD accompanying an Hfe mutation is consistent with the reported role of iron in AD (Connor *et al.* 1992a; Connor *et al.* 1992b).

MTP1

Metal Transport Protein 1 (MTP1), also known as Ireg1 and ferroportin, was first identified as a cellular iron exporter in the duodenum (Abboud & Haile 2000). It has subsequently been identified in neurons of the rat brain (Burdo *et al.* 2001) mouse and human brain. Very little additional information has been established on the presence or function of this protein in the brain. However, the selective expression in neurons indicates these cells have a mechanism for iron efflux that could be very significant in the context of protection from iron induced oxidative stress.

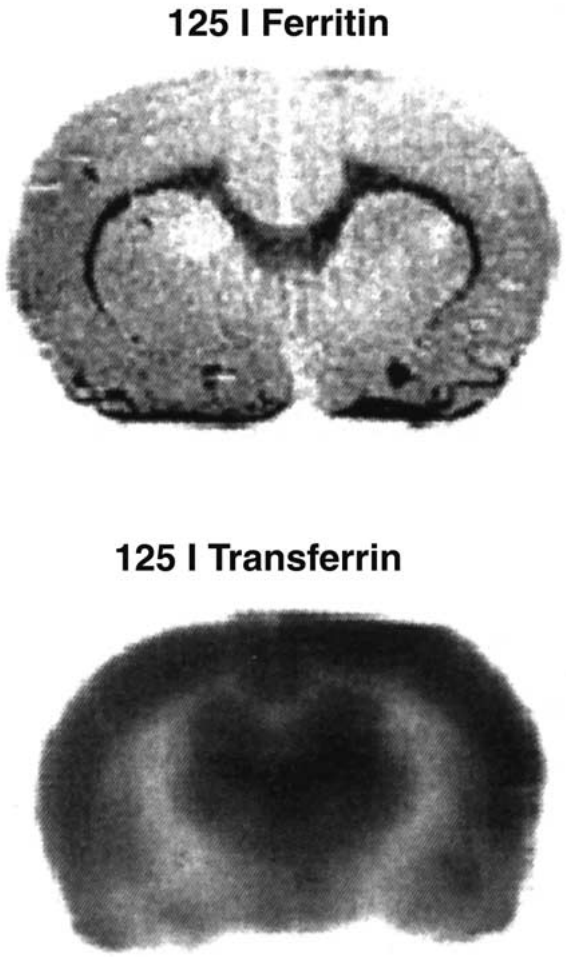

Fig. 2. In this figure, the distribution of ferritin and transferrin binding in the adult rat brain is shown. These are representative autoradiographs showing the differences in the binding distribution of ^{125}I-ferritin and ^{125}I-transferrin. The subcortical white matter is distinct in both micrographs; appearing dark (indicating binding) in the ferritin labeled section and light (no binding) in the transferrin labeled brain section. The anterior commissure is also apparent in cross-section at the base of the brain in the ferritin labeled section as symmetrical black dots near the base of the subcortical white matter. The distribution of the transferrin binding is consistent with other reports for rat brain (Hill *et al.* 1985; Mash *et al.* 1990) and our report in the human brain (Hulet *et al.* 2000). The distribution of ferritin binding in the rat is consistent with our reports in the adult mouse and human (Hulet *et al.* 1998; Hulet *et al.* 1999). The binding studies on the rat brain were performed by Sharon Menzies.

Ferritin

Ferritin has classically been known as an iron storage protein in many types of cells, including those of the brain. The cellular and regional distribution of ferritin in brain has been discussed elsewhere (Benkovic & Connor 1993; Connor *et al.* 1990) and this review is limited to a discussion of ferritin in brain iron transport. Recent reports have indicated the presence of a ferritin receptor in the brain that is predominantly expressed in white matter (Hulet *et al.* 1999a; Hulet *et al.* 1999b). This distribution indicates the presence of two, non-overlapping receptor systems for iron in the brain, as transferrin receptors are mainly located in grey matter regions of the brain (Figure 2).

The selective expression of a ferritin receptor on the myelin producing cells, oligodendrocytes, indicates these cells have developed their own iron uptake system (Hulet *et al.* 1999a, b, 2000). It is well established that iron acquisition by oligodendrocytes is essential for normal production of myelin. Hypomyelination is a consistent and predominant effect of iron deficiency in humans and animal models (Pinero & Connor 2000) and ferritin receptor expression in myelin tracts is altered in Multiple Sclerosis (Hulet *et al.* 1999b).

The expression of a ferritin receptor ensures that oligodendrocytes do not have to compete for Tf delivered iron in the brain and also provides these cells with the opportunity to acquire much more iron than could be delivered by Tf. The uptake of ferritin into oligodendrocytes is ATP dependent and clathrin dependent (Hulet *et al.* 2000). Alterations in ferritin levels in brain could influence iron delivery to the oligodendrocytes. Too little iron via ferritin could promote demyelination but also, elevated ferritin could be a source of iron for inducing iron mediated oxidative injury. Ferritin is present in the CSF and thus could be expected to access the interstitial fluid in the brain. The levels of CSF ferritin are roughly 10% of the levels of ferritin found in the serum (Campbell *et al.* 1986). Normal CSF contains approximately 3 ng/ml ferritin, but this concentration will increase during infectious meningoencephalitis, CNS vascular diseases and dementia without vascular pathology (Sindic *et al.* 1981). Marked elevations of CSF ferritin (30-fold) were observed in patients with bacterial or fungal meningitis in contrast to modest CSF ferritin elevations in patients with viral meningitis. Ferritin is also elevated in the cerebral spinal fluid of MS patients (LeVine *et al.* 1999). Consequently, CSF ferritin

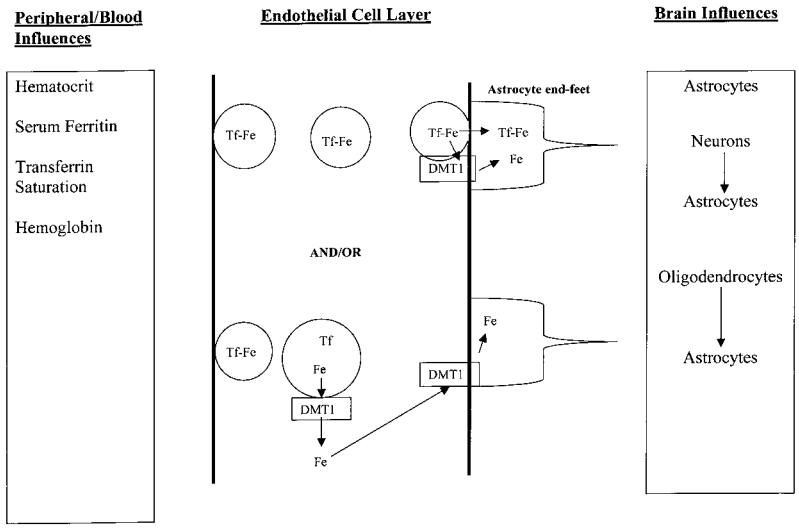

Fig. 3. This is a schematic to demonstrate the possible mechanisms for uptake into the brain across the blood-brain-barrier and the sites of influence upon the regulation of that uptake. Iron uptake at the endothelial cell involves interaction with a transferrin receptor and internalization of the Transferrin (Tf)-Tf receptor complex. The Tf receptor is internalized with Tf bound iron (Fe) but is not shown within the endosome for clarity. The top model shows transport of Tf-iron across the endothelial cell in a transcytotic vesicle. The iron is removed when the vesicle docks at the abluminal membrane. The divalent metal transport protein 1 (DMT1) present on the vesicle or the abluminal membrane assists in the movement of iron out of the endosome and into the glial end foot on the abluminal membrane of the endothelial cell. The bottom model suggests that following endocytosis, iron is removed from the endosome within the endothelial cell via DMT1. The released iron then is escorted by currently unknown mechanisms to ferritin for storage, to the labile iron pool (LIP) for the needs of the endothelial cells or to the abluminal membrane for transport into the glial end-foot process. The two proposed models are not mutually exclusive and the iron status of the brain or the endothelial cell may dictate the predominant system at any given time. This latter point leads to the question of regulation of brain iron uptake. The current status for regulation of cellular iron uptake is that the intracellular iron level (labile iron pool) is maintained by the coordinated expression of ferritin and transferrin receptors. In the case of the endothelial cells of the BBB, these cells are exposed on both the luminal and abluminal membranes by factors that could influence iron uptake and these influences could be competing rather than working in concert. The factors in the serum (box on the left) carry information regarding systemic iron status. In the box on the right are the cells in the brain that have their own iron requirements. There is presumably communication between these cells and the endothelial cells. This communication may be through the astrocytes whose glial end-feet line the abluminal surface of the endothelial cells. Thus, the box on the right also attempts to show a possible hierarchical system where the neurons report their iron status to the astrocytes which then signal the endothelial cells via the glial end-feet. The reader should keep in mind that the influence from the brain is region specific given the different concentrations of iron that are found among the different brain regions.

could be a valuable early clinical tool for differential diagnosis (Campbell *et al.* 1986).

In contrast to serum ferritin, which is highly glycosylated (> 70%), ferritin within the CSF is largely non-glycosylated (< 20% glycosylated). Non-glycosylated ferritin is usually considered as 'tissue ferritin' suggesting that CSF ferritin is derived from cell death and subsequent ferritin release (Zappone *et al.* 1986). However, the concentration of ferritin within the CSF of normal patients is significantly higher than that which would be expected to occur by passive diffusion across the blood-CSF barrier (Keir *et al.* 1993). This suggests that local synthesis and secretion of ferritin by brain cells occurs normally.

Lactoferrin

Receptor-mediated transcytosis of lactoferrin (Lf), an iron binding glycoprotein similar to transferrin, has been demonstrated in primary cultures of bovine vascular endothelial cells (Fillebeen *et al.* 1999). Binding of ^{125}I-Lf to these cells is saturable, concentration dependent, and inhibited by unlabeled Lf. Double labeling of Lf and Fe in this study demonstrated transcytosis of intact holo Lf across the endothelial cell monolayer. An interesting aspect of this experiment is that Lf transport was 70% inhibited by the receptor-associated protein (RAP), a specific antagonist of the low-density lipoprotein receptor-related protein (LRP). This indicates an involvement for LRP in lactoferrin transport. Lactoferrin receptors have also been reported on neuromelanin cells in the substantia

nigra (Faucheux *et al.* 1995). Lactoferrin is present in the normal human cortex, but is present at higher levels in neurodegenerative diseases such as Parkinson's disease (Leveugle *et al.* 1996).

Conclusion

The brain presents unique challenges to the study of iron transport because it resides behind an endothelial cell barrier, has multiple regions with differing metabolic requirements, and has a variety of cell types with specialized functions and hence specialized iron requirements. Despite the importance of iron delivery to the brain, the mechanism by which the brain acquires iron or how the mechanism is regulated is poorly understood. A proposed model must take into account the mechanism for iron uptake and transport across the endothelial cells lining the blood vessels as well as the factors that impinge on this system to modulate uptake. Furthermore, the regulation of uptake is apparently brain region specific because of the heterogeneity of iron distribution in the brain. Our working concept is that iron released from the choroid plexus is transferrin dependent and is responsible for maintaining general brain iron homeostasis. However, meeting iron demands at the regional level requires regional regulation of iron uptake. This regional regulation is maintained by expression of TfR on the endothelial cells of the BBB and input from the specified region. A model is proposed in Figure 3.

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